CHPTER ONE
INTRODUCTION

The Sudan is one of the biggest countries concerning animal numbers especially in camels. ranks first among the Arab countries and second in Africa with respect to camel population. (M.A.R,2008) Camels have demonstrated their high adaptation to the most rigorous environment since the domestication.

Their ability to thrive on feed that is not regarded as sustenance for most other domestic animals made them special components for marginal arid ecosystems (Kaness et al., 1997). Camels are the backbone of the Western tribe economy and are also central to their culture. Large areas in Western Sudan are inhabited by nomadic tribes who believe in camels as animal of choice. Those tribes move all the year from place to another looking for water and better pasture.

Nomadic pastoralists have a global food producing strategy depending on the management of herd animals for meat, milk, wool, skin, manure and transport.

Pastoralism which depends on movements of livestock is economically the most efficient, securing the livelihood for large sections and lessening the ecological impact on the environment, even the return per area of fuzzy nature is higher than well defined property rights or commercial ranching (Moran and Emilio, 2006).

The association of camel with harsh environments and the lack of appropriate genetic management strategies did not help camels to reach better economical status when compared with their counter parts cattle, sheep and goats (Alhadrami and Djemli, 2003). The genetic characterization of domestic animals is the first step in considering the sustainable management or conservation of a particular population. In the early 1990's, molecular markers have played a leading role in the characterization of diversity, which provide relatively rapid and cheap assays in the absence of quality phenotypic measures (Toro et al., 2006). Studies of genetic diversity in domestic animals are based on an evaluation of the genetic variation within breed and genetic relationships among them.(Tapio et al.,2005). Mitochondrial DNA is useful for studying the evolution of closely related species and many studies have focused on the mitochondrial D-loop region , the most variable part of mtDN (Ishida et al., 1994) Mitochondrial DNA (mtDNA) has strictly maternal inheritance, which means mtDNA haplotypes should be shared by all individuals within a maternal family due to a higher substitution rate than in the rest of the mtDNA genome (Cann et al., 1984).

In the current study we aimed to start looking deep in the genetic relationships and genetic diversity between some Sudanese camel breeds under nomadic system so The objective of this study is to:

1- Characterize Kababish, Shanabla and Nyalawi camels by their Mitochondrial DNA.

2- Estimate the genetic relationship between Kababish, Shanabla and Nyalawi camels.

3- Estimate the genetic similarity between and within these types of camels.

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CHAPTER TWO

LITERATURE REVIEW

2:1 World Camels:

There are about 19.4 million camels world wide (FAO, 2003), the dromedary accounts for 95%. The Near East, North Africa and the Sahel region have about 70% (13.5 million) of the world's dromedary population. Somalia and the Sudan together own more than half of this figure (Wardeh, 2004). According to recent estimates of livestock in Sudan, there are about 40 million heads of cattle, 50 million heads of sheep, 43 million heads of goat and 4.5 million heads of camel (18% of the whole population in the world). Camels constitute 22% of the animal biomass in Sudan and 26.3% of the numbers of camel in the Arab world (M.A.R.2008).

Track of historical trends in the Sudan is difficult because of lack of reliable data. The history of the dromedary camel in Sudan is even more obscure. It is believed to have entered Sudan from Egypt. A specimen of camel hair rope of the old kingdom was found at Fayum in Upper Egypt, dating about 2980-2474 B.C.9 indicating that the animal had moved south by that period. In Sudan, the oldest evidence is a bronze figure

of camel with saddle found at Merawi, and estimated to date between 25-15 B.C (Adison,1934). probably camels entered Sudan through the following routes: first North West Africa route during the 4th to 6th century, the second rout was Egyptian route and third was Red Sea route (most recent) (Salman.M, 2002).

2:2 Camel Type:

The Sudanese camels like the Arabi type is a large heavy massive types with slow mobility. Mainly is described as a large heavily built animal with well developed hump, and it has a short hair except in the shoulder and hump. The mature camel weight about 400 – 500kg as a life weight, and can carry about 275kg over 25-30km per day (F. M. El Amin, 1984). The Arab type of camel is well suited for meat and milk production and transportation

2:3 Camel population:

Camels in Sudan are concentrated in two main regions: the Western Regions (Darfour and Kordofan) and the Eastern state, where camels are found in the Butana plains and the Red Sea hills (Eisa and Mustafa 2011).

Kordofan state is considered as the leading one in camel population in the country. The most famous owner camel's tribe in the western Sudan; includes Kababish, Hawaweer and Kawahla. North Kordofan state only has the highest camel population with more than one million heads, representing

approximately 5% of the whole world camel population (Faye, 2009).

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(Table 2:1) Estimate of camel population in Sudan according to states:

State	Camel population	Camel population %
North Kordofan	1,369825	31.09
South Kordofan	252023	5.72
North Darfour	546344	12.40
South Darfour	103100	2.34
West Darfour	394778	8.96
Elgadaref	228231	5.18
Kassala	593488	13.47
Red Sea	308861	7.01
Blue Nile	197389	4.48
Sennar	107947	2.45
Algezira	114115	2.59
White Nile	32604	0.74
Northern	45382	1.03
River Nile	1, 05744	2.40
Khartoum	6168	0.14
Total	**4,406,000**	**100.00**

(M.A.R,2008)

2:4 Camels classification:

Camels in Sudan are owned by tribes that inhabit the dry semi-desert areas, and because of its limited distribution and numbers, there has been no development in identification of different breeds as in case with other types of farm animal. Camels in Sudan and elsewhere are classified as pack (heavy) and riding (light) types according to their function (F. M. El Amin, 1984). Recent studies had been made to classify the camels according to their performance like dairy camels, meat camels, dual purpose camels and racing camels (Wardeh, 1998) and (Kalafalla, 2000). Camel owners usually keep camels due to their appreciated productive potential and adaptability. Productive traits such as growth rate, milk yield and fertility have a high priority as they influence the sale of animals and the use of milk to satisfy family needs (Ishag et al. , 2011).

2:4:1 Kababish camels:

This breed is found in North Kordofan state owned by Kababish tribe. As repoted by (Ishag, et al., 2011). The averages barrel girth, chest girth, height at shoulder and body weight of Kababish camel are 2.25 ± 0.26, 1.98 ± 0.12 m, 1.91 ± 0.09 m and 451 ± 86.0 kg respectively. The main colors of this breed are grey, red and yellow. The hair length is medium

or long, the hump size is small; with erect orientation and located in the middle of the back and the udders and teats size is medium. The Kawahla and Hamar camel have similar features to the Kababish camel. These camels' owners reside near Al-obied town in the dry season and move to the north (Soudari area) in the wet season.

