ASSESSMENT OF ILLEGAL BUSHMEAT USING HIGH THOROUGHPUT MOLECULAR TOOLS IN TRANSBOUNDARY VILLAGES BORDERING SERENGETI ECOSYSTEM IN TARIME DISTRICT, TANZANIA

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ABSTRACT

Detection of illegal bush-meat in Africa has over the years relied on morphological identification technique, which is less effective due to post-hunting procedures. Therefore, the current study aimed at assessing bush-meat dynamics in trans-boundary areas of Tanzania and Kenya in the western part of Serengeti ecosystem. A cross-sectional design with two different methodologies was used. First, semi-structured questionnaires were administered to collect information on socio-demographic and other social factors leading to illegal bush-meat hunting, preference and consumption. Secondly, to augment the information obtained from the questionnaire, qPCR-HRM analysis was employed by using three different molecular markers 16s, Cytb and COI for molecular identification of assumed 138 collected sundried bush-meat samples. The results indicated that hunting occurs mostly in the dry season primarily using snares, and wildebeest was revealed to be the most hunted. Furthermore, young demonstrated high bush-meat consumption whereas immigrants showed high bush-meat preference. Likewise, highly educated young respondents indicated to have a high consumption and preference than less educated old respondents. Regarding molecular identification, 20 species were identified altogether, with zebra constituting the majority (n=51, 49.5%). It is recommended that high surveillance against poaching is needed by wildlife authorities during dry season; proper disposal of the unworthy wire cables by TANESCO; sensitization of both primary and secondary school students on legal harvesting of wildlife were made. Other recommendations focused on the need for proper execution of HRM procedures for bush-meat identification; and that for bush-meat samples to be accepted in court of law as exhibits should be analyzed using molecular procedures that proved to be reliable.

DECLARATION

I, Shadia Ibrahim Kilwanila, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Shadia Ibrahim Kilwanila Date

(MSc. Candidate)

The declaration is hereby confirmed;

_____ _____

Prof. B. Mutayoba Date

(Supervisor)

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Dr. N. E. Mbije Date

(Supervisor)

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DEDICATION

My first dedication goes to the Almighty God. I also dedicate my work to my lovely family for love, encouragement and support during my studies.

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AFLP	Amplified Fragment Length Polymorphism
Cq	Quantification Cycle
Cytb	Cytochrome b
(COI)	Cytochrome C oxidase subunit I gene
HRMA	High Resolution Melting Analysis
MIC	Magnetic Induction Cycler
MtDNA	Mitochondria DNA
PCR	RFLP-Polymerase chain Reaction-Restriction Fragment Length Polymorphism
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
Rrna	Ribosomal RNA
Tm	Melting temperature

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Poaching of wildlife is among the illegal activities that threatens biological diversity (worldwide) (Keane *et al*., 2008; Mateo-Tom as *et al*., 2012). These actions are more intense in the African continent where local people appreciate the role of biodiversity in culture, diets and economy (Hilborn *et al*., 2006; Mfunda and Roskat, 2010). Bush-meat is crucial source of protein to those living adjacent to protected areas and is driven by subsistence, commercial and cultural needs (Nielsen, 2006; Bennett *et al*., 2002). Subsistence hunting is mainly hunting for meat and involves the use of traditional methods such as bows and arrows, snares and pitfalls (Holmern *et al*., 2004; Holmern *et al*., 2006). Commercial hunting is mainly hunting for trophies, and is organized in terms of hunting gears, transportation and marketing in which hunting may take place in one country, processing and selling in the other (Wasser *et al*., 2009).

On a bigger scale, bush-meat hunting is inclined by rise in human population which leads to encroachment of the protected areas due to settlement and agricultural activities which lead to human- wildlife conflicts , poverty, fragile governance, and poor law enforcement (Bohne, 2008; Madulu,2001; Mfunda and Roskat, 2010;Nelson, 2003). These factors make bush-meat hunting a conservation concern because of its hostile effects in wildlife populations and biodiversity hence there is the need to heighten conservation efforts (Hilborn *et al.*, 2006). However, such efforts have been curtailed by the continued illegal exploitation of wildlife species (Campbell *et al*., 2001).

The intensification of illegal bush-meat hunting is a root of biodiversity loss and decline of wildlife populations in Africa (Ripple *et al*., 2015: Mfunda and Roskat, 2010). For

example in West Africa, bush-meat is used as food, commodity to trade, and play a crucial role in rituals (Wilkie *et al*., 2011). In Central Africa bush-meat is a source of income for local people who have limited alternative income sources. In Gabon, hunting accounts for around 15 - 72% of the average household income (Smith *et al*., 2008). In Eastern Africa, unsustainable bush-meat hunting is a conservation concern too (Barnett, 2000). In Kenya, bush-meat hunting occurred in 96% of the protected areas and 25% of the meat in Nairobi butcheries was bush-meat (Okello and Kiringe, 2004; Olupot *et al*., 2009).

In Tanzania about one third of total area is protected (Thirgood *et al*., 2004), but the fact that animals do not have boundaries, moving freely from one place to another exposing them to various calamities such as bush-meat hunting which is an important activity and threatens all categories of protected areas including the Game Reserves, Game Controlled Areas and National Parks in different ecosystems (Baldus *et al*., 2003; Bitanyi *et al*., 2012; Holmern *et al*., 2002; Loibooki *et al*., 2002). Other calamities include road kills and zoonotic diseases (Kideghesho, 2006). These mishaps will remain unabated in this country as the human population continue increasing at the current rate of 3.12% per annum exacerbating illegal hunting and use of bush-meat in these areas (Milner-Gulland *et al*., 2003) thus threatening conservation initiatives close and within wildlife areas (Madulu, 2001). The observed trend in increase in illegal harvests poses a serious threat to biodiversity loss becoming a major management challenge for conservation authorities in Africa (Redmond *et al*., 2006).

The Serengeti ecosystem with a total area of about 25 000 km^2 is well known for its diversity and abundance of wild mammals. The ecosystem encompasses Serengeti National Park (SNP), Ngorongoro Conservation Area (NCA), Grumeti Game Reserve

(GGR), Ikorongo Game Reserve (IGR), Maswa Game Reserve (MGR) and Loliondo Game Controlled Area (LGCA) (Sinclair, 2008). The Serengeti National Park covers the largest area (14 763 km^2) and is highly protected from human activities limited only to photographic tourism. Despite this the area continues to suffer much from illegal wildlife harvest which was worse from 1979-1989 and made the Tanzanian government to launch a major anti-poaching initiative called "operation uhai". The estimate of wildlife loss to illegal hunting for bush-meat was reported to range from 40 000 to 200 000 animals per year with vast majority being wildebeests (*Connochaetes taurinus*) (Mfunda and Roskat, 2010; Bitanyi *et al.,* 2012). The harvesting rates of other animals in Serengeti such as buffalo (*Syncerus caffer*), giraffe (*Giraffa Camelopardalis*), impala (*Aepyceros melampus)* and topi (*Damaliscus lunatus*) are alarming (Kideghesgo *et al*., 2006). Whereby buffalo was reported to be reduced by 50%-90% out of their range (Holmern *et al*., 2002; Holmern *et al*., 2006).

Following the seriousness of illegal hunting problem, the government of Tanzania has taken serious efforts, one being introduction of the paramilitary system in wildlife sector which help to enhance capacity of protection officers in fighting against poaching. The other being enactment and enforcement of wildlife conservation Act of 2009 which is guided by the wildlife policy of 2007 (Ogden *et al.,* 2009). However, the execution of these laws has over years, continued to face challenges as a number of culprits which have been apprehended and brought before justice have failed to be convicted. This is due to two reasons; first, bush-meat is sometimes sold in the form that is difficult to identify morphologically by naked eyes (Bitanyi *et al*., 2012; Campbell *et al*., 2001). Second, lack of standardized protocols put up at the national level relating to wildlife crime apprehension, evidence collection, analysis, investigation and presentation of wildlife evidences in courts of law which result in the dismissal of many evidences since they are

not credible. Therefore, understanding and mitigating the bush-meat market and effective law enforcement requires credible forensic evidence, which first relies on the accurate identification of the bush meat being traded to species level (Ogden *et al.,* 2009).

For court exhibits to which species identification is required, the available methods which have been used mostly over the years depends on identifying bush-meat using anatomical features or/and using information given by local people. Bowen–Jones *et al*. (2002) reported that relying on people's identification alone might not give the precise information needed as the bush-meat trade is sometimes complex and poorly understood. According to the study done by Bitanyi *et al*. (2011), it was reported that the correctness of the species identification given by the bush-meat providers in Tanzania was relatively low (59%) compared to molecular identification methods. This is due to the fact that true species identity of bush-meat samples might be subjected to errors that arise in the chain of trade as the meat passes through many middlemen before reaching the consumers (Ndibalema and Songorwa, 2008).

To enable conservationists and national wildlife corps engaged in the control of bush-meat activities formulate sound conservation strategies, it is usually important to know the composition of harvested species so that sound management plans can be instigated (Thirgood *et al*., 2004). This in turn requires the institution of credible forensic tools which can accurately be used to identify the bush-meat traded to species level (Ogden *et al.,* 2009). Such credible forensic tools are still missing in Tanzania but are currently being developed for both elephant and bush meat species (Bitanyi *et al*., 2011; Bitanyi *et al*., 2012; Wasser *et al*., 2008; Wasser *et al*., 2009).

1.2 Problem Statement and Justification

Wildlife crimes such as illegal bush-meat hunting and trade affects economies negatively by depleting valuable wildlife resources at the other impeding conservation efforts and tourism industry. Illegal hunting and consumption of bush-meat also presents public health concerns due to potential transmission of zoonotic diseases like ebola and anthrax (Karesh *et al*., 2005; Karesh *et al.,* 2009). Detection of illegal bush-meat meat in Tanzania like other African countries has over the years relied on morphological identification, which are destroyed by post-hunting procedures such as drying, de-boning, cooking or other preservation methods (Bitanyi *et al.,* 2011). In order to deter illegal hunting and trade of bush-meat and other wildlife products, the recent advent of molecular forensic science (also known as "silent witness") which is associated with application of DNA for accurate identification of individuals has significantly revolutionized security across the globe (Eaton *et al.,* 2009). To date the use of DNA techniques remains to be an effective diagnostic forensic tool for species identification, overcoming the problems of traditional morphology based identification methods (Wong and Hanner, 2008; Bitanyi *et al.,* 2011).

Molecular markers 16S, cytb and COI (Bitanyi *et al*., 2012; Olayemi *et al.,* 2011; Omondi *et al.,* 2015; Ogolla *et al*., 2017) for DNA analysis have replaced earlier methods of identification, such as restriction fragment length polymorphism (RFLP) (Tannock, 2002), random amplification of polymorphic DNA (RAPD) (Partis and Wells, 1996) and amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995), which suffer poor reproducibility and thereby limit the development of reference databases (Yasui *et al*., 2004; Datwyler and Weiblen, 2006). More recently, Sequence Tag Repeats (STRs) (Ellegran *et al.*, 2012; Nicklas *et al*., 2012) and Single Nucleotide Polymorphism (SNPs) (Liew *et al*., 2004; Reed and Wittwer, 2004) have been used, which are however limited to detecting very specific wildlife species.

Recently, molecular forensic evidence admissible in court for species ID requires the use of standard forensic procedures based on DNA barcodes sequences developed on 650bp region of Cytochrome C oxidase subunit I (COI) gene. 659bp region of COI was proposed as a standard barcode region distinguishing and identifying species with a short standardized gene sequence (http://www.barcodeofwildlife.org/). This procedure entails conducting DNA extraction often using commercial kits following PCR amplification of 650bp region of COI followed by DNA sequencing. Hebert *et al.* (2003) revealed a deep genetic divergence among 13 000 closely related species groups from different animal phyla using 650bp region of COI, thus enabling reliable species identification.

The first work on the use of COI for species identification in Tanzania was reported by Bitanyi *et al*. (2011) who described the use primers targeting the 650bp of COI gene to identify antelope and bovid species using sequences down to 100 base pairs (bp) in bush-meat samples collected from Western Serengeti ecosystem.

However, this approach is costly and time consuming for routine bush meat surveillance hence limits the number of samples that can be analyzed using standard DNA forensic procedures. As a result DNA sequencing remains an uncomprehensive undertaking and less affordable in many countries like Tanzania where court penalties are prohibitively low to pay for the laboratory work involved. In addition, the equipment cost, maintenance and operational expertise requirements prohibits the extensive use of this approach in this country. The reliable detection of species for the monitoring and control of the illegal trade in wildlife products can only be guaranteed by the development of the accurate and cost-effective DNA scanning tools for bush-meat exhibits (Hebert *et al*., 2003). This helps to eliminate the possibility of sequencing every evidence submitted in case of multiple submissions of exhibits which might be originating from the same species as

well as those of domestic origin. However, once this is done, the finally selected exhibit need to do through the barcoding and sequencing procedure.

High-resolution melting (HRM) analysis is a fast, sensitive and specific tool developed for genotyping Polymerase chain reaction (PCR) product sequence variations (Wittwer *et al*., 2003) that employs the use of intercalating fluorescent dyes, such as SYBR Green I (Adaszek *et al*., 2010) or Eva Green (Mao *et al*., 2007). The dyes undergo rapid solvent fluorescence quenching as the duplex DNA is melted. The amplicon melting temperatures (Tm) and specific melt curve shapes are dependent on DNA complementarity, G-C content, and amplicon length (Wittwer, 2009; Vossen *et al*., 2009). While HRM analysis has been used with a number of genes for identification of species in viruses (Villinger *et al*., 2017), bacteria (Li *et al*., 2012), malaria *Plasmodium* (Kipanga *et al*., 2014), mosquitoes and their blood meals (Omondi *et al.*, 2015), plant products (Ganopoulos *et al*., 2013), animals (Naue *et al*., 2014) and humans (Gidlöf *et al*., 2009), it has not been standardized to support forensic pipelines for identifying illegally traded wildlife products.

The current study aims at using HMRA for the first time in bush meat surveillance for analysis of target bush meat samples in Tanzania targeting Tarime district, which boarders Kenya. Although many traditional inhabitants in these areas are known to be involved in illegal bush meat activities. Also the current extent of these activities, and social economic impact of these activities remains less known. Hence information from HRMA and social knowledge will enhance National and trans boundary bush meat surveillance for investigating wildlife poaching and trafficking across the two countries through providing basis for informed review and harmonization of national and cross border policies, laws and penalties against illegal bush meat trade.

1.3 Objectives

1.3.1 Overall objective

Assessment of illegal bush-meat activities using high thorough put molecular tools in transboundary villages bordering Serengeti ecosystem in Tarime district.

1.3.2 Specific objectives

i. To assess the current bush meat dynamics in Gibaso and Kegonga villages which borders Kenya within the Tarime district.

ii. To use qPCR-HRMA approach for accurate species ID for bush-meat samples collected in the study areas lacking diagnostic morphological features.

1.4 Research Questions

1. What is the current bush meat dynamics in Tarime district?
2. What are the game meat animals being targeted in illegal harvesting in Tarime and Ngorongoro districts?
3. What is the suitability and reliability of HRMA approach for future use in bush meat surveillance in Tanzania?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poaching before and after Independence

Most protected areas in Africa share the common characteristics which are lowest support from local communities living around them and historical poor public relations (Kideghesho *et al.*, 2006). The demotion of natives through conventional conservation policies and laws enacted by colonial and later conceded over to post-colonial governments endorsed this situation since they ended all their customary rights and management approaches (Campbel *et al*., 2001). The natives were not only banded from any political discussion relating to wildlife conservation matters (Madden *et al*., 2015), but also from hunting species of socio and economic status like antelopes, buffalo (*Syncerus caffer*) and hippopotamus (*Hippopotamus amphibius*) (Kideghesho *et al*., 2006). Local people were forceful expelled from their land since the enacted policies abstracted them as a problem in conservation initiatives hence fences and fines approach was introduced (Madden *et al.*, 2015). Local people expected that after independence postcolonial government will restore their lost customary right but since they needed economic support they still embraced the colonial policies, hence this justified their resentment towards wildlife resources (Neuman, 2002; Nelson, 2003).

They violated laws and poached wild animals to unsustainable levels, which triggered the Tanzanian government to adopt stricter ways of dealing with people who did not abide to laws but it did not halt illegal activities (Benjaminsen *et al*., 2012; Neuman 2002). In 1970s and 1980s the situation was worse since the natural resource sector of Tanzanian received only 1.2% of national development budget, this underfunding was due to economic recession that many of African countries went through (Kideghesho, 2006).

This situation lead to poor staffing and inadequate equipment, this accelerated the poaching rate and the most affected animals were rhino (*Diceros bicornis*) and elephant (*Loxodonta africana*) (Wasser *et al*., 2008). For example in Selous Game Reserve alone 50% of these species were lost while in Serengeti rhino were driven to the edge of extinction and elephant population reduced by 80% within a period of ten years 1975 – 1986 (Packer *et al*., 2011). Hence failure of fences and fines (Gadd, 2005).

The failure of fences and fines principled the introduction of Community Based Natural Resource Management (CBNM) and paramilitary approaches (Kideghesho *et al*., 2006; Cox *et al*., 2010). CBNM recognizes local community as crucial towards accomplishing any conservation initiative while in paramilitary approach law enforcement unit and other workers of natural resources sector are given military training so as to fight against poaching (Fabricius *et al.,* 2013). Though these approaches are still not sufficient to fight illegal hunting and lead government of Tanzania to make a serious efforts to contain this problem through wildlife law enactment and enforcement (Ogden *et al.,* 2009; Katikiro *et al*., 2015). Law enforcement patrols together with CBNM approaches attempt to control illegal hunting, but expected economic benefits from the sale of bush-meat tend to offset the costs associated with a low probability of arrest due to lack of valid forensic evidence (Hofer *et al*., 2000; Lindsey *et al*., 2015).

2.2 Poaching Impact on Wildlife Population

Illegal hunting affects wild animals either directly or indirectly (Borgerson, 2015). Illegal hunting doesn't only affect prey like herbivores through direct removal from their ecosystem but also through killing of predators like lions (*Panthera leo*) and spotted hyena (*Crocuts crocuta*) due to their attraction to carcass leading them to being accidentally caught in snares (Holmern *et al*., 2007). This lead to population decline of

wild animals in different protected areas in Africa; for example in Comoé National Park in Ivory Coast declined by 60-90% during the 1970s, Niokolo - Koba National Park in Senegal declined by 60-99% between 1991-2006, and in Northern Central African Republic declined by 65% during 1985-2005 (Bouché *et al*., 2010; Fischer and Linsenmair, 2001; Hatton *et al*., 2001; Jambiya *et al*., 2007; Lindsey *et al*., 2012).

Predator animals are also indirectly affected through lack of adequate food which affects their reproduction capacity and the juvenile survival, hence their dwindling population (Foster, 2010; Lindsey *et al*., 2015; Ripple, 2015). Illegal poaching of both large and small mammals increases the risk of extinction for example African elephant (*Loxodonta Africana*) is now classified by IUCN Red list as Vulnerable (IUCN, 2005).

The loss of wild animals as a consequence of illegal hunting can have severe consequences for ecosystem services. For example, the removal of large herbivores and seed dispersing mammals can affect the structure and species composition of woodlands and forests (Gustafsson, *et al*., 2012; Wright *et al*., 2007). In some cases, illegal hunting has resulted in edge-effects and reduced wildlife densities close to park boundaries and/ or human settlement (Ripple *et al*., 2015). For example, in the Serengeti National Park, illegal hunting has caused significant declines of resident herbivores in areas close to the boundaries (Holdo *et al*., 2011).

2.3 Species Identification

Identification of species has several uses including forensics, speciation and the monitoring of illegal animal and their products being traded (Fajardo *et al*., 2010). For this purpose, it is necessary to have a standard and reliable identification system in place. The simplest method for identifying species is through using their initial morphology or

microscopy (Bitanyi *et al.,* 2012). However, this is not always an optional method as many of bush-meat samples are traded in a diverse forms from the original (Eaton *et al*., 2009). This system would need to efficiently identify a number of different species from various sample types including hair, blood and tissue (Prado *et al*., 2007). A highly specialized specialist is needed to do the comparison and there is room for personal interpretation which can lead to individual bias. In cases where the results are to be used in court, samples are often sent for molecular analysis so as to reduce bias and doubt in the evidence.

Molecular methods used in species identification

The common methods used in identification of different species include DNA finger printing, Polymerase chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) , performing analysis using separate species marker panels, real-time PCR, sequencing of mitochondrial genome as well as the 12s and 16s rRNA loci (Caine *et al*., 2006; Eaton *et al*., 2009; Gupta *et al*., 2011; Kitano *et al.*, 2007; Linacre and Tobe 2011; Murugaigh *et al*., 2009; Prado *et al*., 2007; Wasser *et al*., 2008).

DNA finger printing uses random amplification of polymorphic DNA (RAPD), this technique allows a certain fingerprint for each animal to be created (Wasser *et al*., 2008). Highly tainted samples cannot be used, and the DNA samples from more than one different species cannot be used (Partis *et al*., 2000) and low reproducibility are among the setbacks of this technique. Reproducibility can be increased through strictly standardizing PCR conditions between laboratories, which is unrealistic. This process has proved to be time consuming and expensive hence has now been replaced with the use of Restriction Fragment Length Polymorphisms (PCR-RFLPs) and short segment repeats (SSR). These methods has been successful in distinguishing between different animal

products such as meat and milk. Adbel-Rahman and Ahmed, (2007) used a PCR and PCR-RFLP technique to distinguish between buffalo, cattle and sheep meat. However this method, is also expensive and labour intensive, but was the most widely used. PCR-RFLP analysis has been performed on samples such as dog, badger, cattle, human and pigs, among others (Adbel-Rahman and Ahmed, 2007; El-Sayed *et al.,* 2009; Prado *et al.,* 2007).

Short tandem repeat (STR) profiling systems is commonly used for the identification of species (Angleby and Savolainen 2005; Dawnay *et al*., 2008; Eichmann *et al*., 2005). This encompasses the use of genetic markers such as COI and is more suited to identification at the individual level. STR profiling systems work using statistical probabilities that two evidence samples are the same for example linking the ivory to the elephant population (Dawnay *et al.,* 2008; Wasser *et al*., 2008). However there are no guidelines that outline the number of STR loci that produce valid results in terms of the statistics in wild animals and this introduces room for differing interpretation.

Pyrosequencing is based on the detection of pyrophosphate (PPi) that is released from dNTPs during DNA synthesis. As this happens visible light is generated and this is directly related to the number of nucleotides being incorporated. The 12S and 18S regions of rRNA have been used in designing a species-specific DNA pyrosequencing method (Karlsson and Holmlund, 2007).

2.4 DNA (mtDNA)

Mitochondrial DNA (mtDNA) has also played an important role in a number of forensic investigations in both the human and animal forensic fields (Matsuda *et al*., 2005; Nelson and Melton 2007; Rastogi *et al*., 2007). There are a great number of reasons and

advantages for using mtDNA as opposed to nuclear DNA. Firstly mtDNA can be found in samples that contain less nuclear DNA or in which the DNA is degraded (e.g. hair, skeletal tissue and degraded samples). This is important when dealing with cases in which evidentiary material is limited (Nelson *et al.,* 2007). Mitochondrial DNA is hundreds to thousands of times more abundant than genomic DNA (Bellis *et al.,* 2003; Prado *et al.,* 2007). This is because there are about 800-1000 mitochondria per cell and each mitochondrion contains 2-4 mtDNA molecules. mtDNA is therefore a naturally amplifiable source of genetic material and therefore a valuable resource in forensics (Zha *et al*., 2011). According to the studies done by Andreasson *et al.* (2006) on determining the concentrations of nuclear and mtDNA concentrations in different forensic samples, it was revealed that mtDNA levels were substantially higher in all the samples also it was it was found that mtDNA is packaged and protected in mitochondrion and thus is better preserved in degraded samples. Furthermore, it was reported that due to the presence of many copies of mtDNA, it evolves much faster rate than nuclear DNA, causing it to be highly variable (Murugaigh *et al.,* 2009; Prado *et al.,* 2007). Also, Clayton, 2004 reported that inefficient mechanism of DNA repair causes different mtDNA regions to evolve at different mutational rates, additionally mtDNA codes for functional proteins and does not undergo recombination and therefore it cannot mutate unconditionally. This adds variability to the DNA while a certain level of conservation is maintained. Working with mtDNA has only two major limitations, one being that it represents only the martenal lineage of the animal since it is martenally inherited and the second being hetero plasmy (a condition whereby one individual has two different copies of mtDNA due to novel or inherited mutations (Bellis *et al.*,2003; Rastogi *et al.,* 2007).

