Genetic Characterisation of Jaisalmeri Camel Using Microsatellite Markers

जैसलमेरी उष्ट्र में आनुवंशिक चरित्रण हेतु माईक्रोसेटेलाइट चिन्ह द्वारा अध्ययन

Lokesh Gautam
B. V. Sc. & A.H.

THESIS

MASTER OF VETERINARY SCIENCE
(Animal Breeding and Genetics)

उत्तमा वतिस्तु कृषिकमैव

2002

Department of Animal Breeding and Genetics
College of Veterinary and Animal Science
Rajasthan Agricultural University
BIKANER - 334 001

Genetic Characterisation of Jaisalmeri Camel Using Microsatellite Markers

जैसलमेरी उष्ट्र में आनुवंशिक चरित्रण हेतु माइक्रोसेटेलाइट चिन्ह का अध्ययन

THESIS

Submitted to the

Rajasthan Agricultural University, Bikaner

In Partial fulfillment of the requirements

for the degree of

MASTER OF VETERINARY SCIENCE

In the

Faculty of Animal Breeding And Genetics

By

LOKESH GAUTAM

SEPTEMBER, 2002

RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER
COLLEGE OF VETERINARY AND ANIMAL SCIENCE, BIKANER

CERTIFICATE - 1

Dated : 16th Sept, 2002

This is to certify that **Lokesh Gautam** had successfully completed the **Comprehensive Examination** held on June 4, 2002 as required under the regulations for the degree of **MASTER VETERINARY SCIENCE**.

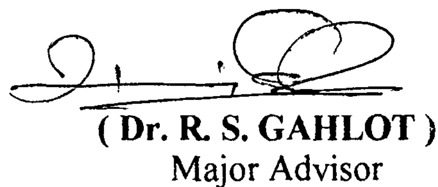
(Dr. B. K. BENIWAL)
Head
Department of Animal Breeding & Genetics
College of Veterinary & Animal Science,
Bikaner

RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER
COLLEGE OF VETERINARY AND ANIMAL SCIENCE, BIKANER

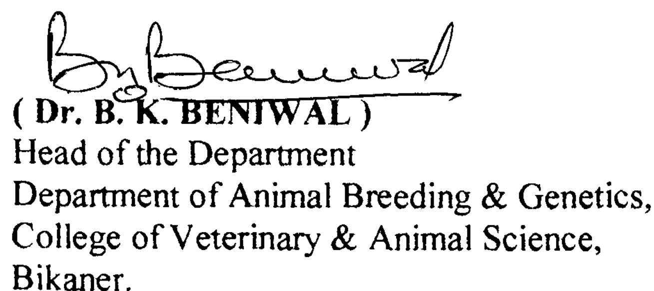
CERTIFICATE - II

Dated : 16th Sept, 2002

This is to certify that this thesis entitled "**GENETIC CHARACTERISATION OF JAISALMERI CAMEL USING MICROSATELLITE MARKERS**" Submitted for the degree of **Master of Veterinary Science** in the subject of **Animal Breeding & Genetics** embodies bonafide research work carried out by **Lokesh Gautam** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of thesis was also approved by the advisory committee on 30th Aug.2002.



(Dr. R. S. GAHLOT)
Major Advisor



(Dr. B. K. BENIWAL)
Head of the Department
Department of Animal Breeding & Genetics,
College of Veterinary & Animal Science,
Bikaner.

(Dr. A. K. GAHLOT)
Dean
College of Veterinary & Animal Science,
Bikaner

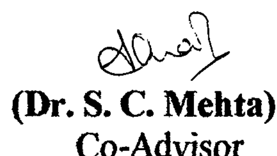
RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER
COLLEGE OF VETERINARY AND ANIMAL SCIENCE, BIKANER

CERTIFICATE -III

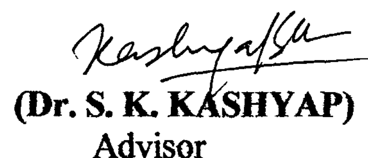
Dated : 18th Oct, 2002

This is to certify that the thesis entitled **"GENETIC CHARACTERISATION OF JAISALMERI CAMEL USING MICROSATELLITE MARKER"** submitted by Lokesh Gautam to Rajasthan Agricultural University, Bikaner in partial fulfillment the requirements for the degree of **Master of Veterinary Science** in the subject of **Animal Breeding & Genetics** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of candidate in the oral examination on his thesis has been found satisfactory, we therefore, recommend that the thesis be approved.