2:4:2 Shanabla camels:

This camel is found in North and South Kordofan states. It is bred by Shanabela and Awamera tribes; who move with their camels between the two states in different seasons. The averages barrel girth, chest girth, height at shoulder and body weight of Shanbali camel are 2.60 ± 0.21 m, 2.06 ± 0.13 m, 1.91 ± 0.08 m and 506 ± 83.0 kg respectively. The main colors of the breed are brown, red, grey and yellow; the hair is straight and long, and the hump is well developed, centrally placed with erect or bent sideways orientation. This camel breed is characterized by heavy weight. The owners of these camels migrate in the dry seasons to South Kordofan state near Kadugli and return to Al-mazroob area in the wet season. (Ishag, et al., 2011).

2:4:3 Nyalawi (Um Gallol) camels:

Darfur camels, like all Arabi type has hair on shoulder and hump the hair removed spontaneously during rainy season and grows again during winter (Wathig 2009).

according to the camel nutrition condition they spend the rainy season in, Kutom, Um buteekh, Jibal adoula and Maleet in Northern Darfour they spend the summer season in Nayala, Aldeain, Eltiboon and Um Guzein in the borderland between Kordofan and Darfour State and then to Komat Elzaiadia and El Fashir in Darfur, all previous names are local places for transient settlements of nomads.

2:5 Why conservation and genetic diversity:

Animal genetic resources have been contributing to food and agriculture for more than 12,000 years, providing meat, milk eggs, fiber and manure. It is estimated that directly or indirectly, domestic animal supplies 30%-40% of total human requirements for food and agriculture (FAO, 2000). Animal husbandry is crucial in ensuring food security and sustaining livelihood for many of the poorest members of rural households. The rural poor often drive a great proportion of their income from livestock production, and with the growing demand of livestock product throughout the world there are opportunities for boosting their income. To ensure that the small holders continue to thrive, improvement in livestock production and production system is vital. Conservation of vast animal genetic resources developed by

farmers through the years is desirable to circumvent the loss of genetic diversity. In Africa, genetic diversity of farm animals is under threat due to diseases, conflicts and migration. Another factor that is equally perceived as a threat is uncontrolled crossbreeding of African breeds with exotic breeds. Most livestock improvement programmes in Africa have resorted to crossbreeding indigenous breeds with imported breeds or directly replacing the indigenous genotypes (Wollny, 2003). These exotic breeds are considered to be more productive, but the problem is that these animals are mainly suited to the conditions of the countries they come from and they have difficulty in adapting to African environment. Among the domesticated populations, it is estimated that 1 to 2 breeds are lost every week according to the report of World Watch List of Domestic Animal Diversity (FAO, 2003). However the impact of these losses on global or local diversity remains undocumented. While it is already too late for many European breeds, the situation is also particularly worrying in the developing world where rapid changes in production systems are leading to replacements of breeds or at best crossbreeding. Therefore there is an urgent need to document the diversity of our livestock genetic resources and to design strategies for their sustainable

Documentation on conservation (Hanotte and Jianlin, 2005). existing genetic resources, including the description of the population phenotypic characteristics, performance, cultural importance and genetic uniqueness is one of the main areas of livestock conservation activities (Alvarez et al., 2009).

2:6 Molecular Makers:

Genetic markers can be used to identify specific regions of chromosomes where genes affecting quantitative traits are located, known as quantitative trait loci (QTL). DNA markers such as mitochondrial DNA markers, RAPD, AFLP, RFLP and microsatellites have been developed and utilized in genetic diversity analysis (Weber & May, 1989). The classification for the Sudanese camels is based on conformational and tribal ownership Information on genetic diversity is essential in optimizing both conservation and utilization strategies for Animal Genetic Resources (AnGR).

As resources for conservation are limited, prioritization is often necessary. New molecular tools hold the promise of allowing the identification of genes involved in a number of traits, including adaptive traits, and polymorphisms causing functional genetic variation (QTN Quantitative Trait Nucleotides). However, there is no sufficient knowledge to prioritize conservation choices on the basis of functional molecular diversity, and alternative measures are still needed. Phenotypic characterization provides a crude average estimate

of the functional variants of genes carried by a given individual or is not recorded.

2:6:1 Polymerase Chain Reactions (PCR):

Molecular biology has been revolutionized by the polymerase chain Reaction (PCR). PCR is an vitro technique Which allows the amplification of aspecific deoxyribonucleic acid (DNA) region that lies between two region of known DNA sequence .PCR is used to amplify asequence of DNA using apair Olignucleotide primer each complementary to one of the DNA using target sequence .This pocess (PCR) has become an essential tool in molecular biology as an aid to cloning and gene analysis because PCR can so efficiently increase any target DNA in a logarithmic and control fashion. The advent of the PCR meant that insufficiencies in the quantity of DNA were no longer a limitation in molecular biology research or diagnostic procedures. The development of PCR also had a major impact on studies of eukaryotic genomes (Nicolas, 1996). The chemistry involved in PCR depends on the complementarity (matching) of the nucleotide bases in the double-stranded DNA helix. When a molecule of DNA is sufficiently heated, the hydrogen bonds holding together the double helix are disrupted and the molecule separates or denatures into single strands. If the DNA solution is allowed to cool, then the complementary base pair can reform to restore the original double helix.

2:6:2 Mitochondrial DNA:

The mitochondrion is a membrane-bound organelle found in most eukaryotic in the cytoplasm of cells(Henze et al., 2003). These organelles have extra nuclear DNA. Animal mtDNA is a small circular double stranded molecule with a size of 15-20kiloobases (Desjardins and Morais, 1990).

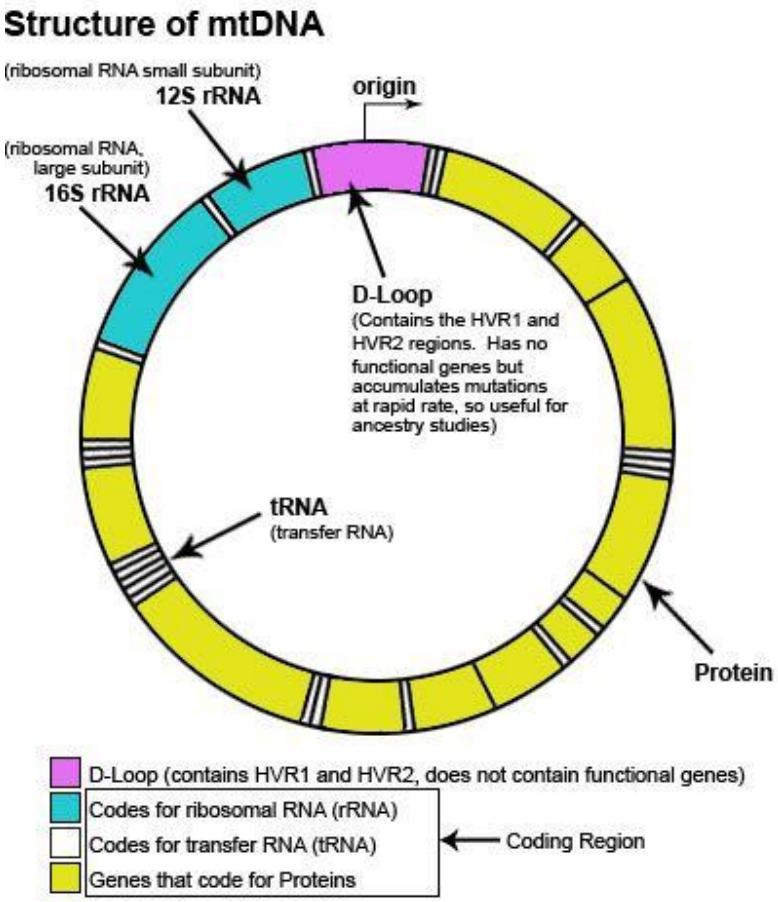
The most prominent roles of mitochondria are to produce the energy currency of the cell, oxidative phosphorination and the formation of ATP. mitochondria may replicate their DNA and divide mainly in response to the energy needs of the cell, rather than in phase with the cell cycle. When the energy needs of a cell are high, mitochondria grow and divide. When the energy use is low, mitochondria are destroyed or become inactive.(Seo AY et al., 2010).