2.5 Cytochrome B and Cytochrome Oxidase 1

Mitochondria has several regions which can be used for identification of species, these include cytochrome *b*, cytochrome oxidase 1 (CO1), 12s and 16s rRNA and the hypervariable or control region (mtDNAHV) located within the D-loop region of the

mitochondrion. Among these regions, the most commonly used in species identification is cytochrome *b* as it is species-specific (Hsieh *et al*., 2001; Linacre and Tobe 2011; Matsuda *et al.,* 2005; Parson *et al.,* 2000) and is therefore a perfect target for identification. It was revealed by Wasser *et al*. (2008) that 385bp region of cytochrome *b* has the widest taxonomic representation in nucleotide databases with over 8000 cytochrome *b* gene sequences for vertebrates available in Gen Bank.

Cytochrome *b* has been mostly used in population genetics, however in the case of species identification, 650 bp of cytochrome oxidase 1 (CO1) is used as gold standard (Herbert *et al*., 2003). The use of CO1 in species identification is based on the sequencing of the CO1 gene in the sample and the comparison of the results to known sequences published on Consortium for the Barcode of Life (CBOL). This leads to a reliance on CBOL and the test is limited to the species that have been uploaded onto CBOL. Researchers in South Africa used this method of sequencing COI in three forensic wildlife cases and it was successful (Dalton and Kotze 2011). Over the years there have been the debate on which gene (cytb and COI) is more applicable in species identification, however cytochrome *b* was found to be more informative when looking at a relatively smaller fragment (Tobe *et al.*, 2009). Furthermore, Parson *et al*. (2000) did a study looking at meat authentication, and was able to produce a 358bp of cytochrome b amplicon from 44 different animal species covering five major vertebrate groups. On the same study Parson *et al*. (2000) did the Restriction analysis and was able to differentiate species successfully. This indicates that there is enough variation within the cytochrome *b* gene for species-specific primers to be designed. Similarly, Bravi *et al.* (2004) used the same 358bp fragment to identify canine samples from a number of contaminating species. They were able to discover enough sequence divergence which can help in differentiating species by using PCR-RFLP method.

2.3 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was the first DNA amplification method developed in 1985 (Irwin, 1991). It has three major steps, (i) denaturation at (92-94 °C) where the double strand is separated into single strand, (ii) annealing at (40-60 °C) where the primers bind to the 5' end of the target and amplification by strand extension through the enzymatic action Light Cycler polymerase, and finally (iii) extension at 72 °C(Bellis, 2003). PCR has been extensively used in bush meat identification by several authors including (Bitanyi *et al.*, 2011; Eaton *et al.*, 2009: Gaubert *et al*., 2015; Olayemi *et al*., 2011). However this technique has many disadvantages, one of them is that it allows some levels of contamination which reduces the quality of DNA produced and it has low level of target DNA (Eaton *et al.*, 2009). Therefore this technique has been modified into many different approaches each having its specific features, principle, materials and equipment .One of the modification is the, Real time PCR to overcome the problem of contamination (Eaton *et al*., 2009).

2.4 Real Time Polymerase Chain Reaction (q-PCR) RT-PCR

Real-time PCR (q-PCR) is a new, improved quantitative and qualitative technology that can amplify and detect the nucleic acid of specific organisms quickly in one run in the same tube or plate. q-PCR overcomes the problem of potential carryover contamination that can occur with traditional Polymerase Chain Reaction (PCR) (Persing *et al*., 2011). In q-PCR, amplification products are detected by specific interactions which allow the accumulation of product to be monitored during the early phase of the reaction and continuously over multiple cycles of amplification. These interactions are DNA binding fluorophores, the 5' endonuclease, adjacent linear, haipin oligoprobes and self-fluorescing amplification (Mackay *et al.,* 2002). There are many advantages of q-PCR especially when compared with traditional PCR. One of the major advantages is that the formation

of the Polymerase Chain Reaction (PCR) product can be monitored continuously and particularly during the early cycles of PCR amplification, where the formation of product is directly related to the amount of starting DNA. This means that it is possible to accurately quantify the amount of DNA in the original sample. In addition, q-PCR is fast, sensitive and specific and it does not rely on time consuming post PCR methods such as gel electrophoresis for analysis of the product (Epsy *et al*., 2006; Persing *et al*., 2011; Liew *et al*., 2004). This technique has been applied in the study of wildlife population especially in bush meat, Eaton *et al.* (2009) they used q-PCR in combination with universal primer to measure individual species content and total meat content respectively.

2.5 High Melting Resolution Analysis (HRMA)

High-resolution melting (HRM) analysis is a relatively and highly sensitive new post-PCR analysis method used for identifying genetic variation in nucleic acid sequences (AB, 2010). This simple, fast method is based on PCR melting (dissociation) curve techniques and is enabled by the recent availability of improved double-stranded DNA (dsDNA) binding dyes along with next-generation real-time PCR instrumentation and analysis software (Ogden *et al*., 2009). HRM analysis can discriminate DNA sequences based on their composition, length, GC content, or strand complementarity (Reed and Wittwer, 2004). HRM analysis starts with PCR amplification of the region of interest in the presence of a dsDNA-binding dye such as SYTO® 9. This binding dye has high fluorescence when bound to dsDNA and low fluorescence in the unbound state.

Amplification is followed by a high-resolution melting step using instrumentation capable of capturing a large number of fluorescence data points per change in temperature, with high precision (Reed and Wittwer, 2004). Ds DNA dissociates (or melts) into single

strands, the dye is released, causing a change in fluorescence. The result is a melt curve profile characteristic of the amplicon (Wasser *et al.,* 2008). It is therefore perfect for single nucleotide polymorphism genotyping, species identification, sequence matching and mutation scanning without the need for any further separation and additional processing after PCR (Olupot *et al*., 2009). HRMA as the name suggests analyses the melt curve over extremely small ranges and can distinguish between different genotype and species based on the variation of the melting curves. HRMA makes it possible to detect small differences based on very small changes in the melting curve due to high resolution offered by this technology. Differentiating between isolates having similar melting temperature but slight differences in sequence is possible, based also on the variation in the shape of melt curve due to its extremely high resolution (Hebert *et al*., 2003).

Advantages of HRMA

HRMA is a closed tube method which minimizes contamination because there is no need to process the sample after DNA extraction, which will then be ready for analysis, and does not require the use of labelled probes instead intercalating dyes such as Eva Green which does not inhibit PCR and has low toxicity are used (Smith *et al*., 2008). After PCR, in order to generate a melting curve (MC), the PCR products are heated with different ranges of temperature starting from lower temperature and gradually increase (From 40°C to 95°C). Recently species identification of bush meat using High Resolution Melting Analysis is currently considered to be the best approach this is because it has more advantages than the other methods such as sequencing and suited for high throughput applications (Packer *et al*., 2011). HRM machines are user friendly, packed with full of intelligent analysis features which help to analyze the samples with in few minutes. The HRMA machine like MIC (Magnetic Induction Cycler) is fast enough to

finish 35 cycles with in 25min[3]. Since the MIC machine can be connected to a Personal Computer via blue tooth, multiple instruments can be run via a single computer and analyzed after each run increasing sample throughput (Herbert, 2003). It is simple to analyze data because alleles produce distinct melting curve shapes and melting temperatures (TM) that can be compared with reference samples so as to know the species and genotype (Palais *et al*., 2005). These variations of HRMA have been developed to enhance resolution (Wittwer *et al*., 2003).

2.6 DNA Quality and Quantity

DNA yield can be assessed by using various methods including Spectrophotometer, absorbance (optical density), agarose gel electrophoresis, or use of DNA-binding dyes. All these methods are convenient, but have varying requirements in terms of equipment needed, ease of use and calculations to consider. After isolation of DNA, quantification and analysis of quality are necessary since High Resolution Melting Analysis (HRMA) is naturally high sensitive to the quality and quantity. Roche, 2008 recommended that given the primers design is well optimized according to the prerequisites of real time PCR, the same extraction procedure for all samples which are highly reproducible should be used and each reaction should start with the same amount of DNA concentration. The DNA concentration for each sample should be measured, then adjusted to the same concentration with the resuspension buffer. Rastogi *et al*., 2007 also reported that salt carryover is one of the biggest factor that can affect HRM results because it can subtly change the thermodynamics of the DNA melting transition**.** Salts affect DNA melting behavior, so it is important that the concentrations of buffer, Mg2+ and other salts in the reaction mix to be as uniform as possible across all samples. In order to know the DNA purity the ratio between absorbance at 260 nm and 280 nm (A260/A280) is used, this is because the DNA absorb light at 260 nm and protein absorbs more strongly at 280 nm.

Pure DNA should have a ratio between 1.8 and 2.0 the value below indicates protein and RNA contamination. Absorption at 230 nm reflects contamination of the sample by substances such as carbohydrates, peptides, phenols or aromatic compounds. The ratio A260/A230 should be approximately 2.2 for pure nucleic acid samples.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area Description

3.1.1 Location and boundaries

The Serengeti ecosystem (Fig. 1), covers an area of about 25 000 km of which 14 763 km^2 is covered by Serengeti National Park (SNP), 1672 km^2 by Mara reserve, 8288 $km^2$by Ngorongoro Conservation Area (NCA), and the remaining 2200 km^2 covers Grumeti Game Reserve (GGR), Ikorongo Game Reserve (IGR), Maswa Game Reserve (MGR) and Loliondo Game Controlled Area (LGCA) (Kideghesho, 2008).

The ecosystem is managed by three different conservation authorities namely Tanzania National Parks Authority (TANAPA which manages SNP), Ngorongoro Conservation Area Authority (NCAA) which manages NCA while Tanzania Wildlife Management Authority (TAWA) manages all the remaining areas under GR and Game Controlled Areas. SNP and NCA allows, only non-consumptive utilization limited only to photographic tourism while TAWA allows both consumptive and non-consumptive utilization. In consumptive utilization, trophy hunting is allowed based on a quota system set out annually in hunting blocks (Baldus *et al*., 2003).

The study was conducted in the eastern part of Tarime district which is found in Mara region. It is Located in Northwestern Tanzania between 01°21′S 34°23′E within Serengeti ecosystem. The district has 73 villages and 399 sub villages. This study had two components i) social component which was conducted in two villages Gibaso and Kegonga ii) molecular component covered wider area so as to get the better picture of how serious illegal hunting is and increase the probability of acquiring assumed bush-

meat samples. The areas covered were both rural (Gobaso, Kegonga, Masurura, Masanga, Kenyamosabi and Bong'eng'e villages) and urban (Lebu, Msati, Legolyo and Kwibanga sub villages).

3.1.2 Study community

The major ethnic group inhabiting this area are Kurya tribe which born and raised within the study area, and other minor tribes include Luo, Sukuma, Jita, Taturu, Isenye and Ikizu in which many of them were migrants. The majority of inhabitants involved in the social component of this study were female with age ranging between 31-40 years. Primary education level was the prominent feature of the respondents followed by respondents with no formal education while minority had college education. Their main economic activities are agriculture (farming and livestock keeping) and small scale gold mining. The main agricultural crops include maize, sorghum, cotton and cassava.

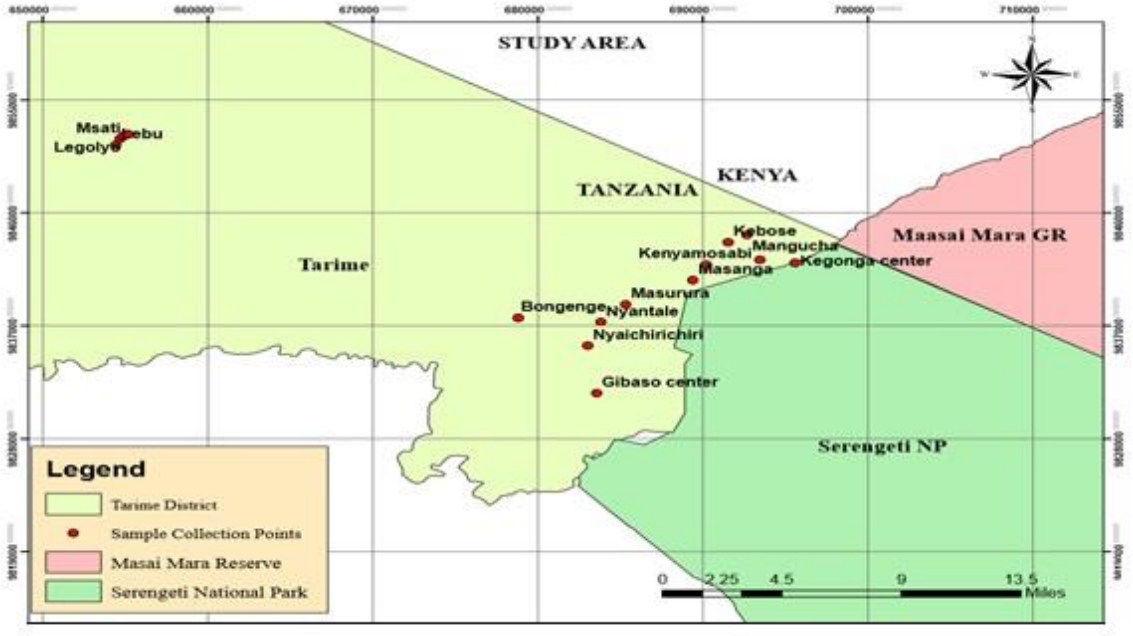

Figure 1: Map showing some parts of Serengeti ecosystem and study sites

3.2 Study Design

Research design

A cross-sectional descriptive design which allows both qualitative and quantitative data to be collected at one point in time was adopted as suggested Kothari (2004) and Saunders *et al*. (2007). In order to conduct active surveillance of bushmeat activities in the area, two methodologies were used (i) descriptive survey based on semi structured questionnaire (Appendix 1) was administered in order to collect information on socio-demographic and other socio factors leading to illegal bush meat hunting, preference and consumption. To augment the information obtained from the questionnaire, another method was used (ii) Collection of assumed sundried bushmeat samples sold by local traders for species identification using qPCR-HRMA approach.

3.3 Bushmeat Dynamics in Gibaso and Kegonga Villages which Borders Kenya within Tarime District

3.3.1 Sampling procedures

A purposive sampling method was used to select two villages namely Gibaso and Kegonga. The criteria being that they are trans-boundary bordering Serengeti and Masai Mara protected areas Prior to administering the questionnaires to the households, informal meetings were held with the village officers to solicit their consent and obtain the household lists in their localities.

The number of households which were provided by village officers were 1252 and 253 from Gibaso and Kegonga respectively. From that list, 200 and 100 households were randomly selected from Gibaso and Kegonga, respectively and used in the social component of this study This is in accordance with Saunders *et al.* (2007) who

recommended the sampling size of 30 and above to be a reasonable sample size for socio-science studies as it is statistically large enough to make scientific conclusions.

According to URT (2006), a household include a single person or group of persons who live together and share living expenses and usually constitutes a husband, wife and children. This household criterion was used in this study because in most rural areas of the developing world, the household is the basic unit of production and reproduction. Moreover, in most of the Africans traditions and customs, the household is the basic unit of social structure (Libida, 2004). The heads of respective households were the main respondents since they acted as spokespersons of the household in all tribes interviewed. In all selected villages, the questionnaires were administered to all selected households.

3.3.2 Administering questionnaire

Questionnaires were administered from November 2017-January 2018. During questionnaire administration, the native local guides were used to introduce the researcher to each household. The questions were mostly directed to parents (heads of the household), but in a few cases other adult members of the family were interviewed if parents were unavailable.

Since, bush-meat consumption is an illegal activity, it usually impossible to get straight answers from respondents by using a questionnaire, and for this reason a different approach was used. A separate questionnaire was developed which started with general demographic questions including occupation and income, and this enabled the respondents to be more at ease. Respondents were further asked about sources of protein in the village, followed by information about access to bush-meat, meat prices and preferences, and finally information about hunters' activities in the village and the bush-

meat market. All questionnaires were administered in Swahili and vernacular language (particularly Kurya) where necessary.

3.3.3 Data analysis

The data collected through questionnaire were coded to facilitate data entry in the computer. Systematic organization of data into categories and in this case numerical codes were assigned to responses (Bobbie, 1995). Data were analyzed by using Statistical Package for Social Sciences (SPSS) to obtain descriptive statistics such as percentages of responses, frequencies, and means of the method used in hunting, hunting season and species hunted. The results were used for construction of figures and tables.

Moreover bush-meat consumption and preference were regressed against all demographic factors (age categories, education levels, gender, occupation, household income, place of birth and tribe of the respondents) to find which of these factors was a significant predictor. The age categories considered in this study were young (18-30 years), middle aged (31-60 years) and old aged (61-100 years) while the education levels considered were no formal education, secondary education (ordinary and advanced levels) and professionals (college and University level). This was followed by ONE WAY ANOVA and Univariate analysis so as to find the significant difference between different variables. Then Pearson correlation was used to establish the relationship between bush-meat consumption and consumers preferences.

3.4 Bush-meat Sample Collection

A total of 188 assumed to be bush-meat samples were purchased from different areas of Tarime district. Snowballing approach was adopted whereby the Village Executive Officer (VEO) and other trusted local villagers selected by VEO were used to collect the samples from traders and middlemen. Also these trusted people helped to refer the

researcher to other people selling bush-meat samples. The sampling was random and all available meat was purchased without any preference. In these area, bush-meat was sold as dry at a price of Tshs 6000/- and was processed by sun drying.

Because meat samples were dry and lacked morphological clues which could be used to identify them at species level, the suggestive species name per sample were provided by middle men and consumers and most names were given in vernacular language. For sample collection, the sampling protocol involved using gloved hands and cutting the dry meat with sterile surgical blade into smaller pieces and thereafter stored in well labeled khaki envelopes.

For each sample, a new pair of gloves, blades and envelopes were used. The bio data information collected at each sampling included the sample name, date of collection, village location and GPS coordinates. At the end of the field work, all samples collected were transported to SUA and frozen at -20°C in the laboratory waiting for DNA analyses.

Sample preparation

For each sample which was DNA analyzed, the external layer which contained debris and dirty was trimmed off and the inner part was scraped carefully into finer powder by using sterile surgical blade. Approximately, 20 mg of powdered meat was transferred into well labeled sterile clean 1.5 ml micro-centrifuge tubes and stored at -20°C waiting for DNA extraction. Gloves were worn throughout the process and before processing a new sample, the benches were carefully cleaned and DNA decontaminated by using 5.85%w/v sodium hypochlorite (bleach) followed by 70% ethanol.

3.5 Optimization

The optimization was pivotal since the qPCR-HRMA approach for bush-meat analysis are based on using the Magnetic Induction Cycler (MIC) (Biomolecular systems, Upper

Coomera, Australia) was recently purchased in the lab, not used previously in determining the species IDs of bush-meat in Tanzania. The collaborating International Centre of Insects Physiology and Ecology (ICIPE), Kenya laboratory used in the initial development and standardization of the qPCR-HRMA assays for bush-meat identification at species level is based on using Rotor-Gene Q PCR thermocycler (QIAGEN, Hannover, Germany) and uses two DNA extraction in-house procedures based on QIA-JHK method with SDS buffer and QIA-JHK method with proteinase K buffer that had not been used at SUA and needed to be standardized as well. The use of these two extraction procedure were aimed at making the DNA extraction cheaper than using commercial DNA extraction kits.

3.5.1 Comparative assessment of three DNA extraction procedures QIA-JHK with SDS buffer, QIA-JHK with Proteinase K buffer and Quick-DNATMMiniprep Plus kit on DNA yield and quality of sun-dried bush-meat samples

We started with the ICIPE DNA extraction procedures, but since they did not produce reproducible results, we were compiled to compare these with the kit. Three different DNA extraction methods described below were initially tested to assess their DNA yield, quality and reproducibility in the PCR_HRMA being developed. QIA-JHK method with SDS buffer and QIA-JHK method with proteinase K buffer and Quick-DNA^{TM}Miniprep Plus kit by Zymo research was used to assess the method which works better for dry bush meat samples and generates high quality as well as good yield DNA which produced reproducible HRMA and melting curves for easy comparison. A negative extraction template (net) with no tissue sample was also included during the extraction protocol. In addition known samples for cattle, sheep, goat and horse were also extracted and used as positive controls in qPCR-HRMA. The isolated DNA was stored at -20^{o}C for later Polymerase Chain Reaction (PCR) and HRMA analysis.

3.5.1.1 Extraction procedures for QIA-JHK method with SDS buffer

In a 1.5ml micro-centrifuge tube containing 20mg of respective bush-meat sample, 300µl of cell lysis buffer containing (10mM Tris (pH8.0),0.5%SDS and 5Mm EDTA) was added and incubated for 2hrs at 65°C in the water bath. Then 100 µl of Protein Precipitation Solution containing (8M Ammonium Acetate and 1mM EDTA) was added at room temperature and vortexed at 10 seconds three times. The tubes were transferred onto ice for 5min and then centrifuged for 3min at 25000 rcf. The supernatant was carefully drawn and placed in a fresh 1.5ml micro-centrifuge tube containing 300 µl of molecular grade 100% isopropanol and the pellets were discarded. The mixture was inverted 100 times by using a Lab quake shaker (Bi Barnstead International, US) followed by centrifuge for 10min at 25 000 rcf. The supernatant was discarded and to a pellet 300 µl of molecular grade 70% ethanol was added and mixed gently by inverting several times to extract the DNA. The mixture was then centrifuged for 1min at 25 000 rcf and the ethanol was gently pipetted off. The DNA pellet adsorbed onto the tubes was dried overnight by inverting over a paper towel. During the next day the DNA was hydrated by adding 50 µl PCR grade water and left to dissolve at 65°C for 2 hrs.

3.5.1.2 Extraction procedures for QIA-JHK method with proteinase K buffer

The same protocol was used but instead of using the cell lysis buffer, Proteinase K was used for cell digestion.

3.5.1.3 Extraction procedures for Quick-DNATM Mini prep Plus kit for tissue samples

Quick-DNA^{TM}Miniprep Plus kit by Zymo research employs Proteinase K digestion and Zymo-spin Technology for effective recovery of DNA. The extraction was done as per manufacturer's instructions with minor modification as follows:

In 1.5 ml micro centrifuge tubes which containing 20mg of respective dry meat samples collected in the study area and assumed to be bush meat tissue sample, 95µl of nuclease free water, 95µl solid buffer and 10µl of Proteinase K were added, vortexed thoroughly and then incubated at 55°C for 3hrs until the samples were completely solubilized. Thereafter, the tubes were centrifuged at 12 000 rcf for 1 min .Then 200µl of aqueous supernatant was transferred to a clean micro centrifuge tube to which 400µl of Genomic Binding Buffer was added and slowly mixed by using pippete. The 600µl of the mixture for each sample was aliquoted and transferred to respective Zymo-spin^{TM}IIC-XL Columns held in a collection tube, centrifuged at 12 000 rcf for 1.0min. The flow through contained in the collection tubes were discarded .Then 400µl of DNA Pre-Wash Buffer was added to each column in a new collection tubes and centrifuged for .1.0 min and collection tubes were emptied. Washing was done twice, first with 700µl of g-DNA Wash Buffer followed by 200µl of g-DNA Wash Buffer. At each stage the tubes were centrifuged for 1.0 min and flow through discarded. To elute the DNA the columns were placed in the clean micro centrifuge tubes and 50µl of nuclease free water was added (instead of elution buffer as described in Roche, 2008), incubated for 5.0 min and then centrifuged for 1.0 min. The eluted DNA was stored at -20 °C waiting for further analyses.