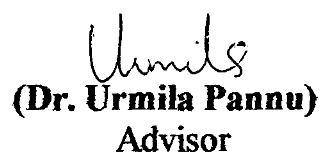
(Dr. R. S. GAHLOT)
Major Advisor



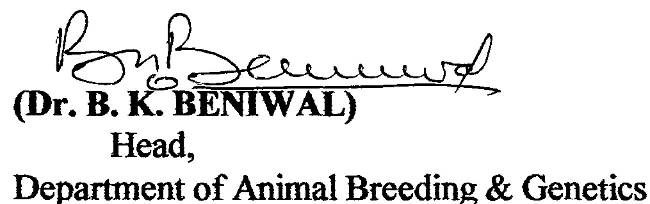
(Dr. S. C. Mehta)
Co-Advisor



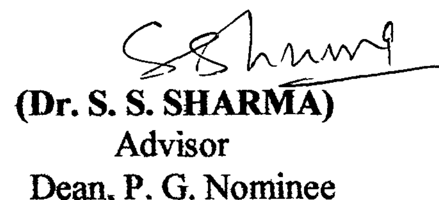
(Dr. S. K. KASHYAP)
Advisor



(Dr. Urmila Pannu)
Advisor

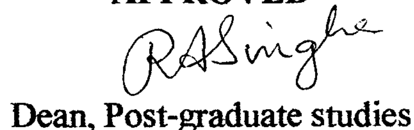


(Dr. B. K. BENIWAL)
Head,
Department of Animal Breeding & Genetics



(Dr. S. S. SHARMA)
Advisor
Dean, P. G. Nominee

APPROVED



Dean, Post-graduate studies

RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER
COLLEGE OF VETERINARY ANIMAL SCIENCE, BIKANER.

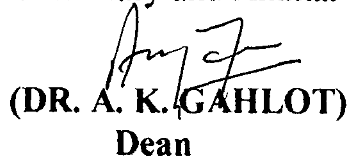
CERTIFICATE - IV

Date: 19th Oct, **2002**

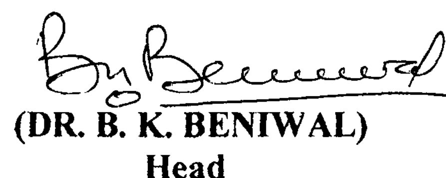
This is to certify that **Lokesh Gautam** of the Department of Animal breeding &Genetics, College of Veterinary & Animal Science, Bikaner has made all correction/modification in the thesis entitled **"GENETIC CHARACTERISATION OF JAISALMERI CAMEL USING MICROSATELLITE MARKER"** which were suggested by the external examiner and the advisory committee in the oral examination held on 18th Oct, 2002. The final copies of the thesis duly bound and corrected were submitted on 19th Oct, 2002 are enclosed herewith for approval.

(Dr. R. S. GAHLOT)
Major Advisor

Enclosed one original and two copies of bound thesis. Forwarded to the Dean, Post Graduate Studies, RAU, Bikaner through the Dean, College of Veterinary and Animal Science.

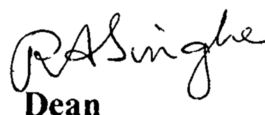


(DR. A. K. GAHLOT)
Dean
College of Veterinary and Animal Science
Bikaner-334001



(DR. B. K. BENIWAL)
Head
Department of Animal Breeding & Genetics,
College of Veterinary and Animal Science
Bikaner – 334001

APPROVED



Dean
Post Graduate Studies
Rajasthan Agricultural University
Bikaner - 334001

ACKNOWLEDGEMENT

I fumble for the genuine words to express my deep sense of gratitude and indebtedness to my esteemed teacher and advisor Dr. R. S. Gahlot, Retired Prof. Department of Animal Breeding and Genetics, for providing expertise and scholastic, intellect guidance during the course of study. I owe to him whole-heartedly my sincerest gratitude and indebtedness for his guidance, encouragement and benevolent attention. I most gratefully acknowledge.

I express my deep sense of gratitude to my co-advisor Dr. S. C Mehta, Scientist, National Research Centre on Camel for his unfathomable devotion, sincere advise and encouragement, deserve all praise for completion this work. The interest and attention he so gracious lavished upon this work. His ready availability at any time that this work has been a success. I most gratefully acknowledge.

It is my proud previlage to express my deep sense of gratitude to my esteemed teacher Dr. B. K Beniwal. Associate Professor and Head, Department of Animal Breeding & Genetics, for providing all facilities needed for this study to carry out and constructive criticism in planning and execution of this work.

I am highly indebted to Dean, College of Veterinary and Animal Science, Bikaner for providing the necessary facilities.

I am also highly indebted to Director, National Research Centre on Camel, Bikaner for providing the necessary facilities.

It is my great pleasure to acknowledge my indebtedness to Dr. S. S Sharma Professor and Head, Department of Obstetrics and Gynaecology and Dr. S. K Kashyap, Associate Professor & Head, Department of Veterinary Microbiology, as members of my advisory

committee for their generous help and constructive suggestions extended during the course of study

It is a pleasant disposition to express gratitude to my venerable advisor Dr. (Mrs.) Urmilla Pannu, Assistant Professor, Department of Animal Breeding and Genetics for her worthy guidance, hearty encouragement and giving valuable suggestion as when required.