mtDNA is maternally inherited in most species mtDNA l genes are not inherited by the same mechanism as nuclear genes. Typically, the mitochondria are inherited from one parent only. when an egg cell is fertilized by a sperm, the egg nucleus and sperm nucleus each contribute equally to the genetic makeup of the zygote nucleus. mitochondria DNA, usually come from the egg only. The sperm's mitochondria enter the egg but do not contribute genetic information to the embryo (Kimball, J.W, 2006).

mtDNA is a powerful tool for tracking ancestry through females (matrilineage) and has been used in this role to track the ancestry of many species back hundreds of generations. generations (Brown et al., 1979).

The mtDNA contains the genetic code for 37 very important genes (13 of the genes are responsible for producing proteins, 22 of the genes hold the genetic code to produce transfer RNA (tRNA), and 2 genes hold the genetic code to produce ribosomal RNA (rRNA)(Anderson et al.,1981)

Figure 2:1


Structure of mtDNA
(ribosomal RNA small subunit)
12S rRNA
origin
(ribosomal RNA, large subunit)
16S rRNA
D-Loop
(Contains the HVR1 and HVR2 regions. Has no functional genes but accumulates mutations at rapid rate, so useful for ancestry studies)
tRNA
(transfer RNA)
Protein
D-Loop (contains HVR1 and HVR2, does not contain functional genes)
Codes for ribosomal RNA (rRNA)
Codes for transfer RNA (tRNA)
Genes that code for Proteins
Coding Region

2:6:2:1 D-loop region:

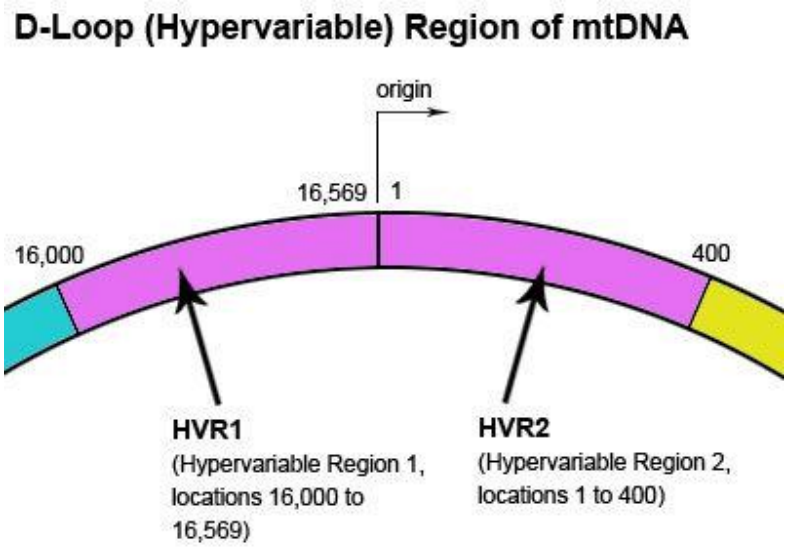
displacement loop or D-loop is a DNA structure where the two strands of a double-stranded DNA molecule are separated for a stretch and held apart by a third strand of DNA. The third strand has a base sequence which is complementary to one of the main strands and pairs with it, thus displacing the other main strand in the region. Within that region the structure is thus a form of triple - stranded DNA. the D-loop with a shape resembling a capital "D", where the displaced strand formed the loop of the "D" (Kasamats et al., 1971).

The D-Loop contains two regions, the HVR1 and HVR2 region. Unlike all of the other regions of the mtDNA, the D-Loop does not contain any functional genes.(Takeshima et at., 2005).

Most of the ancestral markers are found in the D-Loop. The D-Loop is considered a non-vital part of the mtDNA because it does not have a useful biological function. Thus, whenever a mutation occurs in this region the individual does not die and survives to pass the mutation along to future generations. However, the coding region of the mtDNA is considered essential for the survival of the individual, so usually, whenever a mutation occurs in this region, it is often lethal and the organism dies. Thus, mutations which occur in the coding region are usually not passed down to future generations. For this reason, over a period of thousands of years, many mutations accumulate in the D-Loop, but very little are found in the coding region. Mutations are found at a

much lower frequency in the coding region because only the mutations which do not end up being lethal are passed down. When tracing ancestry, scientists usually begin by testing the D-Loop because of its abundance of mutations or “ancestral markers.(Sherer et al., 2002).

Figure 2:2


D-Loop (Hypervariable) Region of mtDNA
origin
16,569
1
16,000
400
HVR1
(Hypervariable Region 1, locations 16,000 to 16,569)
HVR2
(Hypervariable Region 2, locations 1 to 400)

MtDNA is used in livestock genetic studies to produce phylogenetic trees at several taxonomic levels. Since D-loop shows variation within species, it can be used to detect evolution and geographic patterns of diversity in livestock species (Bandelt et al.,1999).

mtDNA has many advantages as a molecular marker for phylogenetic analysis (Moore, 1995). It is highly polymorphic compared to nuclear DNA, evolutionary rates being five to ten times faster than the nuclear genome (Brown et al., 1982).

,Different regions of mtDNA evolve at different rates (Saccone et al., 1991),.. These features mean that each molecule as a whole usually has a single genealogical history through maternal lineages. This made the analysis of mtDNA a powerful tool in evolutionary genetics. mtDNA can be particularly useful in resolving phylogenetic relationship between closely related taxa (Moritz et al., 1987).