3.5.2 Optimization of the PCR-HRMA

3.5.2.1 DNA quality and quantity

DNA yield and purity was measured with a Nano Drop spectrophotometer (ND-1000, Thermo Scientific, and Waltham, MA, USA). Since the purity and quantity of the template can affect the melting behavior of the HRM profiles like shape and melting temperature ™. All samples were diluted to a final concentration of 10 ng/mL as recommended by Vossen *et al*. (2009) so that they can have the same template

concentration before HRM analyses for easy comparison of known with unknown samples.

3.5.2.2 High Resolution Melting Analysis (HRMA)

Three mitochondrial primer pairs targeting vertebrate cytochrome b (*Cytb*), 16S ribosomal RNA (Omondi *et al.*, 2015) and vertebrate COI (Ogolla *et al*., 2017) genes were used in separate PCRs. For each PCR run DNA extracts from cattle, sheep, goat, horse and rabbits served as positive controls, and water and one negative control was used. PCR run were set up in 10 µl reaction volumes comprised of 2 µl of 5x Hot Fire Pol HRM Eva green mix (Solis Bio Dyne, Tartu, Estonia), 0.5 µM of respective forward and reverse primers at 10M concentrations, and 20 ng of DNA template. PCR was performed on a qPCR-HRMA (MIC-4) thermocycler (Biomolecular systems, Upper Coomera, Australia). Reactions were subjected to 95°C initial denaturation for 15 minutes followed by 32 amplification cycles (20 seconds denaturation at 95°C; 10 seconds annealing at 56°C; 30 seconds extension at 72°C) and a final hold at 72°C for six minutes. The amplicons were then melted according to the following conditions: 90 seconds pre-conditioning at 75°C; melting at 0.1°C/second incrementally from 75°C to 90°C. Fluorescence data was acquired after two seconds at each temperature increment.

3.5.2.3 Analysis of HRMA melting profiles

The results from three different extraction procedures was analyzed by using SPSS software version 2.0. Independent one sample T-test was used to compare the quantification cycles (Cq) of the samples extracted by each method. The Cq (Appendix 2) was used because it determines the number of cycle the sample started to amplify, the sample with high quality amplifies faster hence low Cq value while the sample with low

quality delays in amplification hence high Cq value. The identity of the vertebrate species for each sample was deduced by comparing the shapes and peak melting temperatures of each curve of unknown against the known sample by using MIC software version 1.6 and excel book sheet. Also DNA sequencing of some representatives was performed by using Sanger sequencer to confirm the vertebrate species identities.

CHAPTER FOUR

4.0 RESULTS

4.1 Bush Meat Dynamics

Bushmeat dynamics which were considered in this study were hunting season, methods used, species hunted, and bush meat consumption and preferences.

4.1.1 Hunting Season

The majority of respondents agreed that hunting activities occur between July and September (90%, n = 270), while a minority (5.3%, n = 10) reported a period spanning from January to June (Table 1).

Table 1: Hunting season

Month	Strongly disagree	Disagree	Somehow disagree	Neutral	Somehow agree	Agree	Strongly agree
Jan-March	217(72.3)	3(1)	5(1.7)	54(18)	11(3.7)	9(3)	5(3)
Apr-June	214(71.3)	4(1.3)	8(3)	5(2.7)	61(1.7)	7(20.3)	5(2.3)
July-Sept	15(5)	7(2.3)	1(1.5)	1(7)	5(1.7)	1(1.5)	270(90)
Oct-Dec	215(1.7)	4(1.5)	1(1.5)	1(1.5)	42(14)	13(4.3)	20(8.3)

Note: Numbers in brackets are percentage of respondents and outside the bracket are frequencies of the respondents

4.1.2 Hunting methods

Snares were reported to be the main method used in hunting activities (65%, n = 196), whereas firearms featured least (2,7%, n =8). However, the remaining hunting methods together constituted (32.3%, n = 96) of respondents' views (Table 2).

Table 2: Hunting methods

Method	Strongly disagree	Disagree	Somehow disagree	Neutral	Somehow agree	Agree	Strongly agree
Pits	150(50)	3(1)	1(3)	3(1)	13(4.3)	62(20.7)	67(22.3)
Snares	97(32.3)	4(1.3)	1(3)	5(1.7)	11(3.7)	37(12.3)	196(65)
Firearm	273(91)	8(2.7)	4(1)	1(3)	5(1.7)	1(3)	8(2.7)
Poison	227(75.7)	12(4)	1(3)	5(1.7)	10(3.3)	15(5)	29(10)

4.1.3 Hunted species

Among the five categories of questions asked to respondents in regard to the most hunted species in the area under study, Wildebeest were singled as the most hunted species (42%,n=104) followed by Zebra (14.7%, n=41), while warthog (0.7%,n=2) and buffalo(1.7%,n=5) was least hunted. However (18.3%, n=55%) of respondents responded that they hunt any animal available while (16%, n=48) of respondents responded that they don't know (Fig. 2).

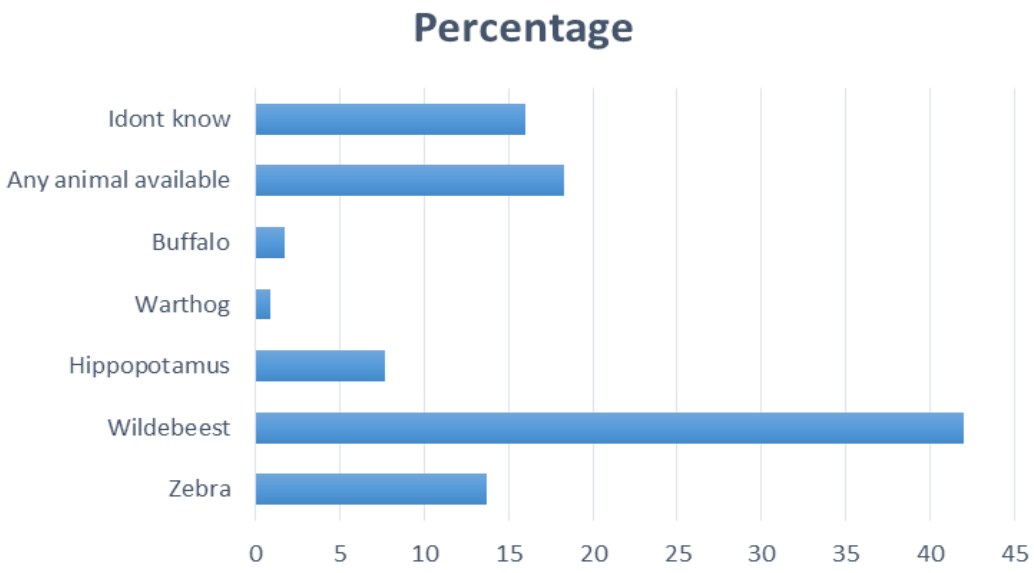

Figure 2: Percentages of hunted species

4.1.4 Relationship between Age and bushmeat consumption

Age categories of the respondents was found to be the only significant predictor (R^2 =0.56, p=0.014) of bushmeat consumption among all demographic factors, whereby different age categories exhibited substantial difference in bushmeat consumption (df =2, f=4.7, p=0.009). Therefore, there were significant difference between young and old aged (p=0.039), young and middle aged (p=0.01) whereas, the difference between middle and old aged was also significant at (p=0.02). Generally the consumption decreased as the age increases, so young consumed more than other age categories (Fig. 3).

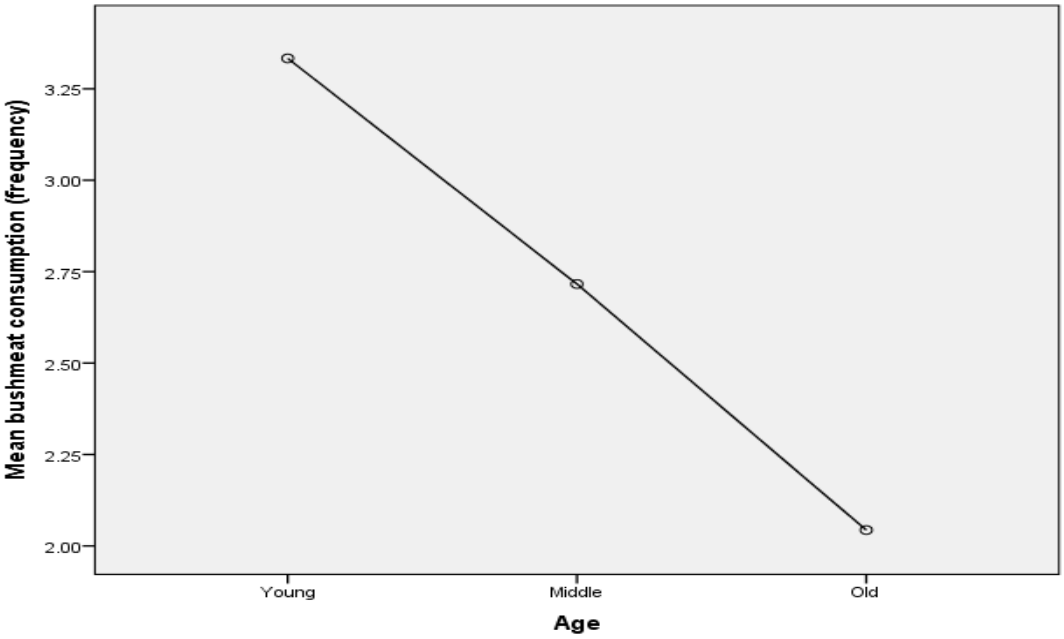

Figure 3: Relationship between Age categories and bushmeat consumption

4.1.5 Relationship between bush meat preference, birth location and education levels

Birth place (R^2=0.039, p=0.034) was found to be the significant predictor of bushmeat preference while education level showed the marginal significance (R^2=0.039, p=0.051). Respondents born outside the village had a significantly greater preference for bushmeat over other protein sources than respondents born within the village (df=1, f=10.3, p=0.002). Bushmeat preference and education level had strong positive correlation (r=1, p=0.022). Likewise, the interaction of birth place and education level effected bushmeat preference significantly (df=2, f=4, p=0.019). Professionals born outside the village had higher bushmeat preferences than those born within the village (p=0.002), whereas place of birth did not significantly affect bushmeat preferences among respondents with secondary or no formal education (p=0.55) (Fig. 4).

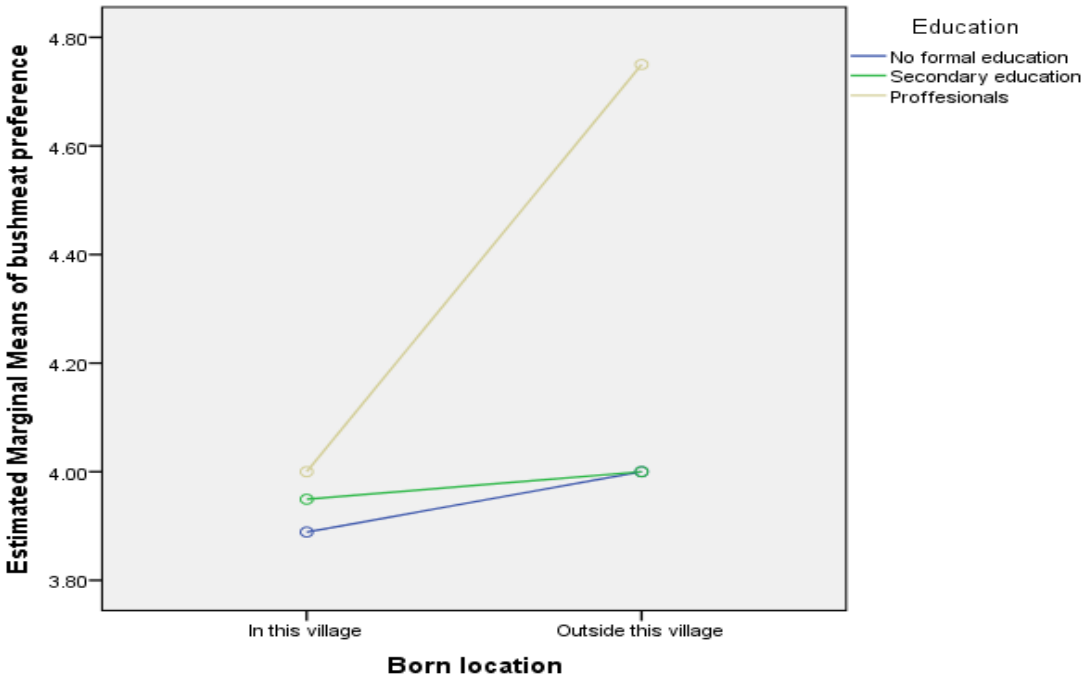

Figure 4: Relationship between bush meat preference, birth location and education levels

4.1.6 Relationship between education levels and different age categories against bushmeat consumption and preference

There was a positive correlation (r=0.07) between bushmeat consumption and preference for bush meat. Education levels and age categories affected both bushmeat consumption and preference significantly (df=2, f=4.9, p=0.008). Subsequently, the significant difference was portrayed in bushmeat consumption and preference between young and old educated respondents (p=0.003) with highly educated young respondents consuming bush meat more than less educated old respondents. While, marginal significant difference (p=0.051) was observed in bush-meat consumption and preference between old and middle educated respondents (Fig. 5).

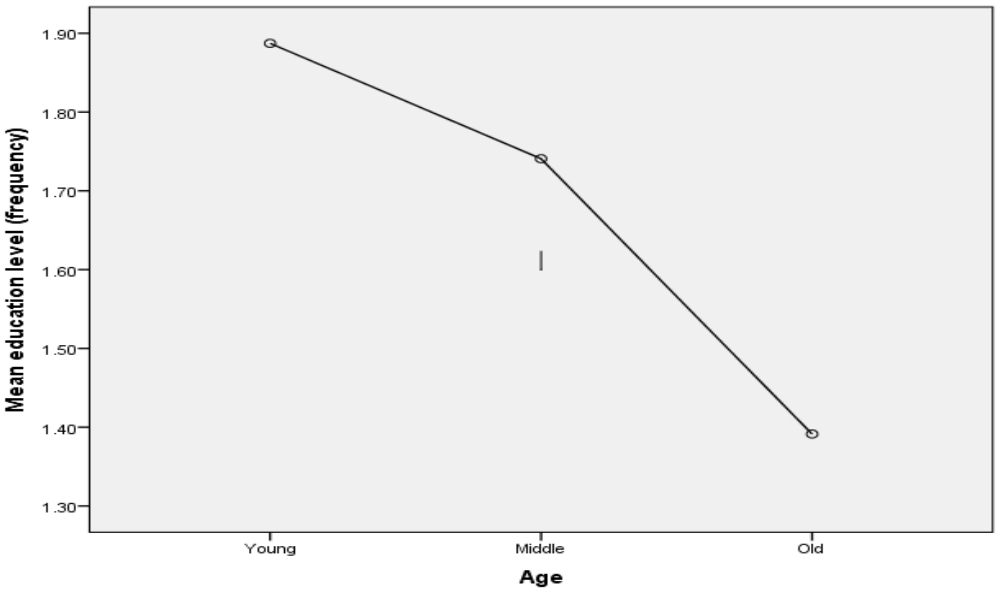

Figure 5: Relationship between education levels and different age categories against bushmeat consumption and preference

4.2 Identification of Species Hunted by Using qPCR-HRMA

Comparative assessment of three DNA extraction procedure (QIA-JHK with SDS buffer, QIA-JHK with Proteinase K and Quick-DNATM Mini prep Plus Kit on DNA yield and quality of sun-dried bushmeat samples

Samples extracted with proteinase k buffer had high DNA concentration 196.96429 ng/µl and high protein contamination 1.7546 when compared with other two methods which had 176.5714 ng/µl and 1.821 of samples extracted with SDS buffer and 48.40714 and 1.896571 of samples extracted with Quick-DNA^{TM} Mini prep Plus Kit. However samples extracted with SDS buffer had high salt concentration with 0.709 when compared with other two methods 1.11507 of proteinase k and 1.296321 of Quick-DNA^{TM} Mini prep Plus Kit. Generally samples extracted with Quick-DNA^{TM} Mini prep Plus kit produced low quantity of DNA but of high quality when compared with other methods (Table 3).

Table 3: DNA quality and quantity of DNA of samples extracted by using three different extraction procedures

Samples			Samples extracted by using Quick-DNA™ Mini prep Plus Kit			Samples extracted by using QIA-JHK method with SDS buffer			Samples extracted by using QIA-JHK method with proteinase k buffer		
Local name	**Common name**	**Scientific name**	**DNA conc ng/µl)**	**260/280**	**230/260**	**DNAconc (ng/µl)**	**260/280**	**260/230**	**DNA Conc (ng/µl)**	**260/280**	**260/230**
Ngitero	Zebra	*Equus burchelii quagga*	60	1.714	1.099	147	1.699	0.41	55.5	1.762	0.933
Inchole	Zebra	*Equus burchelii quagga*	27	2	0.702	46.5	1.661	0.198	56.5	1.569	0.894
Inkonya	Zebra	*Equus burchelii quagga*	146	1.836	1.114	233	1.926	1.678	352.5	1.762	1.772
Mbubuse	Zebra	*Equus burchelii chapman*	56	1.83	0.746	78.5	1.915	0.51	108.5	1.736	1.006
Hanakwe	Zebra	*Equus burchelii quagga*	92	1.822	1.58	426	1.968	1.082	256	1.91	1.813
Isenye	Zebra	*Equus burchelii quagga*	13.4	1.874	1.244	131.5	1.422	0.362	313.5	1.756	0.865
Imkoli	Zebra	*Equus burchelii chapman*	48.5	1.794	1.286	70.5	1.88	0.554	63	1.853	1
Cholo	Zebra	*Equus burchelii chapman*	214.5	1.78	1.456	198.5	1.956	1.254	250.5	1.898	1.245
Imboto	Zebra	*Equus burchelii chapman*	81.5	1.874	1.382	74.5	1.817	1.538	376	1.875	0.788
Kigongo	Zebra	*Equus burchelii chapman*	12.4	2.067	0.381	36	1.333	0.195	15.5	1.367	0.554
Imbuli	Zebra	*Equus burchelii chapman*	94	1.861	1.526	245	1.976	1.607	235	1.888	1.856
Nzombi	Zebra	*Equus burchelii quagga*	35.5	1.868	0.993	248	1.685	0.807	199	1.694	1.109
Ingwe	Zebra	*Equus burchelii chapman*	16.3	1.831	0.52	158	1.975	0.652	161	1.602	1.086
Intele	Zebra	*Equus burchelii chapman*	51	1.889	0.883	77.5	1.742	1.417	189.5	1.692	0.745
Kisokolokobwe	Zebra	*Equus burchelii quagga*	14.8	1.954	1.589	282	1.918	0.964	280.5	1.908	1.714
Inzengwe	Zebra	*Equus burchelii quagga*	8.1	2.051	0.5	125	1.838	0.45	19.5	1.535	1.092
Inkoko	Zebra	*Equus burchelii quagga*	8.8	2.444	0.423	103	1.717	0.359	23.5	1.343	0.592
Maseso	Zebra	*Equus burchelii quagga*	42.5	1.889	1.431	216	1.955	1.197	206	1.856	1.385
Kuncho	Zebra	*Equus burchelii chapman*	27	1.759	1.752	203.5	1.92	0.397	537	1.921	1.59
Ingwele	Zebra	*Equus burchelii chapman*	18.5	1.869	1.025	69	1.865	0.262	104.5	1.771	1.15
Sabala	Zebra	*Equus burchelii chapman*	19.5	1.765	1.066	66.5	1.705	0.236	152.5	1.753	0.559
Indasa	Zebra	*Equus burchelii quagga*	9.7	2.064	0.779	56	1.778	0.473	30	1.765	0.636
Mbichi	Zebra	*Equus burchelii quagga*	194	1.839	1.88	1028	1.955	1.546	834.5	1.842	1.766
Korongo	Zebra	*Equus burchelii quagga*	24	1.605	1.149	122.5	1.815	0.48	162	1.917	0.617
Ingela	Zebra	*Equus burchelii chapman*	5.9	2.145	1.235	119	1.874	0.182	73.5	1.837	1.058
Swala	Zebra	*Equus burchelii chapman*	20	1.766	0.618	51.5	1.907	0.5	27.5	1.735	1
Nungunungu	Zebra	*Equus burchelii quagga*	7.7	1.962	0.765	93.5	1.851	0.219	75	1.667	0.862
Idodo	Zebra	*Equus burchelii chapman*	6.8	2.109	1.573	238	1.935	0.321	357	1.914	1.535
Average			**48.40714**	**1.896571**	**1.296321**	**176.5714**	**1.821**	**0.709**	**196.96429**	**1.7546**	**1.11507**

4.3 Quantification Cycles of Coi Primer

The significant difference was observed in samples extracted with DNA^{TM} Mini prep Plus Kit and those extracted by SDS buffer (F=66, t=12, df=34.75, p=0.000). With samples extracted by kit amplifying earlier with mean quantification cycle of 18.84 while samples extracted with SDS buffer had delays in amplification with mean quantification cycles of 28.98. The significant difference was also observed between samples extracted with kit and those extracted with proteinase K (F=13.37, t=5.74, df=29.34,p=0.001). Again samples extracted with kit amplifying earlier with mean quantification cycle of 18.84 and those extracted with proteinase K showing delay in amplification with mean quantification cycle of 27.19. However, no significant difference was observed in quantification cycle between samples extracted with SDS and proteinase K buffers. Generally samples extracted with kit had early amplification than samples extracted by other two methods (Table 4).

Table 4: Comparison of three extraction methods using their quantification value in COI

Extraction method	Mean Cq value	f	T	df	p
COI_ kit	18.84	66.9	12.23	34.8	0.000
COI_ SDS buffer	28.98				
COI_ kit	18.84	13.4	5.74	29.34	0.001
COI_ proteinase K	27.19				
COI_ SDS buffer	28.98	1.4	1.09	41.67	0.238
COI_ proteinase K	27.19				

4.4 Quantification Values of Cytb Primer

There were no significant difference depicted in the quantification cycle value between all three DNA extraction methods. However the QIA-JHK methods with proteinase k buffer had earlier amplification with mean quantification cycle of 26.79 when compared with samples extracted with SDS buffer and kit, since they had mean quantification cycle of

27.01 and 28.98 respectively. Still the difference between proteinase k buffer and kit was not much big. Samples extracted by kit amplified two cycles late than samples extracted by proteinase k buffer (Table 5).

Table 5: Comparison of three extraction methods using their quantification value in CytB

Extraction method	Mean Cq value	f	T	df	p
CytB_ kit	28.98	1.69	1.29	43.65	0.199
CytB_ SDS buffer	27.01				
CytB_ kit	28.98	2.19	1.41	43.14	0.144
CytB_ proteinase k	26.79				
CytB_ SDS buffer	27.01	0.018	0.12	53.98	0.893
CYytB_ proteinase k	26.79				

4.5 Quantification Values of 16s Primer

The significant difference was observed between samples extracted by kit and SDS buffer method (f=10.77, t=9.95, df=33,p=0.002) with samples extracted by kit amplifying earlier with mean quantification cycle of 8.3 than SDS with mean quantification cycle of 15.18.Also a significant difference was observed between samples extracted by kit and proteinase k buffer methods (f=20.41, t=9.94,df=40,25,p=0.000) again kit had early amplification at 8.3 mean quantification cycle while proteinase k had late amplification of 13.09 mean quantification cycle . No significant difference was observed between samples extracted by SDS buffer and proteinase k. However, proteinase k buffer showed early amplification at 13.09 mean quantification cycle than SDS buffer with 15.18 mean quantification cycle .Generally samples extracted by kit had high quality DNA which amplified early than the samples extracted by the other two QIA-JHK methods. The difference between these three methods was big that the Cq value of samples extracted by kit was almost two times the no of cycles of samples extracted by other two methods (Table 6).