Studies of genetic relationships in livestock provide useful information on the evaluation of breeds, gene pool development, and the magnitude of genetic differentiation. A considerable number of methodologies have been developed to objectively quantify the genetic differences among a set of breeds from allele frequency data. However, only a combination of the methods can provide sufficient information on both the genetic differences between breeds and within breed variation (Alvarez et al., 2005).

2:6:3 Restriction fragment length polymorphisms (RFLPs):

RFLP or restriction enzyme polymorphism is a technique in which the DNA is isolated, cut using restriction enzymes, size fractioned on gels. The advantage of RFLP is that it can be used to screen large number of individuals without requiring complicated molecular techniques (Aquardo et al., 1992).

(RFLP) is the existence of alternative alleles associated with restriction fragment that differ in size from each other. In RFLP analysis, the DNA is digested with restriction enzymes (enzymes that cleave DNA molecules at specific nucleotide sequences) and the resultant fragments are separated by gel electrophoresis. Electrophoresis will be followed by Southern blotting (Southern, 1975) and hybridization to a labelled probe that identifies the locus to be identified. DNA from different individuals will be digested by the same restriction enzymes to give fragments of varying sizes. Specific probe combinations will give highly reproducible restriction fragment patterns for a given individual but in case of mutations, variation between individuals can arise. This is because mutation may alter the sequence in the restriction sites thus preventing the enzyme from cutting. Alternatively, the DNA sequence in fragment between the restriction sites can be changed by insertion or deletion of base pairs which

can also create new restriction sites (Burr et al., 1983; Helentjaris et al., 1985; Evola et al., 1986).

.(Alaa E-ldeein et al., 2011).investigated **(** Identification of Adulteration with Camel Meat Using Polymerase Chain Reaction Assay**)** The primer pair was designed based on mitochondrial D-loop gene for detection of adulteration of camel meat in admixed meat and meat products by polymerase chain reaction (PCR) assay. Amplification of 208-bp DNA fragments was observed from camel, without any cross-reaction with cattle, sheep, goat and chicken. The amplification was further confirmed by endonuclease enzyme Taq I restriction enzymes.

Another study investigated evolutionary relationship between the domestic Bactrian camel and the extant wild two-humped camel and the factual origin of the domestic Bactrian camel remain elusive. determined the sequence of mitochondrial cytb gene from 21 camel samples, including 18 domestic camels (three Camelus bactrianus

Phylogenetic analyses revealed that the extant wild two-humped camel may not share a common ancestor with the domestic Bactrian camel and they are not the same subspecies at least in their maternal origins. Molecular clock analysis based on complete mitochondrial genome sequences indicated that the sub-speciation of the two lineages had begun in the early Pleistocene, about 0.7 million years ago. According to the archaeological dating of the earliest known two-humped

camel domestication (5000-6000 years ago), the study concluded that the extant wild camel is a separate lineage but not the direct progenitor of the domestic Bactrian camel. (Ji R et al., 2009).

CHAPTER THREE

MATERIAL AND METHODS

3:1 Origin of samples:

Different area were visited.

-samples for (Shanabla) collected from Er Rahad town is located in North Kurdufan Province, 30 kilometers south of El Obeid.in dry seaon shanabla camel comes to Khour Abu Habil in Er Rahad and stayed to spend part of summer season. and also collected from ElObied town.

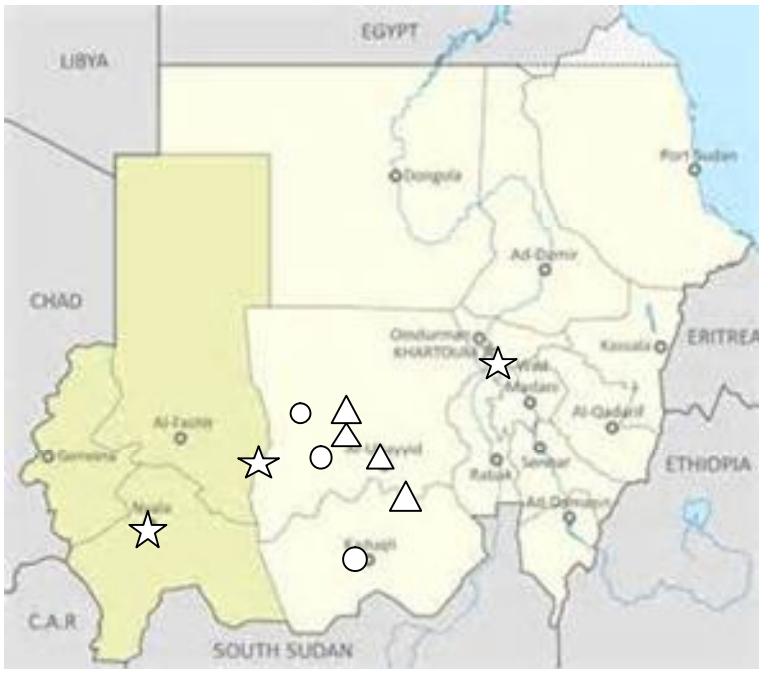
- Samples for **(**Kababish**)** collected from Umpadir area (Soudari**,** Tenna and Um Kraidem**)** it's 190 klm north west El Obeid and spend wet season there.

-Sample for (Nialawy) collected from Tumbool its in Buttana area about 125 klm South east from Capital Khartoum where big market of camels. and Nialawy come to Tumbool the market throw Darb Elarbaeen.

3:2 Blood Samples:

Blood samples (n =45) were collected from jugular vein 5 ml in EDTA tube to prevent clotting of blood from each type of camels (n=15)per each (Kababish, Shanabla and Nialawy camels).

Figure 1:Sudan map (http://images.google.de) edited with location of the different population of camel breed included in the study:



△ **(Kababeish Camel).**

○ **(Shanabla Camel).**

☆ **(NylawiCamel).**

3:3 DNA Extraction:

The following procedures were used to extract the DNA from whole Blood.

3-5 ml bloods were collected in EDTA tubes

10 ml of red blood cell lysis' buffer (RCLB) were added to the collected blood and centrifuged for 5 minutes at 6000 rpm.

This step was repeated until a clear pellet of white blood cells accumulated at the bottom of the tube.

The supernatant was discarded and 800 µl of white cell lysis buffer were added.

To the same mixture, 10 µl of proteinase K (10mg/ml) were added and incubated at 37^{0}C over night.

Equal volume of phenol/chloroform/ iso amyl alcohol was added, mixed and centrifuged at 6000 rpm for 5 minutes.

The upper layer was transferred into a clean tube, and the following were added (double volume of 95% cold ethanol and 1:10 sample volume, Sodium acetate (3M) and incubated for at least 2 hours at -20º C. Then centrifuged for 10 minutes at 12000 rpm then the supernatant was discarded.

2ml of 70% ethanol were then added and centrifuged for 7 minutes at 12000 rpm, the supernatant was discarded.

The previous step was repeated, the supernatant was then discarded and the pellet was allowed to dry for 15 minutes.

100 µl of TE buffer were added and the DNA was stored at -20º C till use.(Sambrook et al., 1989).

(DNA extraction were done in the Central laboratory**)**.

3:4 Primer Pair used:

The primer pair based on mitochondrial D-loop gene (National Center for Biotechnology Information (NCBI)
In this study primer was designed using the published data for camel sequences to amply DNAfragment . DNA fragment of Identical size. Approximately 208bp.
(Forward: 5-AGC CTT CTC TTC AGT CGC ACA C-3).
(Reverse: 5-GCC CAT GAA AGC TGT TGC T-3). ((Alaa El-deein et al., 2011).

3:5 PCR Amplification of DNA Fragment:

To determine mitochondrial D-loop fragments using primer (forward -Reverse) based on mitochondrial D-loop gene.The reaction mixture was prepared in a 25-µl PCR tube (Master Mix), o5-µl each of forward and reverse primer, 1 µl of DNA template-and Volume was completed per reaction mix with free water. the PCR conditions programmed on master cycler gradient thermocycler (Eppendorf, Germany) were as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 91C for 1min, annealing at 54 for 1min, and extension at 72°C for 1.5min then, final extension Was done at 72°C for 10 min the PCR product was Kept at −20°C for further use.

3:6 Analysis of PCR-Amplified DNA Fragments:

Agarose gel electrophoresis was used for analysis of PCR products using 2% agarose gel to make a concentration 1.5g agarose,was put in 60 ml of 1 × TBE buffer was add5 ml in EDTA tube in flask When the gel solution was cold enough (55oC) . Then, 1.5µl (0.2%) of ethidium bromide solution was added as gel visualizing agent and mixed thoroughly. The electrophoresis was done for 50 min at 120V. The PCR product was finally analyzed using gell documentation system.

3:7 Digestions of PCR-Amplified DNA Fragments with Restriction Enzyme (tag1, hinf and scal):

Restriction digestion assay was performed in a final volume of 20 µl by mixing 0.5 units of Restriction enzyme with 1 µl PCR products and add Buffer. The mixture was incubated over night at 37°C.Then, the digested DNA Fragments were run on 2% a grose gel for 50 min at 120v.

CHAPTER FOUR
RESULTS AND DISSCUSION

The diversity of Sudanese camel based on the digestion of D.loop gene region of the mitochondrial DNA using the the amplified PCR product (208 bp) , was subjected for digestion using three different endonuclease enzymes (tag1, hinf & scal) . revealed different results according to digestion undigestion samples

The first endonuclease used was Tag1 which did not cut the PCR product (208bp) of the three breeds. (Table 4:1) and (Figure 4:1)

Whereas Scal Enzyme (Table 4:2) and (Figure 4:2), the same PCR product when digested with hinf (Table 4:3) and (Figure 4:3).

population	No. of animals	cut	Uncut
Kababeish	15	0(0%)	(100%)15
Shanabla	15	0(0%)	(100%)15
Nayalawie	15	0(0%)	(100%) 15

(Table 4:1): Tag1 Enzyme digestion %

population	No .of animals	cut	Uncut
Kababeish	15	2 (6.7%)	13 (93.3%)
Shanabla	15	1 (4.4%)	14 (95.6%)
Nayalawie	15	0 (0.0%)	15 (100.0%)

(Table 4:2)Scal Enzyme digestion %

population	No .of animals	Cut	Uncut
Kababeish	15	14(95.6%)	1(4.4%)
Shanabla	15	15 (100%)	0(0%)
Nayalawie	15	14 (95.6%)	1(4.4%)

(Table 4:3): Hinf Enzyme digestion %

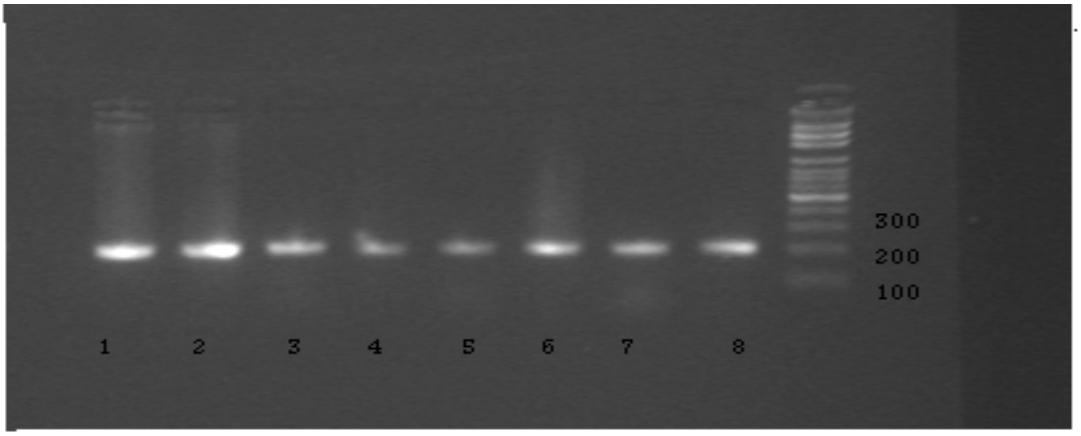


Figure 1:4

- PCR amplification for 208 bp product.

- Lane 1 to 8 for Naylawie camels.

-ladder 100 bp.