Table 6: Comparison of Three Extraction Methods Using Their Quantification Value in 16S

Extraction method	Mean Cq value	f	T	df	p
16S_kit	8.3	10.77	9.95	33	0.002
16S_SDS buffer	15.18				
16S_kit	8.3	20.41	9.94	40.25	0.000
16S_proteinase k	13.09				
16S_SDS buffer	15.18	0.85	2.66	46.58	0.361
16S_proteinase k	13.09				

4.6 Melting Profiles of Samples Extracted by three DNA Extraction Methods

Figure 6-14 shows the profiles of samples extracted by using three methods in three markers. Samples extracted by QIA-JHK method with both buffers (SDS and proteinase K) produced low quality DNA which principled the production low quality profiles. The profiles were characterized by low amplification and lack of repeatability, whereby the sample which represented the single specie produced profiles of different shapes in the same marker .While high quality DNA produced by Quick-DNA^{TM} Mini prep plus kit principled the high quality profiles with high repeatability and good amplification. For these reasons the Quick-DNA^{TM} Mini prep Plus kit was adopted for extraction of all known and unkown samples for easy comparison.

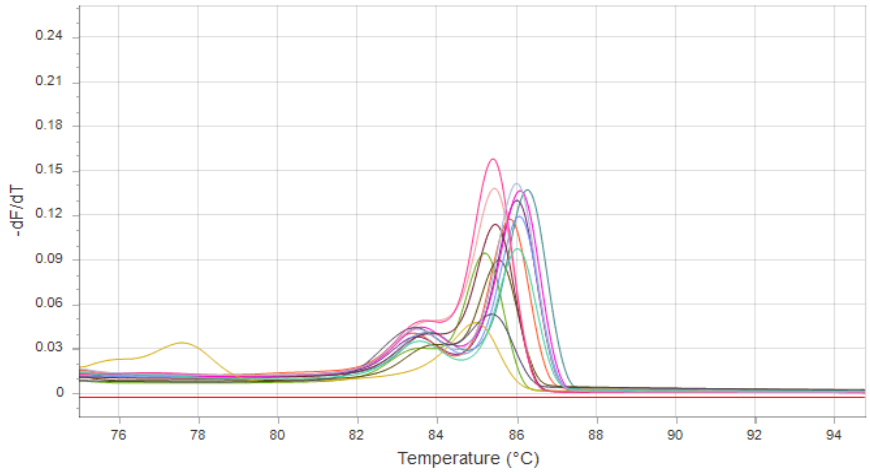

Figure 6: Profiles of samples extracted with QIA-JHK method with SDS buffer (COI)

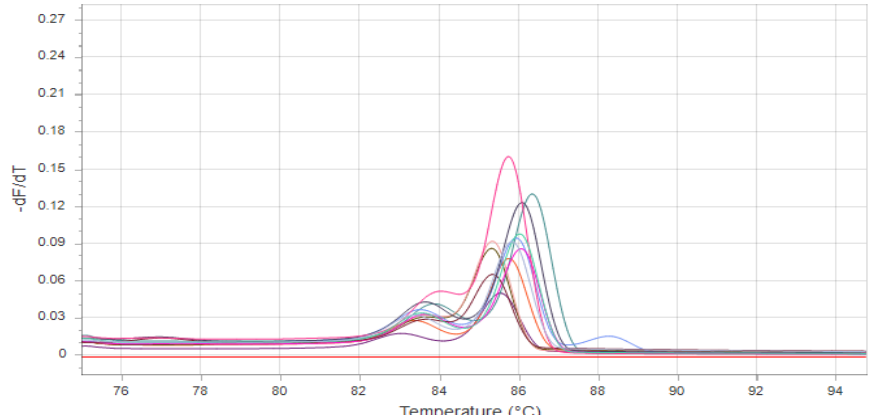

Figure 7: Profiles of samples extracted by QIA-JHK method with proteinase K buffer (COI)

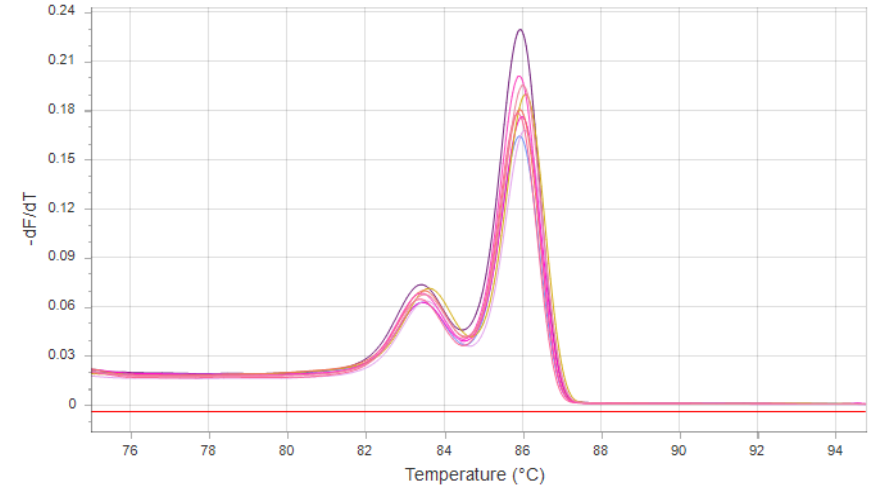

Figure 8: Profiles of samples extracted with Quick-DNA™ Mini prep Plus kit (COI)

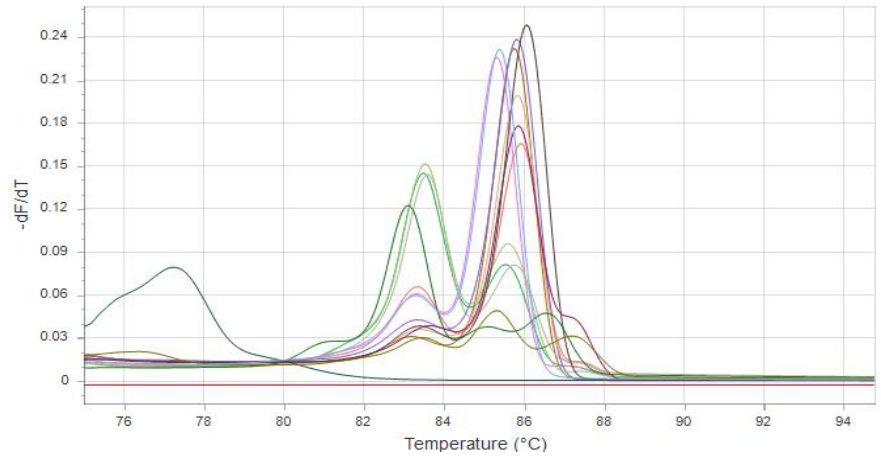

Figure 9: Profiles of samples extracted with QIA-JHK method with SDS buffer (CytB)

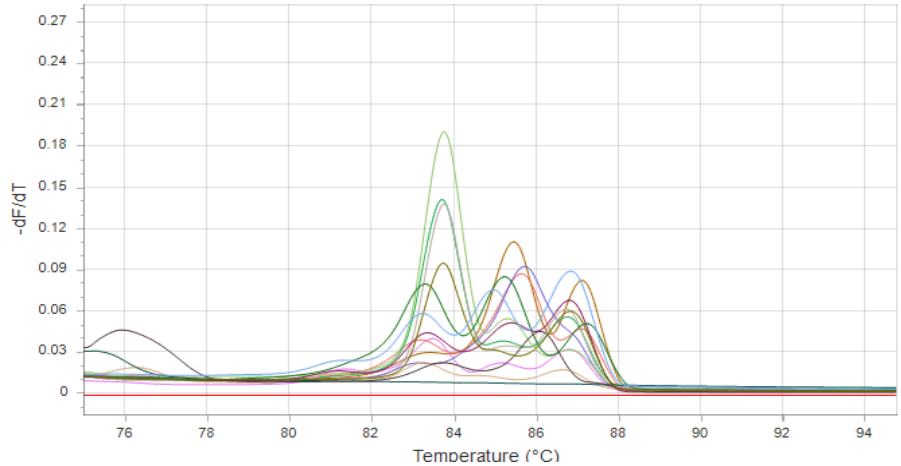

Figure 10: Profiles of samples extracted with QIA-JHK method with proteinase K buffer (CytB)

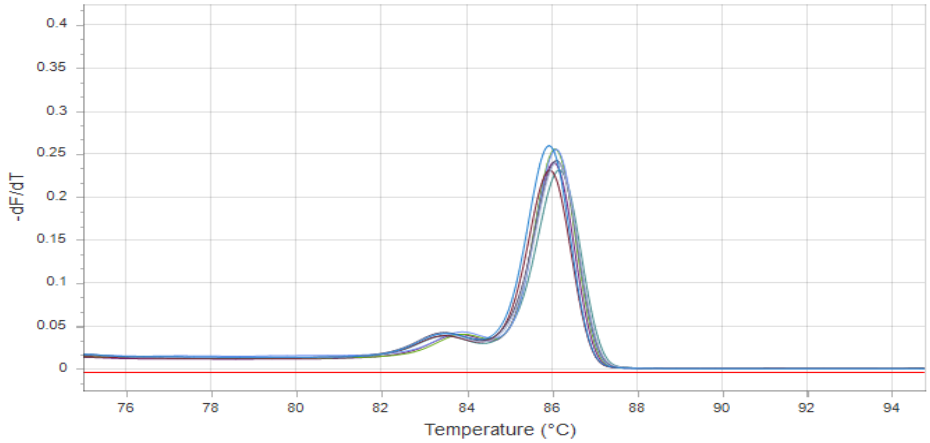

Figure 11: Profiles of samples extracted with Quick-DNA™ Mini prep Plus kit (CytB)

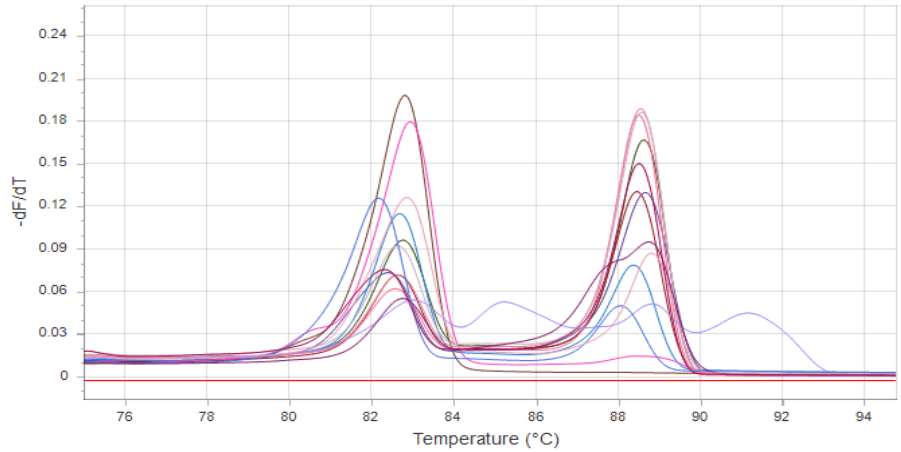

Figure 12: Profiles of samples extracted with QIA-JHK method with SDS buffer (16S)

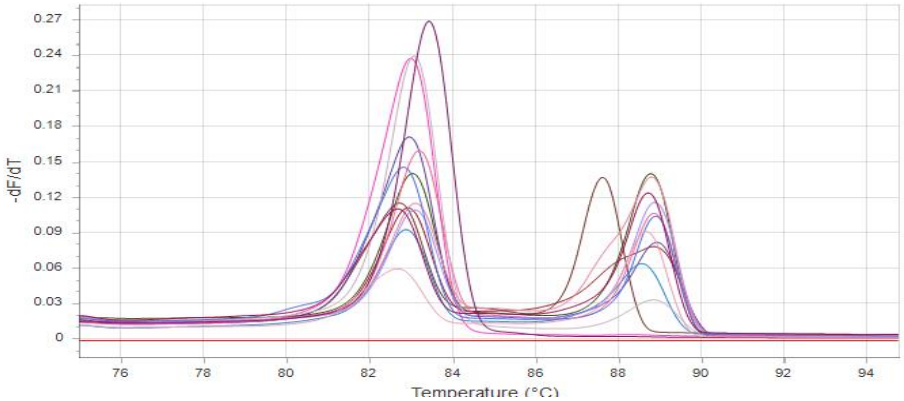

Figure 13: Profiles of samples extracted with QIA-JHK method with proteinase K buffer (16S)

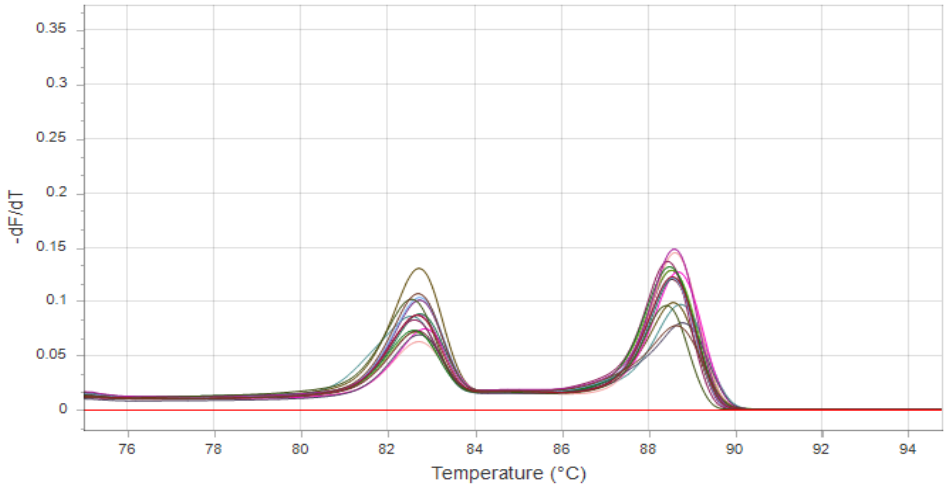

Figure 14: Profiles of samples extracted with Quick-DNA™ Mini prep Plus kit (16S)

4.2.2 Reference samples

The Species ID for samples was confirmed against the existing library available in the laboratory under the USAID-PEER projects (Table 7). Then HRM was run on all sequenced samples to generate profiles which were used as references.

Table 7: HRMA profiles and the melting temperatures of the species used as references in identification of unknown samples for CO1, CytB and 16S primers

Common name	Scientific name	Tm (COI)	Tm (CYTB)	Tm(16S)
Hartebeest	*Alcelaphus baselaphus*	85.50	84.28	82.15
Zebra	*Equus burchelli*	85.95	83.95	82.84
Savvana hare	*Lepus microtis*	82.44	83.62	82.99
Greater cane rat	*Thryonomys swinderianus*	83.97	84.16	80.48
Civet	*Civettictis civetta*	83.98	82.64	82.60
T.gazelle	*Eudorcas thomsonii*	83.30	85.14	82.02
Common warthog	*Phacochoerus africanus*	83.96	84.54	81.52
Y.baboon	*Papio cynocephalus*	82.61	87.09	87.24
Genet	*Genetta genetta*	83.12	83.42	81.12
Spotted hyena	*Crocuta crocuta*	84.34	85.06	88.08
B. backed jackal	*Canis mesomelas*	86.51	84.15	81.66
Giraffe	*Giraffa camelopardalis*	86.44	84.67	84.21
Bohor reedbuck	*Redunca redunca*	81.72	84.70	83.79
Goat	*Capra aegagrus hircus*	84.24	82.73	82.83
Horse	*Equus caballus*	84.79	83.35	82.98
Cattle	*Bos taurus*	85.88	84.79	83.46
Sheep	*Ovis aries*	85.43	83.23	83.48
Chicken	*Gallus gallus domesticus*	85.41	87.98	86.19
Domestic pig	*Sus scrofa domesticus*	82.75	83.69	82.70
Rabbit	*Oryctolagus cuniculus*	82.44	84.16	82.61
Lion	*Panthera leo*	82.21	84.91	83.40
Cerval cat	*Leptailurus serval*	82.29	82.64	88.79
Grant gazelle	*Nanger granti*	81.36	84.51	82.92
Sunni antelope	*Neotragus moschatus*	84.07	84.61	83.81
Wildebeest	*Connochaetus taurinus*	84.39	83.98	82.51
Red forest duiker	*Cephalophus natalensis*	84.43	86.17	84.80
Impala	*Aepyceros melampus*	85.57	84.80	84.10
Eland	*Tragelaphus oryx*	82.54	84.16	83.26
Kirki dik-dik	*Madaqua kirkii*	83.06	85.16	84.87
Topi	*Damaliscus lunatus*	84.47	84.90	81.81
Greater kudu	*Tragelaphus strepsiceros*	85.92	85.83	83.99
Bushbuck	*Tragelaphus scriptus*	83.77	84.35	82.77
Sitatunga	*Tragelaphus spekii*	84.39	83.94	82.52
African buffalo	*Syncerus caffer*	82.56	85.05	83.25
Hippopotamus	*Hippopotamus ampibius*	87.58	86.73	82.43
Black rhino	*Diceros birconis*	75.39	85.60	82.43
African elephant	*Loxodonta aficana*	83.15	83.84	80.80
Lesser kudu	*Tragelaphus imberbis*	84.47	85.71	83.67
Lesser cane rat	*Thryonomys gregorianus*	81.91	84.60	8.69

4.6.1 HRMA for identification of bush meat samples

Identification of bushmeat species hunted by sequencing

The first analyis was done on 28 samples which had different venacular names indicating that they represent different species. All samples in all primers portrayed the profiles with two different shapes (Fig 7). In order to confirm their identities they were sequenced. The results showed that all samples represented two subspecies of the plain zebra specie which are *Equus burchelii quagga* (n=15) and *Equus burchelii chapman* (n=13) (Table 7).

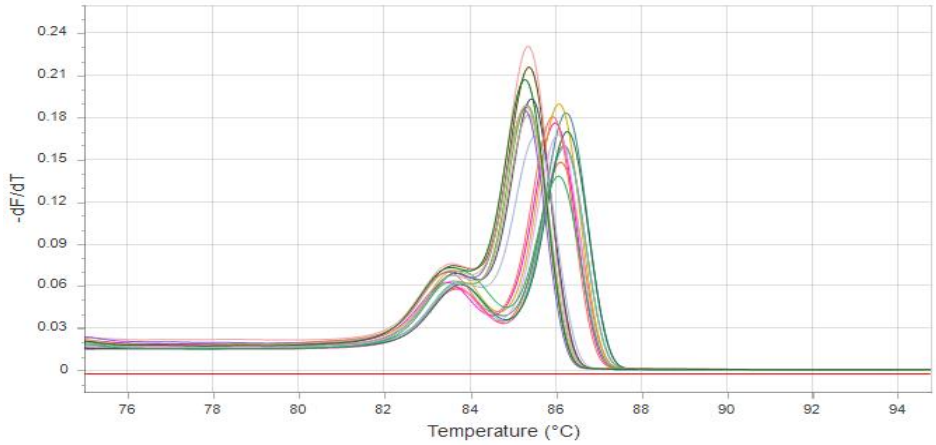

Figure 15: Profiles of COI showing two zebra subspecies

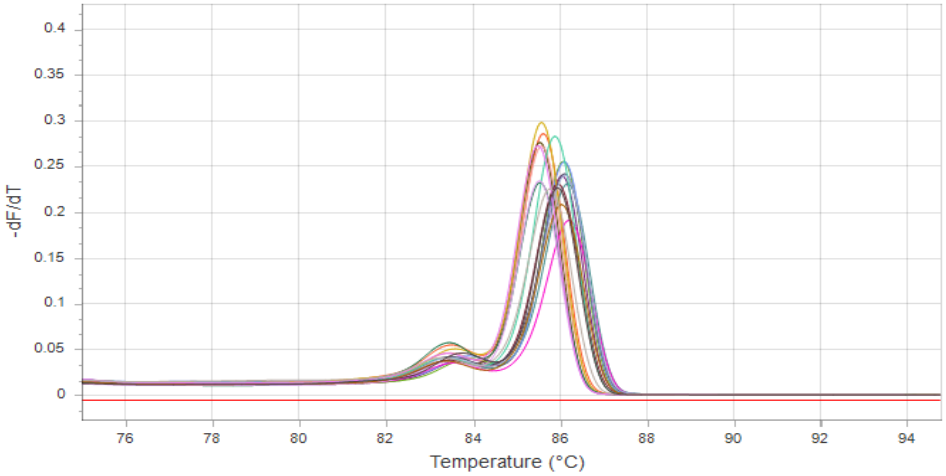

Figure 16: Profiles of Cytb showing two zebra subspecies

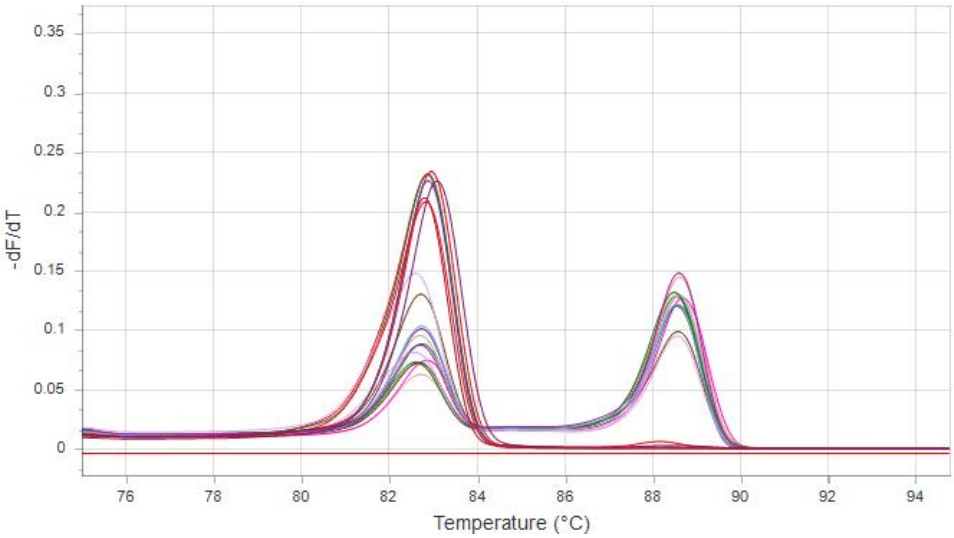

Figure 17: Profiles of 16S showing two zebra subspecies

4.7 Sequences of two subspecies of plain Zebra

ACAATGTCATTGTAACCGCCCACGCATTCGTAATAATTTTCTTCATAGTCATACCCATTATGATCGGAGGATTTGGGAAC

TGATTAGTCCCCTTAATAATTGGAGCACCTGATATAGCTTTTCCCCGAATAAACAACATAAGCTTCTGATTACTTCCCCC

ATCATTCCTACTTCTTCTTGCTTCTTCAATAATTGAAGCGGGCGCTGGAACAGGCTGAACCGTATATCCTCCCCTAGCTG

GAAATCTAGCGCACGCAGGGGCCTCTGTTGACTTAACCATTTTCTCCCTCCACCTGGCTGGTGTATCTTCAATTTTAGGT

GCCATCAATTTCATTACCACAATCATCAACATAAAACCACCAGCTCTATCTCAATACCAAACTCCTCTATTCGTTTGATC

TGTCCTTATTACGGCAGTACTCCTTCTTCTAGCTCTTCCAGTCCTAGCAGCCGGTATTACCATGCTCCTCACAGACCGTA

ATCTAAACACTACCTTCTTCGACCCTGCAGGGGGTGGGGACCCAATCCTTTACCAACACCTATTCTG-

Equusburchelii quagga

CCTACTAGGAGATGACCAGATCTACAATGTCATTGTGACCGCCCACGCATTCGTAATAATTTTCTTCATAGTCATACCCA

TTATGATCGGAGGATTTGGGAACTGATTAGTCCCCTTAATAATTGGAGCACCTGATATAGCTTTTCCCCGAATAAACAAC

ATAAGCTTCTGATTACTTCCCCCATCATTCCTACTTCTTCTTGCTTCTTCAATAATTGAAGCGGGCGCTGGAACAGGCTG

AACCGTATATCCTCCCCTAGCTGGAAATCTAGCGCACGCAGGGGCTTCTGTTGACTTAACCATTTTCTCTCTCCACCTGG

CTGGTGTATCTTCAATTTTAGGTGCCATCAATTTCATTACCACAATCATCAACATAAAACCACCAGCTCTATCTCAATAC

CAAACTCCTCTATTCGTTTGATCTGTCCTTATTACGGCAGTACTCCTTCTTCTAGCCCTTCCAGTCCTAGCAGCCGGTATT

ACCATGCTCCTCACAGACCGTAATCTAAACACTACCTTCTTCGACCCTGCAGGGGGAGGGGACCCAATCCTTTATCAAC

ACCTATTCTGA -***Equus burchelii chapman***

Table 8: Names of the two Zebra subspecies confirmed after sequencing which were given different local names

Lab no	Local name	Common name	Scientific name
7	Ngitero	Zebra	*Equus burchelii quagga*
8	Inchole	Zebra	*Equus burchelii quagga*
9	Inkonya	Zebra	*Equus burchelii quagga*
10	Mbubuse	Zebra	*Equus burchelii chapman*
11	Hanakwe	Zebra	*Equus burchelii quagga*
12	Isenye	Zebra	*Equus burchelii quagga*
13	Imkoli	Zebra	*Equus burchelii chapman*
14	Cholo	Zebra	*Equus burchelii chapman*
15	Imboto	Zebra	*Equus burchelii chapman*
16	Kigongo	Zebra	*Equus burchelii chapman*
17	Imbuli	Zebra	*Equus burchelii chapman*
18	Nzombi	Zebra	*Equus burchelii quagga*
19	Ingwe	Zebra	*Equus burchelii chapman*
20	Intele	Zebra	*Equus burchelii chapman*
21	Kisokolokobwe	Zebra	*Equus burchelii quagga*
22	Inzengwe	Zebra	*Equus burchelii quagga*
23	Inkoko	Zebra	*Equus burchelii quagga*
24	Maseso	Zebra	*Equus burchelii quagga*
25	Kuncho	Zebra	*Equus burchelii chapman*
26	Ingwele	Zebra	*Equus burchelii chapman*
27	Sabala	Zebra	*Equus burchelii chapman*
28	Indasa	Zebra	*Equus burchelii quagga*
29	Mbichi	Zebra	*Equus burchelii quagga*
30	Korongo	Zebra	*Equus burchelii quagga*
31	Ingela	Zebra	*Equus burchelii chapman*
32	Swala	Zebra	*Equus burchelii chapman*
33	Nungununu	Zebra	*Equus burchelii quagga*
34	Idodo	Zebra	*Equus burchelii chapman*

Identification of species hunted by using qPCR-HRMA

All samples which were analyzed and produced the same HRMA melt patterns and Tm values were grouped into one group presumed to be originating from the same species and compared with the reference sample for identification.