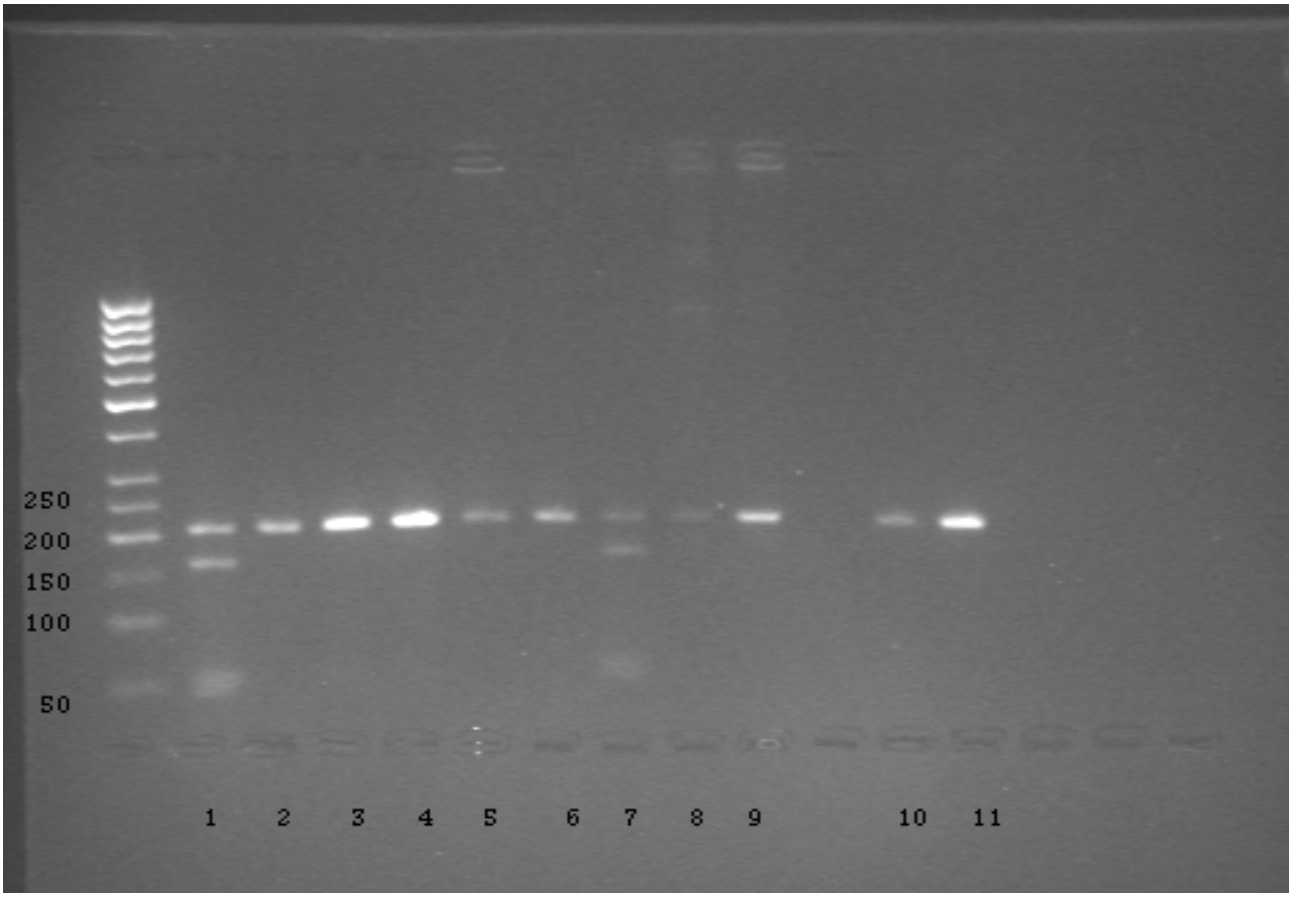


Fig- 4:2

- PCR amplified DNA fragment of D-loop gene digested with Scal enzyme

- lane 1 to11 for Kababeish breed.

- lane 1 and 7 were digested.

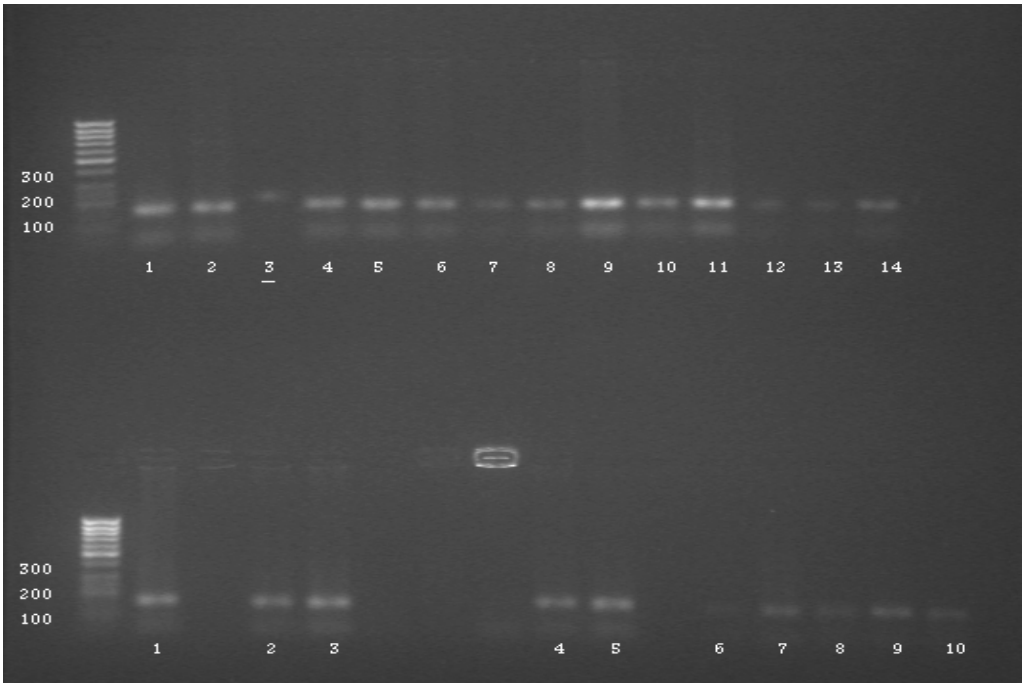


Fig- 4:3

- PCR amplified DNA product 208 bp digested with Hinf enzyme.

- Upper lane(1to14) Kababeish breed. lane3 was digested.

- Lower Shanabla breed lane (1to10) No digested.

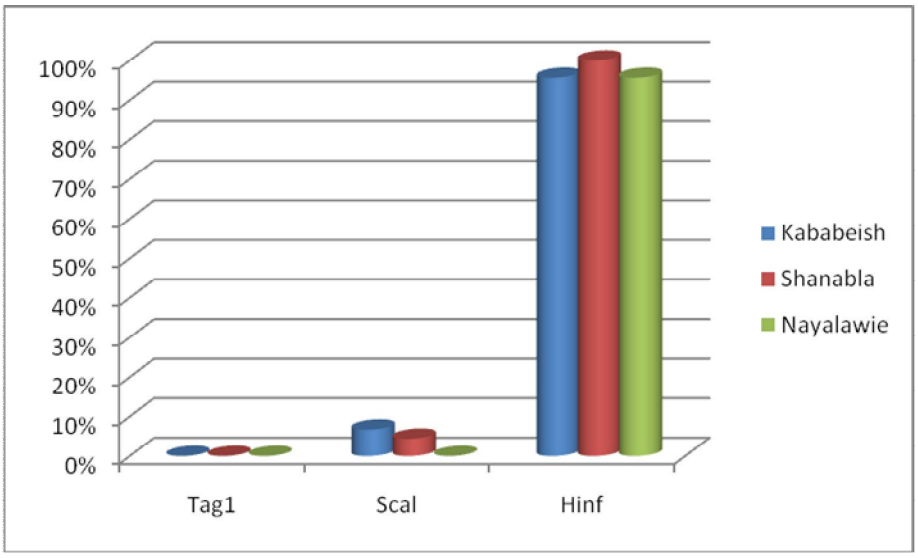


Fig- 4:4

shows differences of frequency within Population.