One sample was selected for sequence as the representative of the group that did not match any reference. A total of 20 groups from CYTB, COI and 16S were obtained. The criteria of grouping was the shape and melting temperature. The species having the melting temperature falling within 10 ± was grouped as the same species (Table 8). Out of those groups, 103 samples were identified into respective species and 35 remained unknown.

Table 9 represents the general results of the species diversity among the bush meat samples with their respective melting temperatures in which a total of 20 species were identified. Zebra constituted the highest number (n=51, 49.5%) of the identified samples followed by hartebeest, which had (n=10, 9.7%) samples. In addition, savanna hare, wildebeest and common duiker constituted (n=4, 3.9%) each, while reedbuck, topi, Grant's gazelle and impala constituted (n=3, 2.9%) each. These were relatively frequently identified among the bush meat samples. Giraffe, hippopotamus, common warthog, civet cat, eland, bushbuck, genet and buffalo constituted (n=2, 1.9%) each while Greater cane rat, suni antelope, Thomson gazelle and giraffe had only one verified sample each. The melting profiles of all identified animals was obtained (Appendix 2). The melting profiles of 10 species (Greater cane rat, warthog, zebra, hartebeest, savannah hare, suni antelope, red forest duiker, genet, civet and hippopotamus) of 16S, COI and *cyt b* are depicted in Fig 8, 9 and 10, respectively.

Table 9: shows 28 samples which were grouped as one group representing the same species in all three primers

Lab no	Local name	Common name	Scientific name	COI™	CYTB™	16s™
7	**Ngitero**	Zebra	*Equus burchelii quagga*	74.42	85.06	74.05
8	Imboto	Zebra	*Equus burchelii quagga*	74.64	85.49	75.25
9	Inchole	Zebra	*Equus burchelii quagga*	74.86	85.52	75.35
10	Kigongo	Zebra	*Equus burchelii chapman*	75.04	85.53	82.41
11	Inkonya	Zebra	*Equus burchelii quagga*	75.05	85.54	82.43
12	Imbuli	Zebra	*Equus burchelii quagga*	75.08	85.57	82.45
13	Mbubuse	Zebra	*Equus burchelii chapman*	75.1	85.57	82.5
14	Nzombi	Zebra	*Equus burchelii chapman*	75.3	85.61	82.5
15	Harakwe	Zebra	*Equus burchelii chapman*	75.34	85.82	82.5
16	Ingwe	Zebra	*Equus burchelii chapman*	75.35	85.86	82.51
17	Insenye	Zebra	*Equus burchelii chapman*	75.36	85.87	82.52
18	Intele	Zebra	*Equus burchelii quagga*	75.36	85.88	82.52
19	Imkole	Zebra	*Equus burchelii chapman*	75.37	85.95	82.54
20	Kisokolokobwe	Zebra	*Equus burchelii chapman*	75.38	85.95	82.55
21	Choro	Zebra	*Equus burchelii quagga*	75.39	85.97	82.55
22	Inzengwe	Zebra	*Equus burchelii quagga*	75.39	85.98	82.56
23	Inkoko	Zebra	*Equus burchelii quagga*	75.4	86.03	82.56
24	Maseso	Zebra	*Equus burchelii quagga*	75.41	86.04	82.59
25	Kuncho	Zebra	*Equus burchelii chapman*	75.42	86.04	82.61
26	Ingwele	Zebra	*Equus burchelii chapman*	75.42	86.06	82.62
27	Sabala	Zebra	*Equus burchelii chapman*	75.42	86.07	82.62
28	Indasa	Zebra	*Equus burchelii quagga*	75.54	86.09	82.63
29	Mbichi	Zebra	*Equus burchelii quagga*	75.72	86.1	82.69
30	Korogwe	Zebra	*Equus burchelii quagga*	75.77	86.11	82.72
31	Ingela	Zebra	*Equus burchelii chapman*	77.08	86.17	82.76
32	Swala paa	Zebra	*Equus burchelii chapman*	77.13	86.17	82.81
33	Idodo	Zebra	*Equus burchelii quagga*	77.14	86.2	82.84
34	Nungunungu	Zebra	*Equus burchelii chapman*	78.04	86.22	82.9

Table 10: **Number (N), percentage (%) and the melting temperature of three markers (COI, CYTB and 16S) of identified species (n=103) among bush meat samples obtained from Tarime district located in Western Serengeti**

Common name	Scientific name	N	%	COI	CYTB	16S
Zebra	*Equus burchelii*	51	49.5	85.95	83.95	82.84
Hartebeest	*Alcelaphus buselaphus*	10	9.7	85.50	84.28	82.15
Savanna hare	*Lepus microtis*	4	3.9	82.44	83.62	82.99
Wildebeest	*Connochaetes taurinus*	4	3.9	84.39	83.98	82.51
Red forest duiker	*Cephalophus natalensis*	4	3.9	84.43	86.17	84.80
Bohor reedbuck	*Redunca redunca*	3	2.9	81.72	84.70	83.79
Topi	*Damaliscus lunatus*	3	2.9	84.47	84.90	81.81
Grant gazelle	Nanger granti	3	2.9	81.36	84.51	82.92
Impala	*Aepyceros melampus*	3	2.9	85.57	84.80	84.10
Hipopotamus	*Hipopotamus amphibius*	2	1.9	87.58	86.73	82.43
Common warthog	*Phacochoerus africanus*	2	1.9	83.96	84.54	81.52
Civet cat	*Civettictis civetta*	2	1.9	83.98	82.64	82.60
Eland	*Tragelaphus oryx*	2	1.9	82.54	84.16	83.26
Bushbuck	*Tragelaphus Scriptus*	2	1.9	83.77	84.35	82.77
Genet	*Genetta genetta*	2	1.9	83.12	83.42	81.12
African buffalo	*Syncerus caffer*	2	1.9	82.56	85.05	83.25
Greater canerat	*Thyronomys sweinderinus*	1	0.97	83.97	84.16	80.48
Sunni antelope	*Neotragus moschatus*	1	0.97	84.07	84.61	83.81
Thomsons gazellle	*Eudorcas thomsonii*	1	0.97	83.00	77.62	81.52
Girrafe	*Girrafa camelopardaris*	1	0.97	86.44	84.67	84.21

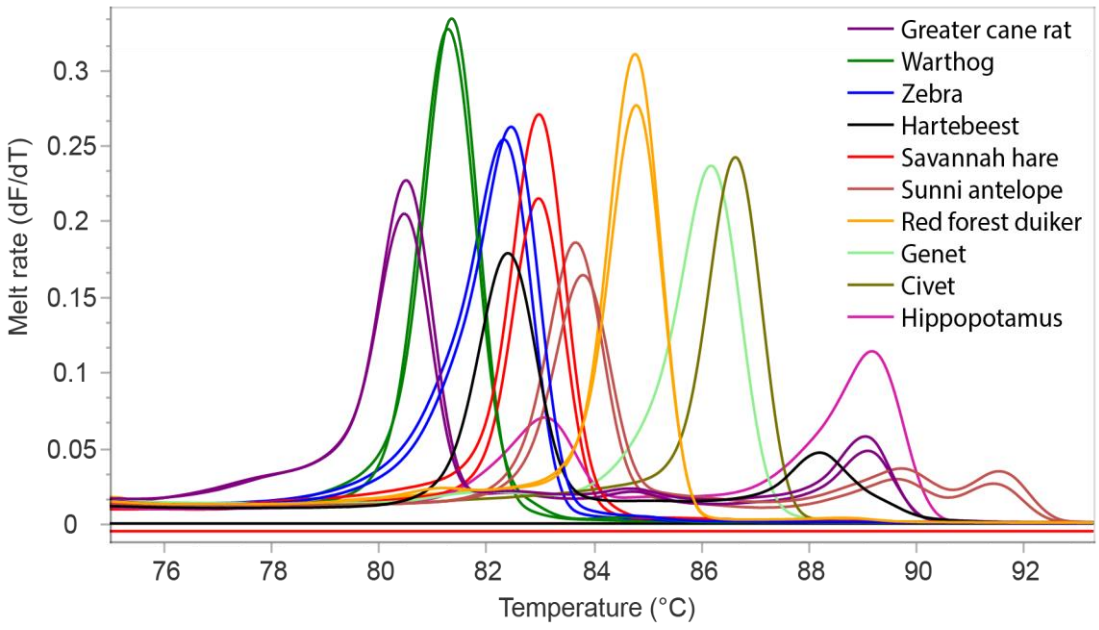

Figure 18: Profiles of 10 species by 16S marker from unknown samples

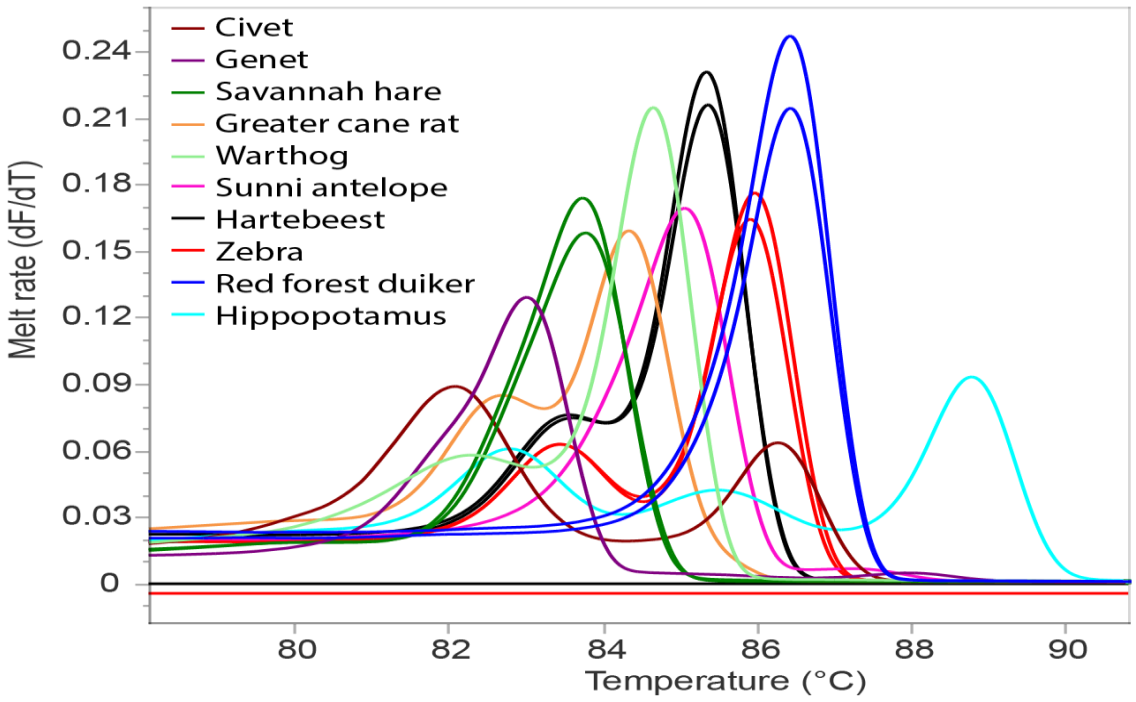

Figure 19: Profiles of 10 species by cytb marker from unkown samples

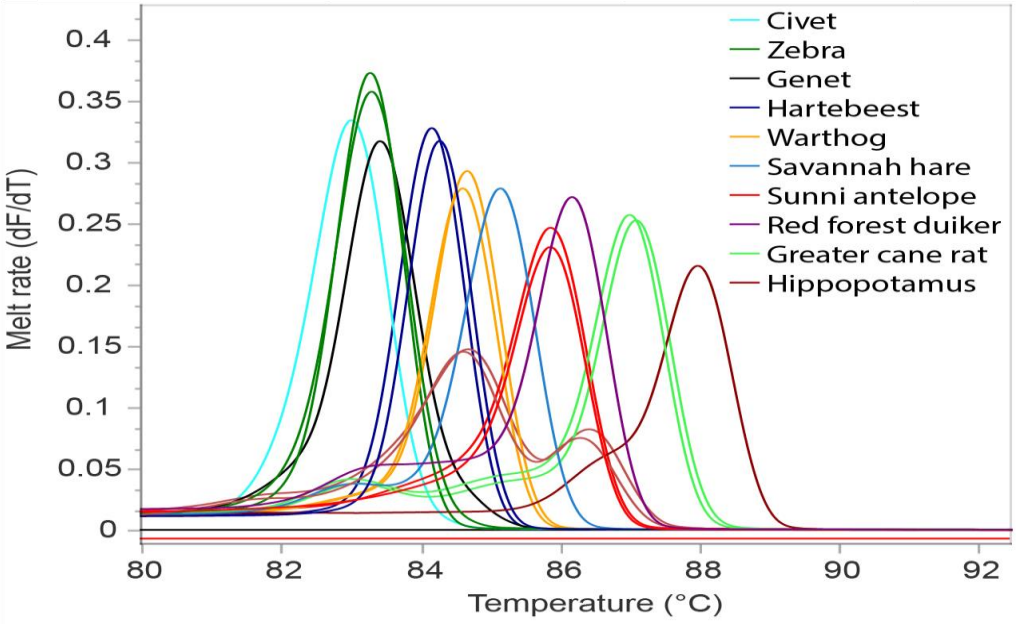

Figure 20: Profiles of 10 species by COI marker from unknown samples

CHAPTER FIVE

5.0 DISCUSSION

5.1 Bush Meat Dynamics

5.1.1 Hunting season

Illegal Hunting in the study area occurs all year round, but it is severe during the dry season (July-September). Four reasons are associated with this pattern: first, during this period animals are moving from Serengeti national park to Masaimara crossing Mara River. During migration animals are in a great panic and confusion spreading up to village lands making it easy to be accessed by hunters. Second this period (July-September) coincides with high season for tourism activities, June-October (https://ww.safaribookings.com/serengeti/best-time). As a result, park authorities put high priority to tourist safety thereby allocating many rangers around different tourism facilities such as campsites and hotels as well as accompanying tourists, for example on walking safaris, mountain hiking and canoeing. This situation may lead to shortage of rangers available for anti-poaching patrols hence giving room for illegal activities including hunting. Third, July- September is the dry spell period, which is associated with ungulate concentration around water sources (Brown, 2007). Such points make wildlife vulnerable to illegal hunting as hunters can easily ambush them at one point than in wet season when they are scattered in various areas including those less accessible due to over-flooded rivers and dirt roads (Holmern *et al*., 2007).

Fourth, in accordance to farmers' calendar of activities July-September is off season, therefore farmers divert to illegal hunting as a dual source of protein and income (Lindsey *et al*., 2012; Holmern *et al*., 2007; Loibooki *et al*., 2002, Nyahongo *et al*., 2005).

Therefore, understanding seasonal hunting patterns, particularly time of the year when illegal hunting is ultimate can help to alleviate poaching thus improve biodiversity conservation. Since energy and funds to run anti-poaching activities are limited, focusing such efforts in the most critical times of the year would make best use of limited resources.

5.2.2 Hunting methods

The most reported hunting method was snaring, which consist of a noose fastened to a support material such as woody substance along an animal pass e.g. along wildlife trails or areas close to water sources (Hofer *et al*., 1996). Animals are caught when they put their head (or a leg) into the snare and pull it tight as they keep moving (Noss, 1998).

The method is preferred to poison, arrows, pits and fire arms due to its readily availability at low cost, and difficult for enforcement agencies to detect (Lindsey *et al*., 2012). Use of snare as the preferred method of illegal hunting has previously been reported in the Serengeti ecosystem by Holmern *et al*. (2007).

Following recent socio-economic transformations, including increased pressures from mushrooming human populations in the western edge of Serengeti ecosystem such as Bunda, Magu, Bariadi, Tarime, Maswa and Meatu districts, and the generalized use of firearms, the magnitude of illegal bush meat hunting has reached unsustainable levels (Nyahongo *et al*., 2005; Gaubert *et al*., 2015; Jenkins *et al*., 2011; Nasi *et al*., 2008; Noss, 1997).

5.2.3 Species hunted

From the questionnaire surveys, wildebeests appeared to be the most hunted ungulate. This finding corresponds with previous results in the same ecosystem (Bitanyi *et al*. 2012;

Lindsey *et al*., 2012). This could be linked to being easy to hunt, high abundance,wide distribution together with the fact that . Other regularly hunted species also reported are zebra, eland, buffalo and Thompson's gazelle (Ndibalema and Songorwa, 2008; Bitanyi *et al.,* 2012). Generally, hunting of wildlife species is governed by availability of species, taste of meat and motives for hunting (Kaltenborn *et al*., 2006; Loibooki, 2002; Ndibalema and Songorwa, 2008; Mfunda and Røskaft, 2010).

5.2.4 Bush meat consumption and preference

In Africa most people regard bush meat as a vital dietary item for a complex combination of reasons such as lack of alternate sources of protein, financial limitations, preference and cultural values (Morsello *et al*., 2015). However, the observed higher bush-meat consumption and preference for people with high education level in this study may be due to their knowledge that bush meat has higher nutrient contents, and it is relatively less exposed to antibiotics as a result of medical services rendered. These attributes make bush-meat to be considered healthier than domestic meat thereby creating its higher demand. The nutritional value of bush meat is widely acknowledged in different studies. Available evidence indicates that bush-meat compares favorably well with domestic meat in higher mineral and protein, but less fat contents (Bennett *et al*., 2002; Fa *et al*., 1995; Loibooki *et al.*, 2002) and higher caloric (energy) value (Okiwelu *et al*., 2010).

From this study, age had effect on bush-meat consumption, but not preference. In this context, young and middle aged people had high bush-meat consumption than old aged people. The fact that young and middle aged people are the ones that go hunting provides them additional access to bush-meat consumption than those who remain behind. Therefore, results of this study contradict with Hema *et al*. (2017) who reported that younger people have low bush meat consumption than older people due to partly growing

‘westernization’ of the lifestyles, especially among the middle classes. According to them, this age class of the community often do not see bush-meat consumption as ‘socially acceptable’ as it is perceived by them as a sign of ‘being very local’ (i.e. not culturally advanced). Equally, results of this study disagree with Le Breton *et al*. (2006) and Fa *et al*. (2003) who found no effect of age on both bush meat consumption and preference while Morsello *et al*. (2015) found effect of individual characteristics, specifically age and sex, relevant to preference but not to consumption.

Further, immigrants had high bush meat preference than native people in the study area. This may be associated with lack of cultures and norms that might be inhibitive on their side in respect to bush-meat consumption. For example while the Kurya who are the native in habitants of western Serengeti do not eat elephants because they believe that they are their deceased chiefs (kideghesho, 2008), immigrants such as chagga eat a range of bush-meat including the elephant itself, and therefore immigrants frequently involved in illegal hunting (Mfunda and Røskaft, 2010).

5.2 Species identification by HRM

The use of HRMA provides a rapid and cheaper surveillance molecular tool allowing a more wide collection and analysis of suspected bush meat samples and therefore accelerate the forensic analysis, increase prosecution, thereby deterring crimes against wildlife. Application of HRMA can also reduce the costs and increase thorough put of molecular surveillance saving a lot of money and labour time for generating forensic evidence for bushmeat prosecution. From conservation point of view, this technique can be very useful since it can distinguish the closely related wildlife and domestic species belonging to the same families such plain’s zebra, grevy’s zebra and domestic donkey (family Equidae) or buffalo and domestic cow (family bovidae).

The species found to be locally hunted for bush-meat in the study area by using HRMA includes zebra, giraffes, bush pig, hare, warthogs, a wide range of antelope species as well as civet and genet. However several other species are not hunted for reasons associated with cultural beliefs (Ndibalema and Songorwa, 2008; Kideghesho, 2008). For instance, most households in western Serengeti do not eat bushbuck *(Tragelaohus scriptus)* because it is believed to cause leprosy and is therefore culturally forbidden (Mfunda 2001; Kideghesho, 2008). Likewise, the consumption of Roan antelope *(Hippotragus equinus)* is forbidden because killing it would bring bad luck and possibly death to the family (Mfunda, 2010) while elephants are not consumed because they believe that they are deceased chiefs (Kideghesho, 2008) hence contribute to biodiversity conservation. Though cultural beliefs lessen extent of bush-meat hunting, the activity remains among conservation challenges facing the ecosystem given that immigrants are less bound by the cultures related to illegal hunting.

Further, in this study Zebra was clearly identified as the most common hunted species while wildebeest and buffalo were reported to be the most hunted species in the previous studies in the same ecosystem (Bitanyi *et al*., 2012; Campbell and Hofer, 1995; Holmern *et al*., 2006; Lindsey *et al*., 2012; Ndibalema and Songorwa, 2008). This may be due to the fact that consumption and preference pattern of bush-meat change from time to time because hunters go for the specie which is easy to hunt and available at a given time of the year. In addition, this pattern may also be associated with taste and motives for hunting (Barnett, 2000; Kaltenborn *et al*., 2006; Ndibalema and Songorwa, 2008; Mfunda and Røskaft, 2010). For example, the deeply rooted hunting practice in western Serengeti synchronizes with annual migration of the herbivores (Kaltenborn *et al*., 2005) whereas the motive for hunting of medium to big sized wildlife such as zebra, wildebeest and buffalo is trading (Morselo *et al.,* 2015).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on investigations made on the current bush meat dynamics and the use qPCR-HRMA approach for accurate species identification this study draws up the following conclusions.