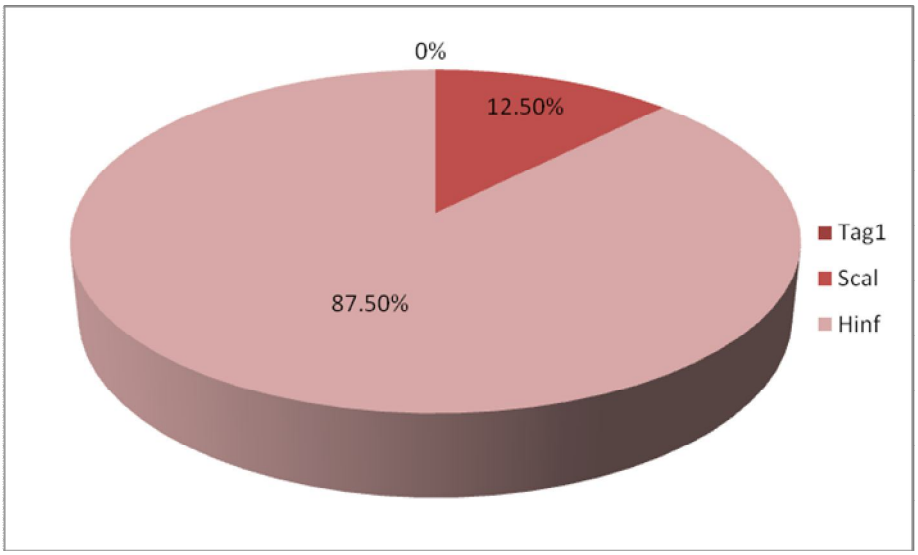


Fig- 4:5

shows the relationship between three Enzymes in Kababeish Breed.

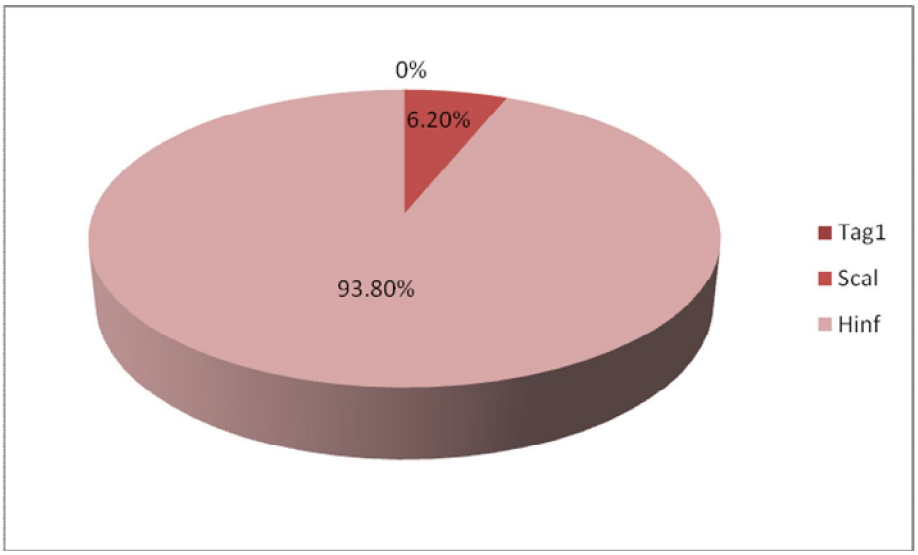


Fig- 4:6

shows the relationship between three Enzymes in Shanabla breed .

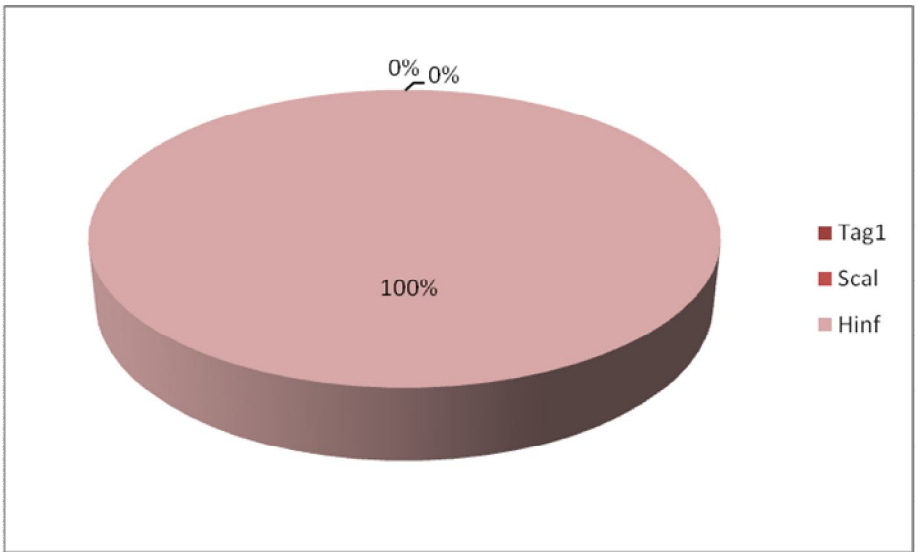


Fig- 4:7

shows the relationship between three Enzymes in Nayalawie breed.

The first restriction enzyme (Tag 1) No recognise site in three species, as shown(Table 4:1) and (Figure 4:1)that means their no genetic back ground within breeds kabebish shanabla and Nyalawi in this enzyme this result was agree with(Alaa Eldeein et al., 2011) in Egypt when they used the same restriction enzyme tag1 as the enzyme of choice to detect the camel meat from different animal meats it seems that there might be a genetic marker for them, also this result raised a question about the difference between Sudanese and Egyptian camel breeds.

The second restriction enzyme (scal1) (Table 4:2) and (Figure 4:2), few recognise number in two breeds without any effect to Nyalawi breeds and left the rest of samples uncut, this result revealed that the genetic diversity started to differentiate within breed might be as a result of cross breeding with outsider animals. These two breeds were kabebish and shanabla, those two tribes share adjacent areas in Kordofan region and it make sense that they share genetic back ground in their animals. that what (Ishag, et al.,2011) dedicated there are significant differences between (Kababish, Shanabla, Maalia, and Maganeen)these four ecotypes in body measurement , howere these differences might be due to continues inbreeding among these ecotypes and migration routes adopted by camel –keeper which may

also reflects the genetic variation among these ecotypes

Strikingly, the third enzymes (hinf1) (Table 4:3) and (Figure 4:3). which have the high number of recognize site in the

amplified sequences in kababeish and shanabla and also in the genome of Nyalawei camels. The first two are owned by tribes which inhabit two adjacent areas, but the Nyalawi breed inhabit different region far in the west, which might be due to the direct impact of Darfur conflicts which enforced the tribe which own Nyalawi camel to move to the borders of Kordofan area, and this resulted in cross breeding with camels in that area. Drought and desertification which struck parts of Africa have led to tremendous losses of livestock markedly in cattle and to lesser extend in sheep and goats. Camels population, on the other hand, appeared only marginally affected (Schwartz and Dioli, 1992). During the famous drought of 1984 in the Sudan camels seemed to be the only animal that survived and mildly affected. They have migrated south-wards to area which were not known to be normal habitat for camels, (Yagoub, 2003).