Illegal bush-meat hunting occurs mostly in the dry season (July-September), the period in which wildlife are known to disperse at night to village land in search of green pastures and water sources hence easily ambushed by poachers without being noticed by anti-poaching unit. Crop raiding by wildlife at night encourages bush-meat hunting as retaliatory act for their ravaged crops.

Snares appeared to be the most used hunting method due to easy availability (not bought, but simply corrected by community members) of the snare material in the village land originating from remains of electric cables which are improperly disposed by electricity authorities following construction of power lines.

Younger community members appeared to have more bush-meat in their menu compared to older people because they have addition access to bush-meat right from the field to home as they are involved in hunting events.

Educated immigrants showed high bush-meat preference as they are not bound by any indigenous culture against consumption of any wildlife species. Moreover, younger educated respondents had high bush-meat preference and consumption based on

knowledge on health safety (little antibiotics and alike) and high nutritional values of wildlife meat.

Molecular technique proved to be more authentic technique in bush-meat identification which have lost morphological features as it could refute 28 samples collected independently having different local names to a single specie i. e plains zebra. This affirm the reliability of the technique in resolving wildlife forensic cases.

6.2 Recommendations

i. High surveillance against poaching is needed by wildlife authorities during dry season to combat rampart illegal hunting for bush-meat that occurs during the period.
ii. TANESCO should improvise proper disposal of the unworthy wire cables in a way that they will not be accessed by local communities to use them against wildlife.
iii. Sensitization of both primary and secondary school students should be enhanced by community based wildlife conservation officers on the effects of illegal hunting to biodiversity and economy of the country at large through tourism related activities.
iv. For the HRM to be used in identification of species all samples should be extracted by using the same extraction procedures and each run should start with the same concentration of the DNA template.
v. Since HRMA is a rapid and cheap screening tool it can be used in many developing countries like Tanzania in the court of law to facilitate screening of samples taken as exhibits and hence reduce time taken for prosecution of wildlife related cases.

vi. HRMA can be used in the airports by either cites or the ministry of natural resource in screening all meat samples that are suspected to be bushmeat of both endangered and non-endangered.

vii. For the bush-meat samples to be accepted in court of law as exhibits should be analyzed using molecular procedures proved to reliable

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APPENDICES

Appendix 1: Household survey questionnaire

Part1: Particulars of respondent

Questionnaire number……

Date……..

Village name……..

Ward……..

Sex:1 Female……. 2 Male……..

Age……

1.1 Education level

1) No formal education 2) Primary education

3) Secondary education 4) College/university

1.2Marital status

1) Single 2) Married

3) Divorced 4) widowed/widower

1.3 Total number of people in the household

1) 1-2 2) 3-5

3) 6-8 4) 9 and above

1.4 Household income per annum (in Tshs)

1) Less than 500,000 2)500000-800000

3) 800000-1000000 4) Above 1000000

1.5 What is your occupation?

1) Crop production 2) livestock keeping

3) Hunting 4) Crop production and livestock keeping

5) Crop production and business 6) Crop production and hunting

7) Livestock keeping and business 8) other (mention)

Part II Source of protein

2.0 Meat consumption as protein source for the household.

1 cattle 2 sheep 3 goat 4 pig 5 fish 6 chicken 7 bushmeat

Others mention……..

2.1 Meat prices in the village

Meat type consumed	Price per kg
cattle	
sheep	
goat	
pig	
fish	
chicken	
bushmeat	
others	

2.2 Meat preferred

Type of meat	**Meat preference**
cattle	
sheep	
goat	
Pig	
fish	
chicken	
bushmeat	
others	

1 high 2 medium 3 low

2.3 Why **do you prefer such meat?**

Part III Motivation for hunting

3.0 Source of meat protein in the village

1) Beef 2)fish 3) pork 4) egg 5) chicken 6)bushmeat

7) others (mention)

3.1 The following part consist of statements. Please indicate to which level you agree or disagree with the statement by ticking in the appropriate number.

1) Strongly agree 2) Disagree

3) Slightly disagree 4) Neutral

5) Slightly agree 6) Agree

7) Strongly agree

I pouch because of …….

1=strongly agree,2=Disagree,3= Slightly disagree,4= Neutral,5= Slightly agre,6= Agree,7= Strongly agree	1	2	3	4	5	6	7
For economic gain							
Household consumption							
Animal destroy my crops							
Animals predate my livestock							
My cultural belief							
For cash/trade							

2.2 Which species are usually targeted for bush meat

1) Zebra 2) Wildebeest

3) Impala 4) Warthog

5) Buffalo 6) Dik-dik

7) Other mention…….……

2.4 What is the bushmeat price in the area?

Part IV Information about hunter's activities

3.0 Tools /methods preferred in poaching activities are ……..

1=strongly agree,2=Disagree,3= Slightly disagree,4= Neutral,5= Slightly agre,6= Agree,7= Strongly agree	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Snares							
Digging pits							
Fire arms							
Poison							

3.5 Season does hunting occur……..

1=strongly agree,2=Disagree,3= Slightly disagree,4= Neutral,5= Slightly agre,6= Agree,7= Strongly agree	**1**	**2**	**3**	**4**	**5**	**6**	**7**
January to March							
April to June							
July to September							
October to December							

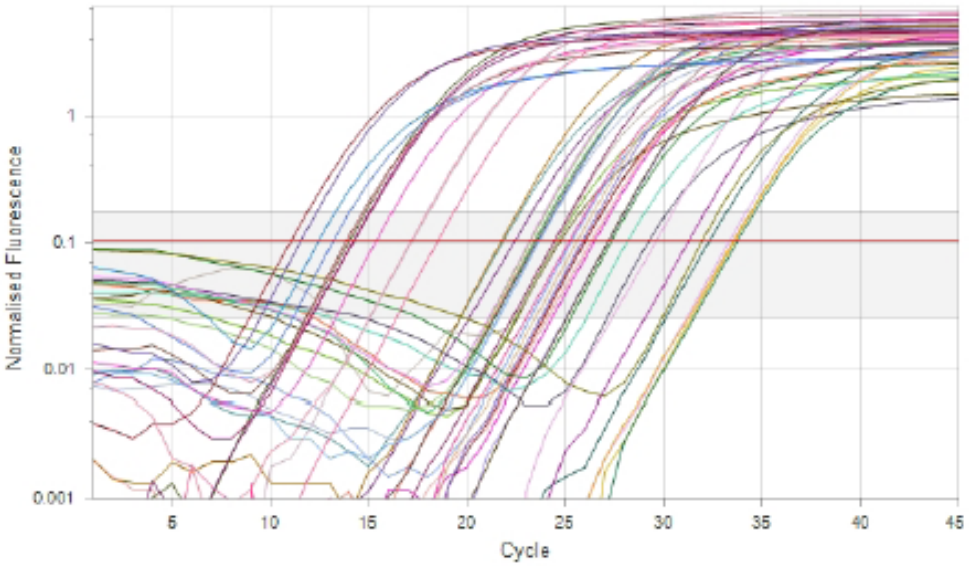
Why…….

Appendix 2: Quantification cycles (Cq) of the samples (7-34) extracted by using SDS buffer, proteinase k buffer and extraction kit respectively using three molecular markers (CO1 Cytb and 16S respectively).

Report

Cycling: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Normalisation	LinRegPCR
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.107 (Automatic) starting at cycle 1



Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
1		NGITERO_7_SDS_COI	22.41	0.83	0.99999	
2		INCHOLE_8_SDS_COI	24.59	0.89	0.99904	
3		INKONYA_9_SDS_COI	26.03	0.94	0.99947	
4		MBUBUSE_10_SDS_COI	21.86	0.89	0.99989	
5		HANAKWE_11_SDS_COI	24.76	0.83	0.99685	
6		ISENYE_12_SDS_COI	26.31	0.87	0.99995	
7		IMKOLI_13_SDS_COI	25.29	0.93	0.99980	
8		CHOLO_14_SDS_COI	26.46	0.90	0.99991	
9		IMBOTO_15_SDS_COI	26.05	0.90	0.99967	
10		KIGONGO_16_SDS_COI	33.61	0.88	0.99995	
11		IMBULI_17_SDS_COI	29.25	0.87	0.99844	
12		NZOMBI_18_SDS_COI	25.21	0.89	0.99999	
13		INGWE_19_SDS_COI	28.18	0.90	0.99903	
14		INTELE_20_SDS_COI	23.65	0.92	0.99979	

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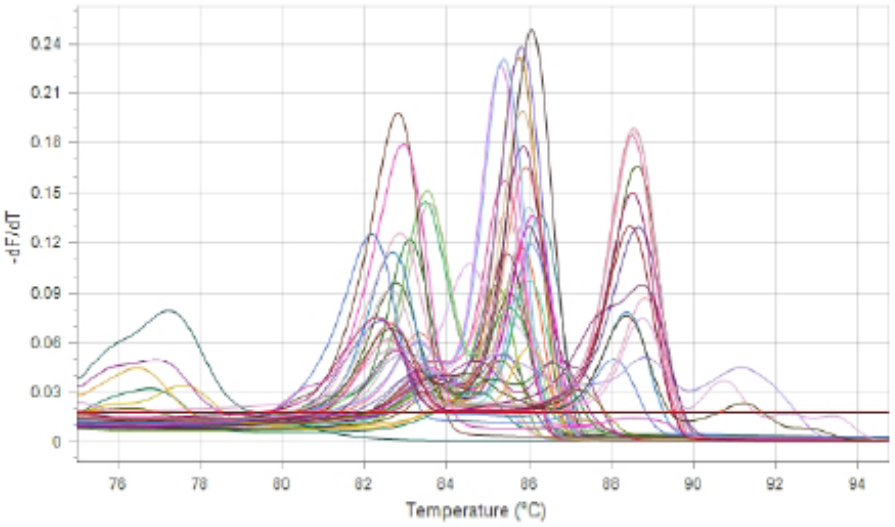
Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
15		NTC	33.77	0.87	0.99995	
16		H2O	33.33	0.89	0.99997	
17		NGITERO_7_SDS_CYTB	21.75	0.92	0.99983	
18		INCHOLE_8_SDS_CYTB	23.41	0.91	0.99981	
19		INKONYA_9_SDS_CYTB	23.43	0.96	0.99990	
20		MBUBUSE_10_SDS_CYTB	23.17	0.96	0.99985	
21		HANAKWE_11_SDS_CYTB	24.56	0.91	0.99798	
22		ISENYE_12_SDS_CYTB	25.89	0.92	0.99994	
23		IMKOLI_13_SDS_CYTB	23.22	0.97	0.99995	
24		CHOLO_14_SDS_CYTB	25.58	0.92	0.99994	
25		IMBOTO_15_SDS_CYTB	32.32	0.86	0.99983	
26		KIGONGO_16_SDS_CYTB	24.42	0.91	0.99996	
27		IMBULI_17_SDS_CYTB	27.32	0.95	0.99982	
28		NZOMBI_18_SDS_CYTB	23.55	0.89	0.99999	
29		INGWE_19_SDS_CYTB	32.01	0.97	0.99980	
30		INTELE_20_SDS_CYTB	23.65	0.96	0.99994	
31		NTC	31.26	0.88	0.99994	
32		H2O	33.54	0.84	0.99997	
33		NGITERO_7_SDS_16S	14.05	0.90	1.00000	
34		INCHOLE_8_SDS_16S	11.07	0.92	0.99983	
35		INKONYA_9_SDS_16S	13.88	0.95	0.99963	
36		MBUBUSE_10_SDS_16S	11.51	0.96	0.99988	
37		HANAKWE_11_SDS_16S	12.42	0.93	0.99957	
38		ISENYE_12_SDS_16S	18.48	0.92	0.99988	
39		IMKOLI_13_SDS_16S	15.35	0.94	0.99981	
40		CHOLO_14_SDS_16S	17.16	0.93	0.99986	
41		IMBOTO_15_SDS_16S	27.20	0.89	0.99994	
42		KIGONGO_16_SDS_16S	17.21	0.90	0.99990	
43		IMBULI_17_SDS_16S	13.19	0.96	0.99963	
44		NZOMBI_18_SDS_16S	14.24	0.87	1.00000	
45		INGWE_19_SDS_16S	13.96	0.96	0.99973	
46		INTELE_20_SDS_16S	14.21	0.90	0.99999	
47		NTC	29.60	0.87	0.99995	
48		H2O	27.18	0.93	0.99998	

Melt: CO1_CYTB_16S Genes

Target	HRMA For Surveillance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Invert Data	No
Threshold	0.017 starting at 75°C



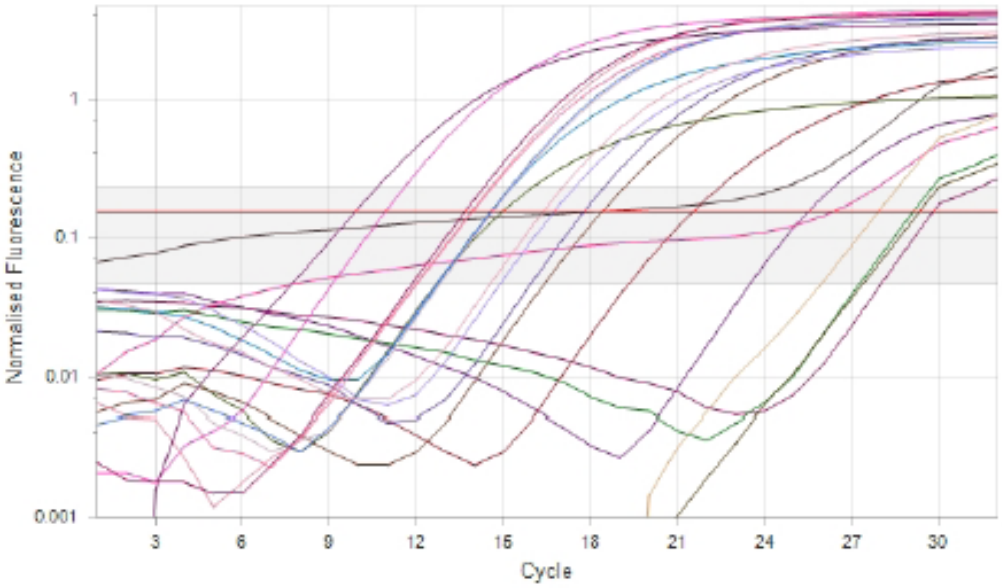
Well	Colour	Sample Name	Tm (°C)	Genotype
1		NGITERO_7_SDS_COI	83.50, 86.01	
2		INCHOLE_8_SDS_COI	85.19	
3		INKONYA_9_SDS_COI	83.41, 85.83	
4		MBUBUSE_10_SDS_COI	83.82, 86.27	
5		HANAKWE_11_SDS_COI	85.55	
6		ISENYE_12_SDS_COI	85.43	
7		IMKOLI_13_SDS_COI	83.66, 86.06	
8		CHOLO_14_SDS_COI	83.62, 86.08	
9		IMBOTO_15_SDS_COI	85.46	
10		KIGONGO_16_SDS_COI	77.57, 84.96	
11		IMBULI_17_SDS_COI	83.45, 85.35	
12		NZOMBI_18_SDS_COI	83.78, 85.40	
13		INGWE_19_SDS_COI	83.57, 86.02	
14		INTELE_20_SDS_COI	83.51, 86.00	
15		NTC	76.74, 84.72	
16		H2O	84.54	
17		NGITERO_7_SDS_CYTB	83.20, 85.76	

Well	Colour	Sample Name	Tm (°C)	Genotype
18		INCHOLE_8_SDS_CYTB	83.33, 85.91	
19		INKONYA_9_SDS_CYTB	83.48, 85.53	
20		MBUBUSE_10_SDS_CYTB	83.32, 85.31	
21		HANAKWE_11_SDS_CYTB	83.58, 85.74	
22		ISENYE_12_SDS_CYTB	83.35, 85.82	
23		IMKOLI_13_SDS_CYTB	83.52, 85.60	
24		CHOLO_14_SDS_CYTB	83.43, 85.83	
25		IMBOTO_15_SDS_CYTB	77.22	
26		KIGONGO_16_SDS_CYTB	83.67, 85.85	
27		IMBULI_17_SDS_CYTB	83.10, 85.12, 86.54	
28		NZOMBI_18_SDS_CYTB	83.40, 86.06	
29		INGWE_19_SDS_CYTB	76.29, 83.46, 85.31, 87.18	
30		INTELE_20_SDS_CYTB	83.30, 85.38	
31		NTC	76.88, 84.69, 86.68	
32		H2O	76.45, 84.12, 86.00	
33		NGITERO_7_SDS_16S	82.75, 84.69, 88.63	
34		INCHOLE_8_SDS_16S	82.62, 84.87, 88.46	
35		INKONYA_9_SDS_16S	82.80	
36		MBUBUSE_10_SDS_16S	82.38, 88.66	
37		HANAKWE_11_SDS_16S	82.68, 88.37	
38		ISENYE_12_SDS_16S	82.57, 84.69, 88.50	
39		IMKOLI_13_SDS_16S	82.93	
40		CHOLO_14_SDS_16S	82.65, 84.95, 88.55	
41		IMBOTO_15_SDS_16S	83.02, 85.26, 88.82, 91.17	
42		KIGONGO_16_SDS_16S	82.63, 84.85, 88.58	
43		IMBULI_17_SDS_16S	82.16, 88.06	
44		NZOMBI_18_SDS_16S	82.75, 88.71	
45		INGWE_19_SDS_16S	82.85, 88.81	
46		INTELE_20_SDS_16S	82.29, 84.61, 88.51	
47		NTC	75.33, 82.71, 85.21, 88.76, 90.74	
48		H2O	82.64, 84.52, 88.37, 91.21	

Report

Cycling: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Normalisation	LinRegPCR
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.154 (Automatic) starting at cycle 1

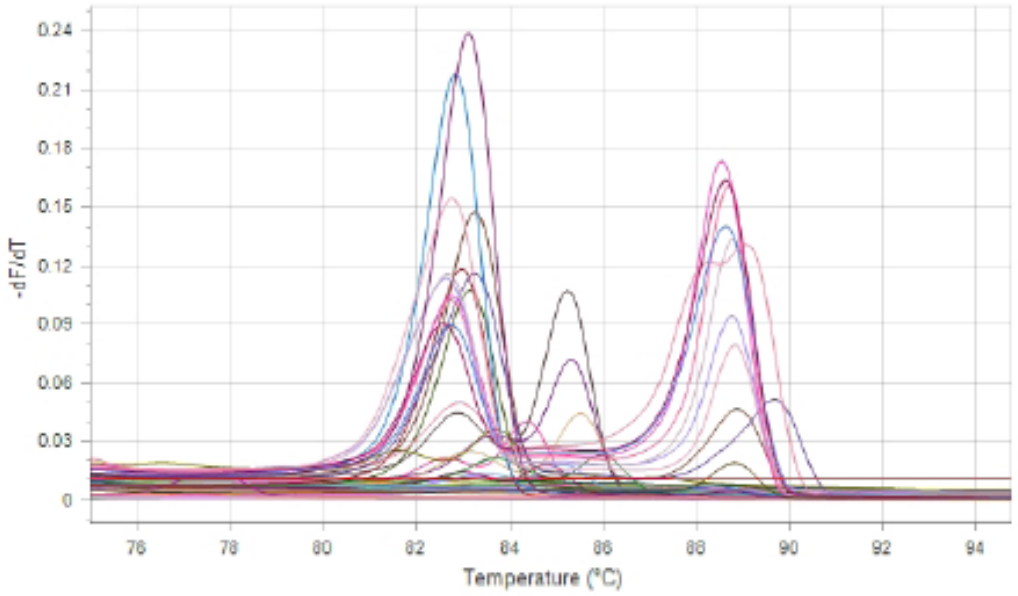


Well	Colour	Sample Name	Cq	Efficiency	Efficiency R^2	Result
1		KISOKOLOKOBWE_21_SDS_COI	25.44	0.75	0.99580	
2		INZENGWE_22_SDS_COI	-	-	-	Excluded
3		INKOKO_23_SDS_COI	-	-	-	Excluded
4		MASESO_24_SDS_COI	-	-	-	Excluded
5		KUNCHO_25_SDS_COI	-	-	-	Excluded
6		INGWELE_26_SDS_COI	-	-	-	Excluded
7		SABALA_27_SDS_COI	-	-	-	Excluded
8		INDASA_28_SDS_COI	-	-	-	Excluded
9		MBICHI_29_SDS_COI	-	-	-	Excluded
10		KORONGO_30_SDS_COI	-	-	-	Excluded
11		INGELA_31_SDS_COI	-	-	-	Excluded
12		SWALA PAA_32_SDS_COI	26.34	0.06	0.93084	
13		NUNGUNUNGU_33_SDS_COI	-	-	-	Excluded
14		IDODO_34_SDS_COI	-	-	-	Excluded

Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
15		NTC	-	-	-	Excluded
16		H20	-	-	-	Excluded
17		KISOKOLOKOBWE_21_SDS_CYTB	-	-	-	Excluded
18		INZENGWE_22_SDS_CYTB	-	-	-	Excluded
19		INKOKO_23_SDS_CYTB	-	-	-	Excluded
20		MASESO_24_SDS_CYTB	-	-	-	Excluded
21		KUNCHO_25_SDS_CYTB	-	-	-	Excluded
22		INGWELE_26_SDS_CYTB	-	-	-	Excluded
23		SABALA_27_SDS_CYTB	-	-	-	Excluded
24		INDASA_28_SDS_CYTB	27.89	0.84	0.99999	
25		MBICHI_29_SDS_CYTB	-	-	-	Excluded
26		KORONGO_30_SDS_CYTB	29.75	0.63	0.95670	
27		INGELA_31_SDS_CYTB	29.09	0.90	0.99979	
28		SWALA PAA_32_SDS_CYTB	18.38	0.05	0.90126	
29		NUNGUNUNGU_33_SDS_CYTB	-	-	-	Excluded
30		IDODO_34_SDS_CYTB	-	-	-	Excluded
31		NTC	-	-	-	Excluded
32		H20	-	-	-	Excluded
33		KISOKOLOKOBWE_21_SDS_16S	14.96	0.63	0.99409	
34		INZENGWE_22_SDS_16S	21.48	0.67	0.99711	
35		INKOKO_23_SDS_16S	18.35	0.78	0.99888	
36		MASESO_24_SDS_16S	17.72	0.89	0.99934	
37		KUNCHO_25_SDS_16S	14.50	0.88	0.99883	
38		INGWELE_26_SDS_16S	13.91	0.85	0.99986	
39		SABALA_27_SDS_16S	10.91	0.91	0.99980	
40		INDASA_28_SDS_16S	13.86	0.86	0.99993	
41		MBICHI_29_SDS_16S	16.69	0.87	0.99903	
42		KORONGO_30_SDS_16S	14.55	0.92	0.99965	
43		INGELA_31_SDS_16S	14.49	0.89	0.99988	
44		SWALA PAA_32_SDS_16S	9.87	0.73	0.99995	
45		NUNGUNUNGU_33_SDS_16S	16.31	0.90	0.99954	
46		IDODO_34_SDS_16S	13.60	0.91	0.99988	
47		NTC	-	-	-	Excluded
48		H20	29.27	0.85	0.99999	

Melt: CO1_CYTB_16S Genes

Target	HRMA For Surveillance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Invert Data	No
Threshold	0.010 starting at 75°C