Fig (4:4) show the percentage of three restriction Enzymes (tag 1 scal hinf) in three breeds (kababeish shanabla and Nyalawei). Fig (4:5) show differences frequncy whithin breeds tag 1did not find recognised site to cut, scal cut two samples and hinf cut 14 samples,the frequency pattern in this breeds mean that similarity in breed aboud 87.5% and varaition 12.5%.

Fig (4:6) show the relationship between the three enzymes in shanabla camel, tag1 did not find recognised site to cut, scal have one recognised site. and hinfcut all samples the

percentage between enzymes in this breed mean that the similarity is 93.8% and varaition 6.2% in this breed.

Fig (4:7) Nayalawie camel, tag1 and also scal did not cut any samples but hinf cut 14 samples that mean the similarity is 100% and there is no different in this breed.

Chi-Square Tests

			Breeds			Total	P-value
			Kababiesh	Shanabla	Nayalawie		
	Scal	Count	2	1	0	3	
Enzymes		% within Enzymes	66.7%	33.3%	0.0%	100.0%	0.384
		% within Breeds	12.5%	6.2%	0.0%	6.5%	
	Hinf	Count	14	15	14	43	
		% within Enzymes	32.6%	34.9%	32.6%	100.0%	
		% within Breeds	87.5%	93.8%	100.0%	93.5%	
Total		Count	16	16	14	46	
		% within Enzymes	34.8%	34.8%	30.4%	100.0%	
		% within Breeds	100.0%	100.0%	100.0%	100.0%	

(Table 4:4):

Enzymes * Breeds Crosstabulation

P-value = 0.384 (non significant difference)

(Sig<0.05)

.

In table (4:4) the P-value = 0.384(Sig<0.05) (non significant difference) this mean there is no different between three (Kababeish ,Shananbla Nayalawie) that means the diversity of those breeds and shared genetic background which might be due to the shared nomadic knowledge about traditional breeding, as they follow safe ways of selection for the male to ensure the conservation of the colour and general phenotypic features of the animals which belong to their tribes are. what (Yagoub, 2003) dedicated the Camel of northern Darfur which inhabit an environmental similar to that of Kordofan and have also similar seasonal migration system.

The observed obtained in the study of Silbermayr k, et al., (2010) to differentiate between wild and domestic camel. Eight mitochondrial haplotypes and found high sequence divergence (1.9%) by Silbermayrk, et al., (2010) on 81 individuals from Mongolia, China and Austria. (PCR-RFLP) assay to differentiate between wild and domestic camel samples the results demonstrate high levels of mitochondrial differentiation between wild and domestic Bactrian camels almost the study indicates difference result due to difference of breed in current study between wild and domestic camel and environment Asia and Austria and in this study all breed from one environment t west Sudan and classified as pack camel.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

This study was the first study in Sudan concerning Mitochondrial DNA of camel. The number of samples included in this study although gives limited results, but it highlighted some dark areas, which may lead us to recommend:

- Further studies on Sudanese camel genomic and mitochondrial DNA and the impact of nomadic system on the genetic diversity of the Sudanese Livestock.
- More molecular marker should be used to get more information based on molecular level (sequences, micro satellites and single nucleotide polymorphism).
- Proper management and conservation of breed resource to protect local genetic reproduction from extinction.
- Further studies using large number of samples to carry out deep molecular characterization of Sudanese camels for the purpose of proper designed program of camels' conservation.

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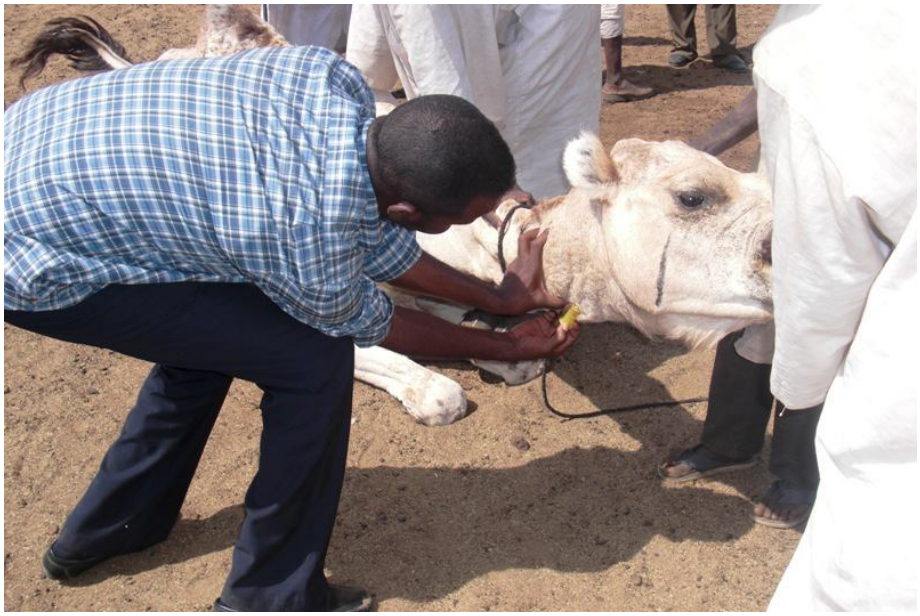
Picture (1:1) Kababish camel in Sodary aria Kordofan state

Picture (1:2) Shanabla camel in Elrahad aria Kordofan state.

Picture (1:3) Nialawy camels Tamboul Area.



Picture (4) Blood samples collected from jugular vein

Picture (5) shows Vortexer

Picture (6) shows the Gel Electrophoresis

Picture (7) shows the Gel documentation system

A privation:

AnGR: Animal Genetic Resources.

bp: base pair.

D-loop: Displacement loop.

DNA: Deoxy Ribonucleic Acid.

EDTA: Ethylene Diamine Tetra Acetic acid.

FAO: Food Agriculture Organization.

mtDNA: Mitochonderial DNA.

PCI: Phenol Chloroform Iso amyle alcohol.

NCBI: National Centre for Biotechnology Information.

PCR: Polymerase Chain Reaction.

QTN: Quantitive Trait Nucleotides.

RCLB: Red Blood Cell Lysis Buffer.

RFLP: Restriction Fragment Length Polymorphisms.

TBE: Tris Boric EDTA.

WCLB: White Cell Lysis Buffer.