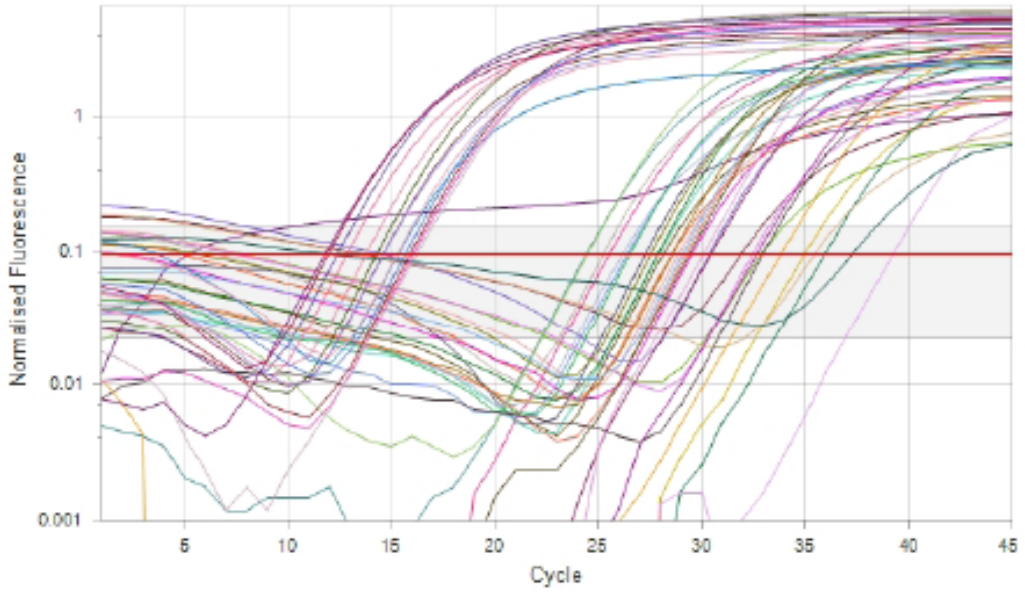
Well	Colour	Sample Name	Tm (°C)	Genotype
1		KISOKOLOKOBWE_21_SDS_COI	75.34, 78.05, 83.68, 85.31	
2		INZENGWE_22_SDS_COI		
3		INKOKO_23_SDS_COI		
4		MASESO_24_SDS_COI		
5		KUNCHO_25_SDS_COI		
6		INGWELE_26_SDS_COI		
7		SABALA_27_SDS_COI		
8		INDASA_28_SDS_COI	84.53	
9		MBICHI_29_SDS_COI		
10		KORONGO_30_SDS_COI	84.85	
11		INGELA_31_SDS_COI		
12		SWALA PAA_32_SDS_COI	82.64, 84.36	
13		NUNGUNUNGU_33_SDS_COI		
14		IDODO_34_SDS_COI		
15		NTC		
16		H20		

Well	Colour	Sample Name	Tm (°C)	Genotype
17		KISOKOLOKOBWE_21_SDS_CYTB	75.06	
18		INZENGWE_22_SDS_CYTB		
19		INKOKO_23_SDS_CYTB		
20		MASESO_24_SDS_CYTB	75.37, 81.22, 83.33	
21		KUNCHO_25_SDS_CYTB		
22		INGWELE_26_SDS_CYTB		
23		SABALA_27_SDS_CYTB		
24		INDASA_28_SDS_CYTB	83.15, 85.51	
25		MBICHI_29_SDS_CYTB	75.37, 78.06, 80.78, 82.73	
26		KORONGO_30_SDS_CYTB	83.00, 84.78	
27		INGELA_31_SDS_CYTB	83.71, 85.97	
28		SWALA PAA_32_SDS_CYTB	75.38, 82.88, 85.23	
29		NUNGUNUNGU_33_SDS_CYTB	75.16, 76.55, 81.63, 83.69, 87.04	
30		IDODO_34_SDS_CYTB	83.45, 85.13	
31		NTC	77.61	
32		H20		
33		KISOKOLOKOBWE_21_SDS_16S	83.14	
34		INZENGWE_22_SDS_16S	82.95	
35		INKOKO_23_SDS_16S	75.41, 83.25, 85.24, 88.87	
36		MASESO_24_SDS_16S	76.91, 78.23, 83.23, 89.66	
37		KUNCHO_25_SDS_16S	75.35, 82.82	
38		INGWELE_26_SDS_16S	75.37, 82.71, 84.88, 88.68	
39		SABALA_27_SDS_16S	75.22, 82.75, 84.64, 88.54	
40		INDASA_28_SDS_16S	82.92, 89.01	
41		MBICHI_29_SDS_16S	75.38, 82.61, 88.75	
42		KORONGO_30_SDS_16S	75.39, 82.65, 84.99, 88.77	
43		INGELA_31_SDS_16S	75.40, 82.73, 85.03, 88.62	
44		SWALA PAA_32_SDS_16S	75.40, 83.10	
45		NUNGUNUNGU_33_SDS_16S	75.37, 82.74, 88.82	
46		IDODO_34_SDS_16S	75.41, 82.56, 84.66, 88.61	
47		NTC		
48		H20	88.82	

Report

Cycling: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Normalisation	LinRegPCR
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.095 (Automatic) starting at cycle 1



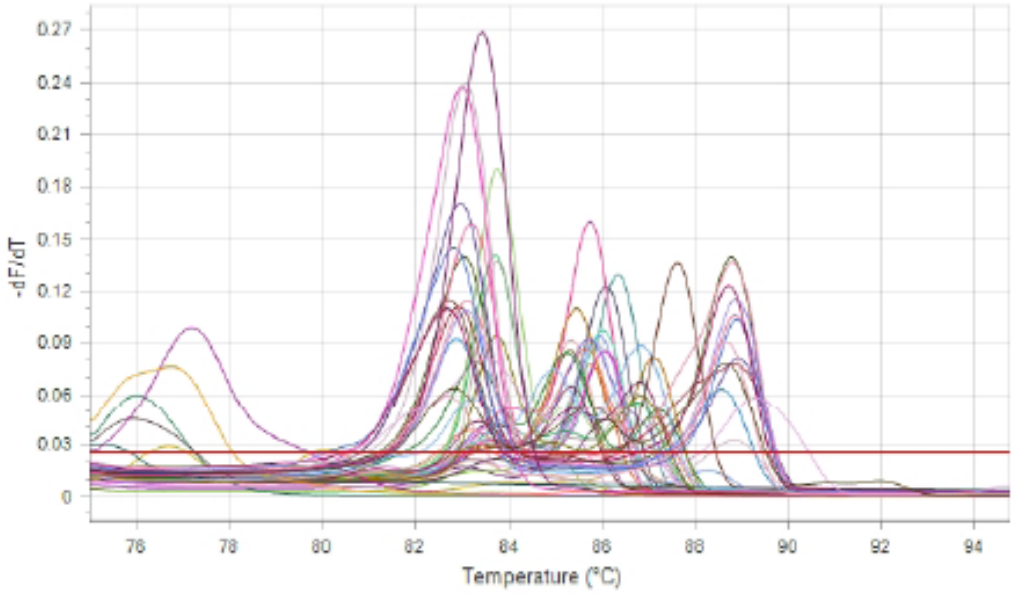
Well	Colour	Sample Name	Cq	Efficiency	Efficiency R^2	Result
1		NGITERO_7_PK_COI	-	-	-	Excluded
2		INCHOLE_8_PK_COI	-	-	-	Excluded
3		INKONYA_9_PK_COI	28.51	0.87	0.99792	
4		MBUBUSE_10_PK_COI	24.35	0.92	0.99952	
5		HANAKWE_11_PK_COI	28.11	0.89	0.99871	
6		ISENYE_12_PK_COI	28.84	0.93	0.99903	
7		IMKOLI_13_PK_COI	26.33	0.90	0.99984	
8		CHOLO_14_PK_COI	29.45	0.93	0.99925	
9		IMBOTO_15_PK_COI	-	-	-	Excluded
10		KIGONGO_16_PK_COI	34.96	0.83	0.99966	
11		IMBULI_17_PK_COI	27.22	0.97	0.99989	
12		NZOMBI_18_PK_COI	25.46	0.91	0.99982	
13		INGWE_19_PK_COI	27.90	0.92	0.99956	
14		INTELE_20_PK_COI	26.14	0.87	0.99877	

Report *COI_CYTB_16S_26_01_2018_PROTENASE K QIA*

Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
15		NTC	35.94	0.82	0.99994	
16		H2O	39.20	0.87	0.99993	
17		NGITERO_7_PK_CYTB	28.50	0.97	0.99990	
18		INCHOLE_8_PK_CYTB	29.19	0.96	0.99984	
19		INKONYA_9_PK_CYTB	26.45	0.96	0.99983	
20		MBUBUSE_10_PK_CYTB	32.39	0.93	0.99941	
21		HANAKWE_11_PK_CYTB	25.19	0.98	0.99993	
22		ISENYE_12_PK_CYTB	30.30	0.94	0.99950	
23		IMKOLI_13_PK_CYTB	24.40	0.93	0.99987	
24		CHOLO_14_PK_CYTB	-	-	-	Excluded
25		IMBOTO_15_PK_CYTB	-	-	-	Excluded
26		KIGONGO_16_PK_CYTB	30.37	0.86	0.99996	
27		IMBULI_17_PK_CYTB	27.85	0.97	0.99992	
28		NZOMBI_18_PK_CYTB	32.94	0.95	0.99964	
29		INGWE_19_PK_CYTB	27.46	0.96	0.99987	
30		INTELE_20_PK_CYTB	29.06	0.97	0.99992	
31		NTC	32.60	0.85	0.99984	
32		H2O	33.80	0.83	0.99994	
33		NGITERO_7_PK_16S	14.23	0.97	0.99996	
34		INCHOLE_8_PK_16S	15.86	0.96	0.99989	
35		INKONYA_9_PK_16S	14.53	0.96	0.99986	
36		MBUBUSE_10_PK_16S	12.31	0.95	0.99986	
37		HANAKWE_11_PK_16S	15.26	0.93	0.99974	
38		ISENYE_12_PK_16S	12.80	0.96	0.99982	
39		IMKOLI_13_PK_16S	16.04	0.96	0.99984	
40		CHOLO_14_PK_16S	13.71	0.96	0.99945	
41		IMBOTO_15_PK_16S	14.55	0.95	0.99979	
42		KIGONGO_16_PK_16S	16.17	0.86	0.99992	
43		IMBULI_17_PK_16S	15.60	0.93	0.99912	
44		NZOMBI_18_PK_16S	11.73	0.89	0.99976	
45		INGWE_19_PK_16S	15.23	0.93	0.99940	
46		INTELE_20_PK_16S	11.92	0.95	0.99963	
47		NTC	30.06	0.89	0.99999	
48		H2O	29.32	0.91	0.99996	

Melt: CO1_CYTB_16S Genes

Target	HRMA For Surveillance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Invert Data	No
Threshold	0.026 starting at 75°C



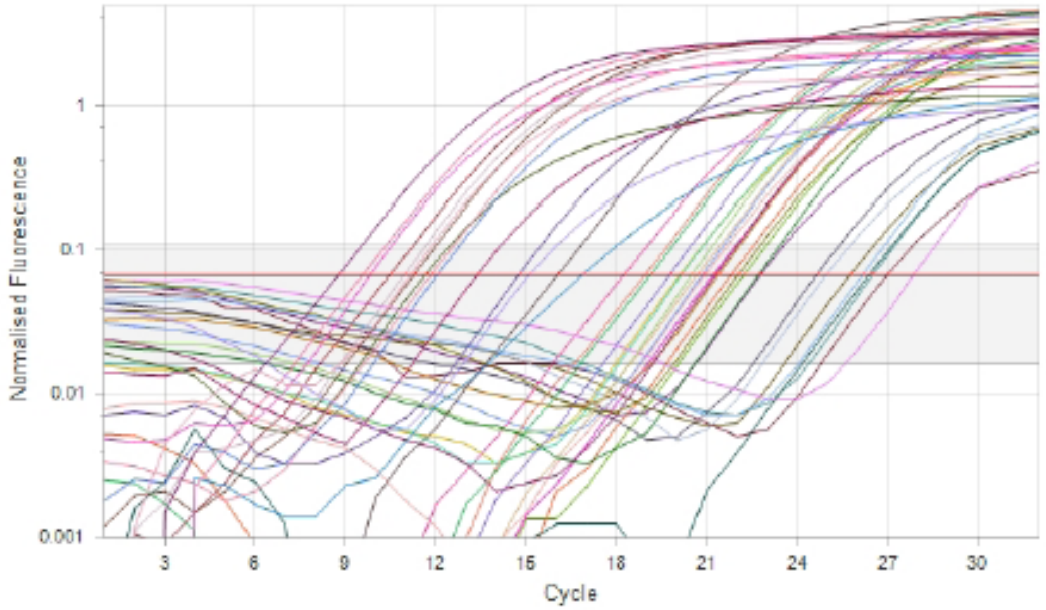
Well	Colour	Sample Name	Tm (°C)	Genotype
1		NGITERO_7_PK_COI	85.54	
2		INCHOLE_8_PK_COI	84.79	
3		INKONYA_9_PK_COI	83.38, 85.75	
4		MBUBUSE_10_PK_COI	83.89, 86.34	
5		HANAKWE_11_PK_COI	83.76, 85.31	
6		ISENYE_12_PK_COI	83.74, 85.33	
7		IMKOLI_13_PK_COI	83.53, 85.93	
8		CHOLO_14_PK_COI	83.65, 86.05	
9		IMBOTO_15_PK_COI	83.71, 85.33	
10		KIGONGO_16_PK_COI	76.69, 80.10	
11		IMBULI_17_PK_COI	83.66, 86.08	
12		NZOMBI_18_PK_COI	84.09, 85.74	
13		INGWE_19_PK_COI	83.57, 86.02	
14		INTELE_20_PK_COI	83.38, 85.83	
15		NTC	76.03	
16		H2O	85.25	
17		NGITERO_7_PK_CYTB	83.46, 85.45, 87.10	

Well	Colour	Sample Name	Tm (°C)	Genotype
18		INCHOLE_8_PK_CYTB	83.20, 85.63, 87.06	
19		INKONYA_9_PK_CYTB	83.70, 85.19, 86.74	
20		MBUBUSE_10_PK_CYTB	83.46, 86.79	
21		HANAKWE_11_PK_CYTB	83.76, 85.33, 86.71	
22		ISENYE_12_PK_CYTB	85.72	
23		IMKOLI_13_PK_CYTB	83.76, 85.30, 86.79	
24		CHOLO_14_PK_CYTB		
25		IMBOTO_15_PK_CYTB		
26		KIGONGO_16_PK_CYTB	83.38, 85.42, 86.79	
27		IMBULI_17_PK_CYTB	83.29, 85.22, 87.21	
28		NZOMBI_18_PK_CYTB	75.95, 86.09	
29		INGWE_19_PK_CYTB	83.74, 86.82	
30		INTELE_20_PK_CYTB	83.25, 84.95, 86.83	
31		NTC	77.19	
32		H2O	76.70	
33		NGITERO_7_PK_16S	83.03, 88.78	
34		INCHOLE_8_PK_16S	82.92, 88.81	
35		INKONYA_9_PK_16S	82.70, 87.61	
36		MBUBUSE_10_PK_16S	82.95, 88.92	
37		HANAKWE_11_PK_16S	82.87, 88.56	
38		ISENYE_12_PK_16S	83.19, 88.86	
39		IMKOLI_13_PK_16S	82.98	
40		CHOLO_14_PK_16S	83.10, 88.79	
41		IMBOTO_15_PK_16S	83.08, 88.88	
42		KIGONGO_16_PK_16S	83.07, 88.83	
43		IMBULI_17_PK_16S	82.80, 88.89	
44		NZOMBI_18_PK_16S	83.43	
45		INGWE_19_PK_16S	82.65, 88.67	
46		INTELE_20_PK_16S	82.66, 88.72	
47		NTC	83.52, 85.82, 89.33	
48		H2O	82.82, 85.74, 88.64	

Report

Cycling: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Normalisation	LinRegPCR
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.068 (Automatic) starting at cycle 1

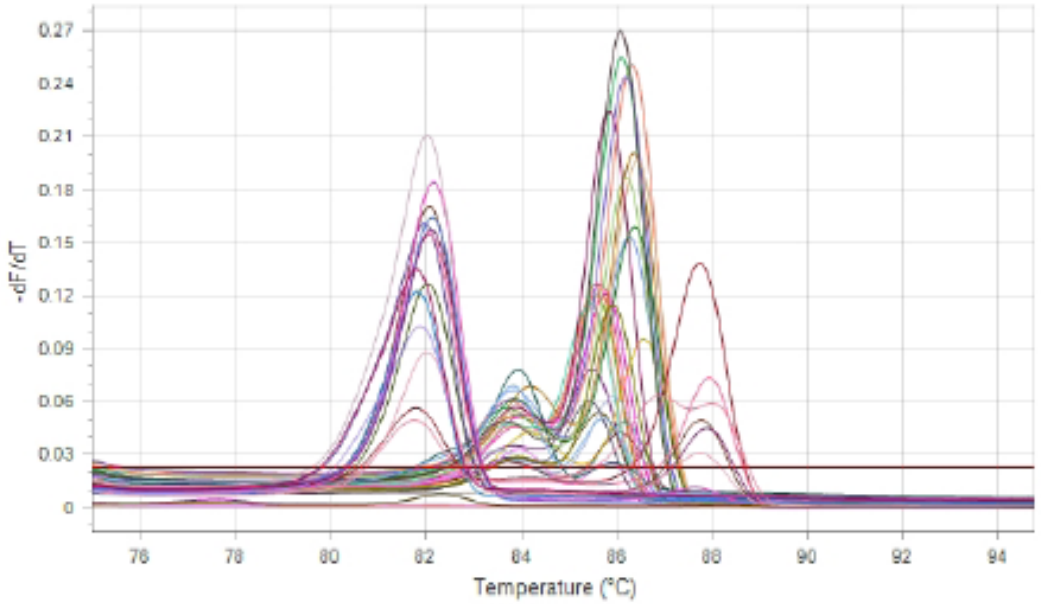


Well	Colour	Sample Name	Cq	Efficiency	Efficiency R^2	Result
1		KISOKOLOKOBWE_21_PK_COI	22.70	0.93	0.99947	
2		INZENGWE_22_PK_COI	22.16	0.92	0.99969	
3		INKOKO_23_PK_COI	21.73	0.93	0.99987	
4		MASESO_24_PK_COI	26.41	0.96	0.99987	
5		KUNCHO_25_PK_COI	25.67	0.90	0.99932	
6		INGWELE_26_PK_COI	20.90	0.94	0.99972	
7		SABALA_27_PK_COI	20.79	0.98	0.99997	
8		INDASA_28_PK_COI	21.15	0.91	0.99984	
9		MBICHI_29_PK_COI	26.95	0.87	0.99840	
10		KORONGO_30_PK_COI	20.07	0.93	0.99953	
11		INGELA_31_PK_COI	24.55	0.95	0.99973	
12		SWALA PAA_32_PK_COI	18.18	0.83	0.99996	
13		IDODO_33-PK_COI	20.11	0.97	0.99978	
14		NUNGUNUNGU_34_PK_COI	24.91	0.92	0.99932	

Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
15		NTC	-	-	-	Excluded
16		H20	-	-	-	Excluded
17		KISOKOLOKOBWE_21_PK_CYTB	21.29	0.92	0.99858	
18		INZENGWE_22_PK_CYTB	18.90	0.85	0.99970	
19		INKOKO_23_PK_CYTB	19.08	0.85	0.99988	
20		MASESO_24_PK_CYTB	27.80	0.89	0.99862	
21		KUNCHO_25_PK_CYTB	-	-	-	Excluded
22		INGWELE_26_PK_CYTB	19.68	0.91	0.99994	
23		SABALA_27_PK_CYTB	20.40	0.98	0.99999	
24		INDASA_28_PK_CYTB	20.60	0.89	0.99992	
25		MBICHI_29_PK_CYTB	26.30	0.89	0.99979	
26		KORONGO_30_PK_CYTB	21.21	0.97	0.99991	
27		INGELA_31_PK_CYTB	22.62	0.97	0.99997	
28		SWALA PAA_32_PK_CYTB	16.02	0.82	0.99994	
29		IDODO_33-PK_CYTB	22.01	0.95	0.99982	
30		NUNGUNUNGU_34_PK_CYTB	26.11	0.96	0.99986	
31		NTC	-	-	-	Excluded
32		H20	-	-	-	Excluded
33		KISOKOLOKOBWE_21_PK_16S	11.57	0.90	0.99869	
34		INZENGWE_22_PK_16S	10.39	0.85	0.99994	
35		INKOKO_23_PK_16S	11.20	0.89	0.99991	
36		MASESO_24_PK_16S	14.67	0.96	0.99979	
37		KUNCHO_25_PK_16S	16.82	0.56	0.99948	
38		INGWELE_26_PK_16S	9.49	0.87	0.99986	
39		SABALA_27_PK_16S	9.75	0.97	0.99986	
40		INDASA_28_PK_16S	11.78	0.90	0.99986	
41		MBICHI_29_PK_16S	15.07	0.87	0.99709	
42		KORONGO_30_PK_16S	10.92	0.95	0.99998	
43		INGELA_31_PK_16S	12.03	0.94	0.99977	
44		SWALA PAA_32_PK_16S	8.70	0.82	0.99980	
45		IDODO_33-PK_16S	11.11	0.87	0.99705	
46		NUNGUNUNGU_34_PK_16S	13.30	0.91	0.99937	
47		NTC	-	-	-	Excluded
48		H20	-	-	-	Excluded

Melt: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Invert Data	No
Threshold	0.023 starting at 75°C



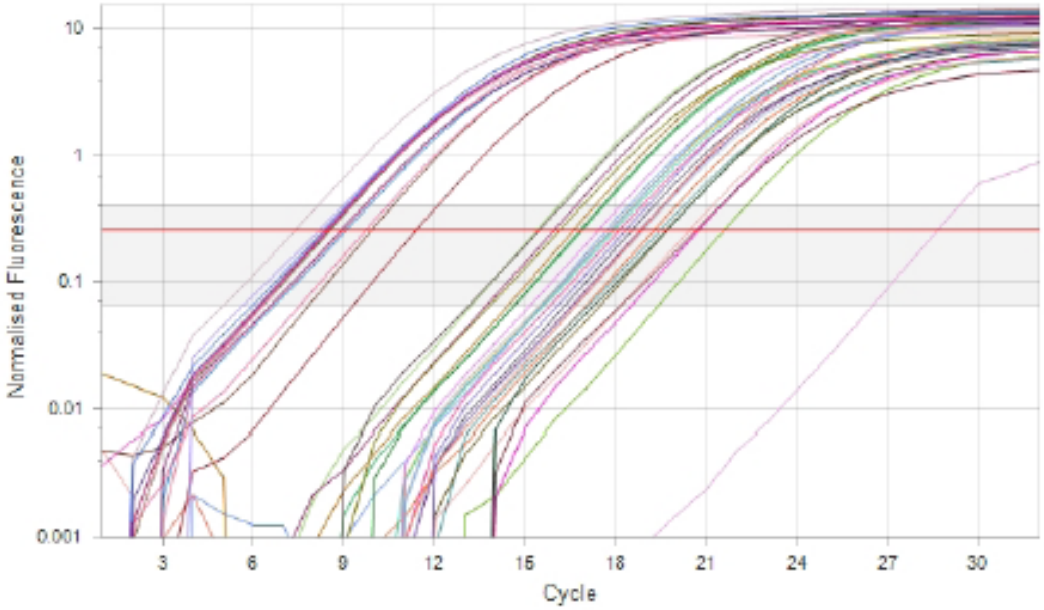
Well	Colour	Sample Name	Tm (°C)	Genotype
1		KISOKOLOKOBWE_21_PK_COI	83.87, 85.47	
2		INZENGWE_22_PK_COI	83.89, 85.56	
3		INKOKO_23_PK_COI	83.90, 85.56	
4		MASESO_24_PK_COI	83.76, 86.15	
5		KUNCHO_25_PK_COI	83.98, 85.62	
6		INGWELE_26_PK_COI	83.80, 85.48	
7		SABALA_27_PK_COI	83.85, 86.26	
8		INDASA_28_PK_COI	84.11, 85.76	
9		MBICHI_29_PK_COI	83.72, 86.07	
10		KORONGO_30_PK_COI	84.20, 86.56	
11		INGELA_31_PK_COI	83.78, 85.41	
12		SWALA PAA_32_PK_COI	84.02, 85.64	
13		IDODO_33-PK_COI	83.81, 85.43	
14		NUNGUNUNGU_34_PK_COI	83.53, 85.93	
15		NTC		
16		H20		
17		KISOKOLOKOBWE_21_PK_CYTB	84.20, 86.35	

Well	Colour	Sample Name	Tm (°C)	Genotype
18		INZENGWE_22_PK_CYTB	83.75, 86.32	
19		INKOKO_23_PK_CYTB	83.70, 86.10	
20		MASESO_24_PK_CYTB	83.95, 85.83	
21		KUNCHO_25_PK_CYTB		
22		INGWELE_26_PK_CYTB	75.29, 83.82, 86.18	
23		SABALA_27_PK_CYTB	83.87, 86.19	
24		INDASA_28_PK_CYTB	83.91, 86.43	
25		MBICHI_29_PK_CYTB	83.92, 85.93	
26		KORONGO_30_PK_CYTB	83.91, 85.83	
27		INGELA_31_PK_CYTB	83.89, 86.37	
28		SWALA PAA_32_PK_CYTB	83.67, 86.08	
29		IDODO_33-PK_CYTB	83.95, 85.92	
30		NUNGUNUNGU_34_PK_CYTB	83.82, 85.72	
31		NTC		
32		H20		
33		KISOKOLOKOBWE_21_PK_16S	82.03	
34		INZENGWE_22_PK_16S	81.79, 87.74	
35		INKOKO_23_PK_16S	82.07, 87.77	
36		MASESO_24_PK_16S	81.95	
37		KUNCHO_25_PK_16S	81.81	
38		INGWELE_26_PK_16S	82.06, 87.93	
39		SABALA_27_PK_16S	82.15	
40		INDASA_28_PK_16S	81.75, 86.94, 87.93	
41		MBICHI_29_PK_16S	81.86	
42		KORONGO_30_PK_16S	82.02	
43		INGELA_31_PK_16S	82.12	
44		SWALA PAA_32_PK_16S	82.10, 87.89	
45		IDODO_33-PK_16S	82.02, 87.74	
46		NUNGUNUNGU_34_PK_16S	81.78	
47		NTC		
48		H20		

Report

Cycling: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Normalisation	LinRegPCR
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.254 (Automatic) starting at cycle 1



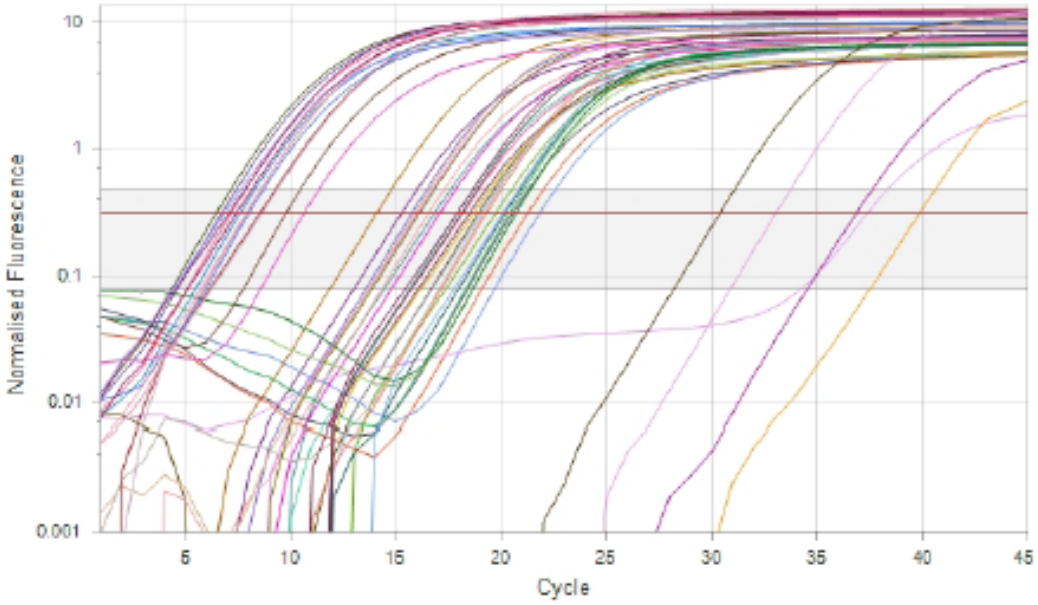
Well	Colour	Sample Name	Cq	Efficiency	Efficiency R^2	Result
1		KISOKOLOKOBWE_21_KIT_COI	18.72	0.84	0.99999	
2		INZENGWE_22_KIT_COI	21.48	0.87	0.99995	
3		INKOKO_23_KIT_COI	19.18	0.85	0.99999	
4		MASESO_24_KIT_COI	19.37	0.78	0.99995	
5		KUNCHO_25_KIT_COI	19.60	0.85	0.99995	
6		INGWELE_26_KIT_COI	20.34	0.83	0.99998	
7		SABALA_27_KIT_COI	17.59	0.88	0.99993	
8		INDASA_28_KIT_COI	20.65	0.85	0.99998	
9		MBICHI_29_KIT_COI	20.57	0.74	0.99995	
10		KORONGO_30_KIT_COI	17.75	0.83	1.00000	
11		INGELA_31_KIT_COI	18.42	0.86	0.99998	
12		SWALA PAA_32_KIT_COI	18.07	0.82	0.99999	
13		IDODO_33-KIT_COI	17.90	0.81	0.99993	
14		NUNGUNUNGU_34_KIT_COI	18.82	0.84	0.99997	

Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
15		NTC	-	-	-	Excluded
16		H20	-	-	-	Excluded
17		KISOKOLOKOBWE_21_KIT_CYTB	16.49	0.88	0.99999	
18		INZENGWE_22_KIT_CYTB	18.77	0.88	0.99999	
19		INKOKO_23_KIT_CYTB	16.81	0.85	0.99998	
20		MASESO_24_KIT_CYTB	17.33	0.84	0.99998	
21		KUNCHO_25_KIT_CYTB	17.76	0.80	0.99989	
22		INGWELE_26_KIT_CYTB	18.24	0.84	0.99997	
23		SABALA_27_KIT_CYTB	15.29	0.88	0.99999	
24		INDASA_28_KIT_CYTB	19.56	0.78	0.99997	
25		MBICHI_29_KIT_CYTB	19.58	0.77	0.99991	
26		KORONGO_30_KIT_CYTB	15.90	0.85	1.00000	
27		INGELA_31_KIT_CYTB	16.74	0.87	0.99999	
28		SWALA PAA_32_KIT_CYTB	15.36	0.81	0.99995	
29		IDODO_33-KIT_CYTB	16.09	0.82	0.99999	
30		NUNGUNUNGU_34_KIT_CYTB	17.77	0.83	0.99995	
31		NTC	-	-	-	Excluded
32		H20	-	-	-	Excluded
33		KISOKOLOKOBWE_21_KIT_16S	8.43	0.86	0.99997	
34		INZENGWE_22_KIT_16S	11.29	0.92	0.99989	
35		INKOKO_23_KIT_16S	9.85	0.93	0.99994	
36		MASESO_24_KIT_16S	8.29	0.85	1.00000	
37		KUNCHO_25_KIT_16S	9.03	0.79	0.99984	
38		INGWELE_26_KIT_16S	9.63	0.87	0.99998	
39		SABALA_27_KIT_16S	8.34	0.89	0.99998	
40		INDASA_28_KIT_16S	8.93	0.79	0.99990	
41		MBICHI_29_KIT_16S	8.12	0.73	0.99983	
42		KORONGO_30_KIT_16S	7.29	0.80	0.99985	
43		INGELA_31_KIT_16S	8.24	0.80	0.99993	
44		SWALA PAA_32_KIT_16S	8.84	0.80	0.99995	
45		IDODO_33-KIT_16S	8.39	0.87	0.99997	
46		NUNGUNUNGU_34_KIT_16S	8.37	0.84	1.00000	
47		NTC	28.54	0.89	0.99998	
48		H20	-	-	-	Excluded

Report

Cycling: CO1_CYTB_16S Genes

Target	HRMA For Surveilance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Normalisation	LinRegPCR
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.305 (Automatic) starting at cycle 1

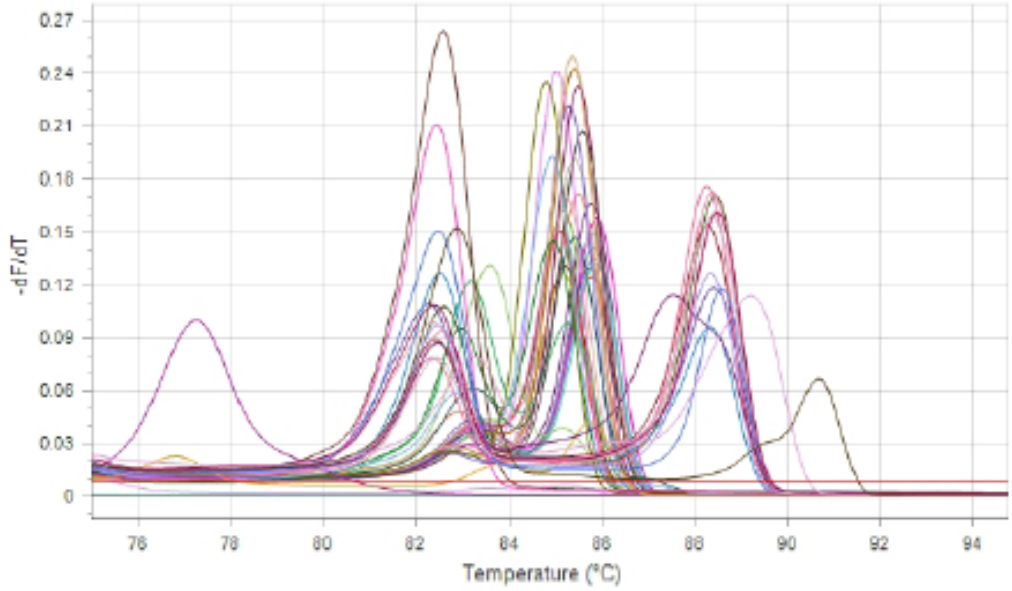


Well	Colour	Sample Name	Cq	Efficiency	Efficiency R^2	Result
1		NGITERO_7_KIT_COI	15.18	0.82	0.99997	
2		INCHOLE_8_KIT_COI	19.63	0.78	0.99996	
3		INKONYA_9_KIT_COI	21.17	0.85	0.99937	
4		MBUBUSE_10_KIT_COI	16.74	0.78	0.99992	
5		HANAKWE_11_KIT_COI	18.83	0.84	0.99983	
6		ISENYE_12_KIT_COI	16.44	0.84	0.99979	
7		IMKOLI_13_KIT_COI	21.73	0.87	0.99922	
8		CHOLO_14_KIT_COI	17.03	0.81	0.99993	
9		IMBOTO_15_KIT_COI	18.38	0.76	0.99991	
10		KIGONGO_16_KIT_COI	18.57	0.74	0.99991	
11		IMBULI_17_KIT_COI	20.43	0.87	0.99904	
12		NZOMBI_18_KIT_COI	18.09	0.77	0.99998	
13		INGWE_19_KIT_COI	17.85	0.82	0.99977	
14		INTELE_20_KIT_COI	18.99	0.81	0.99989	

Well	Colour	Sample Name	Cq	Efficiency	Efficiency R^2	Result
15		NTC	-	-	-	Excluded
16		H2O	37.29	0.57	0.99771	
17		NGITERO_7_KIT_CYTB	14.03	0.85	0.99998	
18		INCHOLE_8_KIT_CYTB	19.20	0.89	0.99978	
19		INKONYA_9_KIT_CYTB	20.28	0.92	0.99972	
20		MBUBUSE_10_KIT_CYTB	16.00	0.83	0.99999	
21		HANAKWE_11_KIT_CYTB	19.10	0.91	0.99975	
22		ISENYE_12_KIT_CYTB	15.65	0.87	0.99993	
23		IMKOLI_13_KIT_CYTB	20.59	0.93	0.99957	
24		CHOLO_14_KIT_CYTB	15.83	0.88	0.99994	
25		IMBOTO_15_KIT_CYTB	20.11	0.85	0.99982	
26		KIGONGO_16_KIT_CYTB	17.82	0.76	0.99991	
27		IMBULI_17_KIT_CYTB	20.59	0.94	0.99977	
28		NZOMBI_18_KIT_CYTB	18.06	0.77	0.99996	
29		INGWE_19_KIT_CYTB	16.06	0.84	0.99997	
30		INTELE_20_KIT_CYTB	20.03	0.81	0.99999	
31		NTC	36.79	0.80	0.99981	
32		H2O	39.79	0.80	0.99997	
33		NGITERO_7_KIT_16S	6.35	0.90	0.99991	
34		INCHOLE_8_KIT_16S	8.42	0.78	0.99998	
35		INKONYA_9_KIT_16S	9.68	0.95	0.99970	
36		MBUBUSE_10_KIT_16S	6.62	0.90	0.99993	
37		HANAKWE_11_KIT_16S	7.48	0.90	0.99978	
38		ISENYE_12_KIT_16S	7.10	0.92	0.99983	
39		IMKOLI_13_KIT_16S	10.53	0.92	0.99963	
40		CHOLO_14_KIT_16S	7.82	0.93	0.99979	
41		IMBOTO_15_KIT_16S	6.78	0.74	0.99993	
42		KIGONGO_16_KIT_16S	8.51	0.79	0.99998	
43		IMBULI_17_KIT_16S	7.96	0.95	0.99957	
44		NZOMBI_18_KIT_16S	7.02	0.72	0.99979	
45		INGWE_19_KIT_16S	7.80	0.88	0.99993	
46		INTELE_20_KIT_16S	7.31	0.82	0.99997	
47		NTC	33.01	0.88	0.99998	
48		H2O	30.28	0.88	0.99999	

Melt: CO1_CYTB_16S Genes

Target	HRMA For Surveillance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Invert Data	No
Threshold	0.009 starting at 75°C



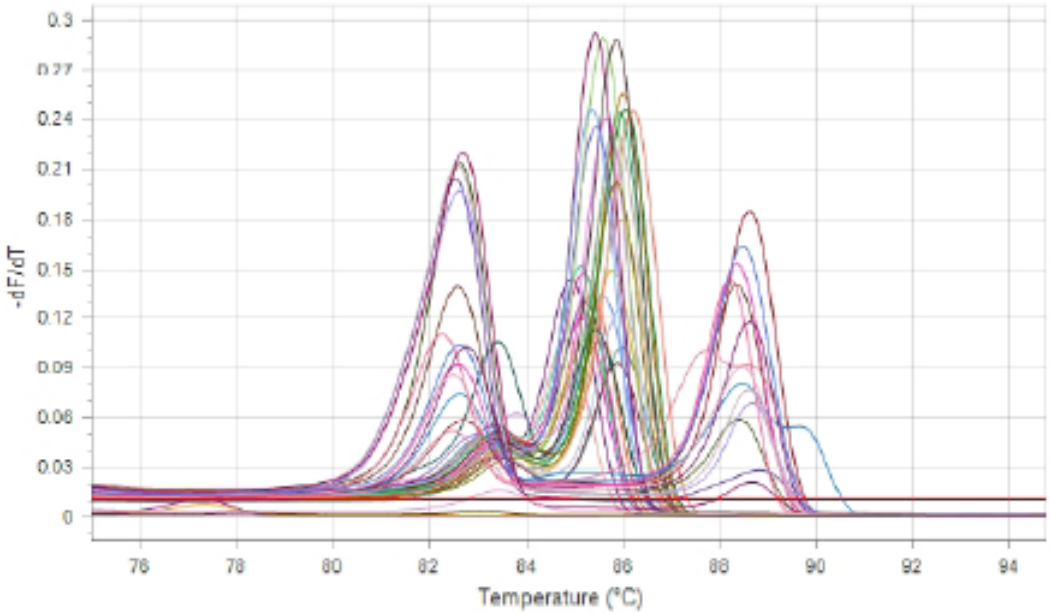
Well	Colour	Sample Name	Tm (°C)	Genotype
1		NGITERO_7_KIT_COI	75.40, 78.61, 83.25, 85.76	
2		INCHOLE_8_KIT_COI	75.37, 77.74, 78.70, 85.16	
3		INKONYA_9_KIT_COI	75.37, 83.32, 85.77	
4		MBUBUSE_10_KIT_COI	75.27, 80.08, 83.29, 85.82	
5		HANAKWE_11_KIT_COI	75.36, 78.08, 83.52, 85.11	
6		ISENYE_12_KIT_COI	75.37, 85.23	
7		IMKOLI_13_KIT_COI	75.08, 83.39, 85.83	
8		CHOLO_14_KIT_COI	77.86, 83.36, 85.88	
9		IMBOTO_15_KIT_COI	75.29, 77.77, 83.52, 85.20	
10		KIGONGO_16_KIT_COI	83.52, 85.18	
11		IMBULI_17_KIT_COI	77.97, 83.21, 85.72	
12		NZOMBI_18_KIT_COI	75.34, 77.36, 83.40, 85.08	
13		INGWE_19_KIT_COI	75.38, 83.36, 85.87	

Well	Colour	Sample Name	Tm (°C)	Genotype
14		INTELE_20_KIT_COI	78.34, 83.27, 85.78	
15		NTC		
16		H2O		
17		NGITERO_7_KIT_CYTB	75.11, 78.19, 82.81, 85.40	
18		INCHOLE_8_KIT_CYTB	82.92, 85.49	
19		INKONYA_9_KIT_CYTB	83.17, 85.27	
20		MBUBUSE_10_KIT_CYTB	78.32, 82.94, 85.02	
21		HANAKWE_11_KIT_CYTB	75.21, 78.14, 83.04, 85.41	
22		ISENYE_12_KIT_CYTB	75.34, 79.28, 82.72, 85.29	
23		IMKOLI_13_KIT_CYTB	83.57, 85.13	
24		CHOLO_14_KIT_CYTB	79.30, 82.78, 85.36	
25		IMBOTO_15_KIT_CYTB	83.23, 85.40	
26		KIGONGO_16_KIT_CYTB	78.14, 83.26, 85.49	
27		IMBULI_17_KIT_CYTB	75.32, 82.97, 84.93	
28		NZOMBI_18_KIT_CYTB	75.34, 76.99, 78.16, 82.85, 85.58	
29		INGWE_19_KIT_CYTB	82.65, 84.80	
30		INTELE_20_KIT_CYTB	82.89, 84.92	
31		NTC	77.26	
32		H2O	76.80, 83.89, 85.75	
33		NGITERO_7_KIT_16S	78.14, 82.58, 88.43	
34		INCHOLE_8_KIT_16S	82.40, 84.65, 88.27	
35		INKONYA_9_KIT_16S	82.56	
36		MBUBUSE_10_KIT_16S	82.24, 84.38, 88.40	
37		HANAKWE_11_KIT_16S	82.50, 88.32	
38		ISENYE_12_KIT_16S	82.33, 88.26	
39		IMKOLI_13_KIT_16S	82.42	
40		CHOLO_14_KIT_16S	78.60, 82.54, 88.38	
41		IMBOTO_15_KIT_16S	82.46, 88.34	
42		KIGONGO_16_KIT_16S	82.44, 84.40, 88.38	
43		IMBULI_17_KIT_16S	75.23, 82.46, 88.58	
44		NZOMBI_18_KIT_16S	75.21, 82.46, 87.56	
45		INGWE_19_KIT_16S	82.33, 84.38, 88.47	

Well	Colour	Sample Name	Tm (°C)	Genotype
46		INTELE_20_KIT_16S	82.35, 88.48	
47		NTC	83.24, 89.19	
48		H2O	82.86, 90.66	

Melt: CO1_CYTB_16S Genes

Target	HRMA For Surveillance and Forensic Screening of Illegal Bushmeat Trade in Tanzania → CO1_CYTB_16S Genes
Invert Data	No
Threshold	0.011 starting at 75°C



Well	Colour	Sample Name	Tm (°C)	Genotype
1		KISOKOLOKOBWE_21_KIT_COI	77.88, 83.16, 84.92	
2		INZENGWE_22_KIT_COI	83.53, 85.25	
3		INKOKO_23_KIT_COI	75.39, 77.13, 78.84, 79.05, 83.63, 85.34	
4		MASESO_24_KIT_COI	83.48, 85.97	
5		KUNCHO_25_KIT_COI	75.35, 83.85, 85.49	
6		INGWELE_26_KIT_COI	75.39, 83.09, 84.82	
7		SABALA_27_KIT_COI	83.07, 85.57	
8		INDASA_28_KIT_COI	78.33, 83.41, 85.14	
9		MBICHI_29_KIT_COI	83.39, 85.88	
10		KORONGO_30_KIT_COI	77.32, 83.26, 85.77	
11		INGELA_31_KIT_COI	83.52, 85.20	
12		SWALA PAA_32_KIT_COI	77.64, 83.45, 85.18	
13		IDODO_33-KIT_COI	83.44, 85.12	
14		NUNGUNUNGU_34_KIT_COI	76.93, 79.92, 83.47, 85.95	

Well	Colour	Sample Name	Tm (°C)	Genotype
15		NTC		
16		H20		
17		KISOKOLOKOBWE_21_KIT_CYTB	75.39, 83.83, 86.00	
18		INZENGWE_22_KIT_CYTB	75.39, 83.59, 86.20	
19		INKOKO_23_KIT_CYTB	75.38, 78.47, 83.46, 85.91	
20		MASESO_24_KIT_CYTB	77.47, 83.80, 85.66	
21		KUNCHO_25_KIT_CYTB	78.23, 83.25, 85.87	
22		INGWELE_26_KIT_CYTB	77.23, 79.25, 83.00, 85.44	
23		SABALA_27_KIT_CYTB	77.05, 78.95, 79.35, 83.11, 85.59	
24		INDASA_28_KIT_CYTB	75.39, 79.26, 83.36, 85.95	
25		MBICHI_29_KIT_CYTB	83.40, 85.43	
26		KORONGO_30_KIT_CYTB	83.35, 85.42	
27		INGELA_31_KIT_CYTB	83.41, 86.04	
28		SWALA PAA_32_KIT_CYTB	83.39, 85.85	
29		IDODO_33-KIT_CYTB	83.75, 85.85	
30		NUNGUNUNGU_34_KIT_CYTB	83.30, 85.35	
31		NTC		
32		H20		
33		KISOKOLOKOBWE_21_KIT_16S	75.37, 82.62, 88.38	
34		INZENGWE_22_KIT_16S	75.40, 82.69, 84.97, 88.62	
35		INKOKO_23_KIT_16S	75.34, 82.57, 84.66, 88.35	
36		MASESO_24_KIT_16S	82.52, 88.80	
37		KUNCHO_25_KIT_16S	77.85, 82.62, 85.19, 88.47	
38		INGWELE_26_KIT_16S	82.25, 84.45, 88.21	
39		SABALA_27_KIT_16S	82.58, 84.68, 88.35	
40		INDASA_28_KIT_16S	75.37, 82.46, 87.77	
41		MBICHI_29_KIT_16S	75.34, 82.60, 88.66	
42		KORONGO_30_KIT_16S	82.56, 88.59	
43		INGELA_31_KIT_16S	82.61, 84.78, 88.48	
44		SWALA PAA_32_KIT_16S	75.46, 82.75, 84.81, 88.62	
45		IDODO_33-KIT_16S	75.39, 82.48, 88.45	

Well	Colour	Sample Name	Tm (°C)	Genotype
46		NUNGUNUNGU_34_KIT_16S	75.36, 82.67, 88.66	
47		NTC	83.43	
48		H20		