I am also greatly indebted to Department of Science and technology for providing fellowship during the research of this study.

Very humbly, I owe my thanks to Dr. C. K Murdia, Associate Professor, Dr. P. D Swami, Associate Professor, and Dr. V. K. Chaudhary, Department of Animal Breeding & Genetics for providing valuable blood samples and all possible help during the research work.

My sincere thanks are also extended to Dr. G. C Gahlot, Assistant Professor and Dr. Rajeev Joshi, Assistant Professor, Department of Animal Breeding & Genetics.

I owe sincere thanks to my seniors Dr. Kuldeep Singh Nehra and Dr. Naresh Sharma for their co-operation, constant encouragement, taking interest in my task and support throughout the course of study.

The help extended by my Colleagues especially, Drs. Mohan Singh Jat, Praveen Garhwal, Rasbihari Yadav, Ajay Goyal and Vinod Poddhar during the period of study is thankfully acknowledged.

I also owe special thanks to Neelam Sharma and my juniors Dharmendra Soan, Ashwni Tambi for encouragement, all types of possible help during period of stress.

I appreciably acknowledge the Rameshwar Vyas and Nand Kishore Chauhan for technical assistance and Vikram for laboratory assistance.

I thank the technical staff of the Department Mr. Deep chand Gahlot, Mr. Durga Shanker Tanwer and non-technical staff for their co-operation and assistance during study.

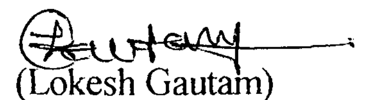
I express my profound sense of gratitude and indebtedness to my revered parents for their blessings, continued encouragement, unstinted moral support.

I wish to dedicate my thesis to my elder brother Late Shri Devendra Gautam whose blessings worked like a candle and showed me path.

I pridefully acknowledge my indebtedness to my elder brothers and Bhabhi for their profound moral support, affection and love.

A word of praise will not be sufficient to appreciate the deep understanding, sense of responsibility and patience shown by my wife Kavita, during entire period of course study.

I am grateful to god, the most merciful and the greatest benefactor, for giving me patience and strength in accomplishment of this endeavor.



(Lokesh Gautam)

CONTENTS

CHAPTER		PAGES
I.	INTRODUCTION	1-5
II.	REVIEW OF LITERATURE	6-27
III.	MATERIALS AND METHODS	28-42
IV.	RESULTS	43-50
V.	DISCUSSION	51-55
VI.	SUMMARY	56-58
VII.	LITERATURE CITED	59-70
VIII	THESIS ABSTRACT (English and Hindi)	i-ii
IX	APPENDIX-I (Composition of Buffers and Reagents)	iii-vi
X	APPENDIX-II (Abbreviation)	vii

LIST OF TABLES

Table No.	Title	Page No.
1.	Annealing temperatures for different microsatellite primers.	48
2.	Allele number, Allele size and Allele frequency at six microsatellite loci Jaisalmeri camel.	49
3.	Heterozygosity and polymorphic information contents at six microsatellite loci in Jaisalmeri camel.	50

LIST OF FIGURES

Figure No.	Title	Pages
1.	Photograph showing alleles at microsatellite locus (VOLP – 10) in Jaisalmeri camels.	45
2.	Photograph showing alleles at microsatellite locus (VOLP – 08) in Jaisalmeri camels.	45
3.	Photograph showing alleles at microsatellite locus (YWLL – 09) in Jaisalmeri camels.	46
4.	Photograph showing alleles at microsatellite locus (YWLL – 58) in Jaisalmeri camels.	46
5.	Photograph showing alleles at microsatellite locus (YWLL – 44) in Jaisalmeri camels.	47
6.	Photograph showing alleles at microsatellite locus (YWLL – 59) in Jaisalmeri camels.	47

INTRODUCTION

CHAPTER - I

INTRODUCTION

Camel is a very versatile animal suitable for riding, load carrying, many types of traction work, short distance transport and agricultural operations. In the arid and semiarid zones, the camel is an important working animal. The camel is ideally suited to desert life and is found in hot as well as cold deserts. The ecological efficiency of this species in service of mankind in desertic and semidesertic zones is very well established. No wonder the camel is known as the ship of the desert traversing long distances on sandy stretches carrying man and material and providing bioenergy for agricultural operations. It's principal use being draught, transport and agricultural operations. It's milk, meat, hide and hair are also of economic importance. Despite it's potential to thrive well on meager sources under extremes of desert climatic conditions. The population of camel in Rajasthan is 0.668m (Livestock Census, 1997). The population of camel at national and world level (1.52m and 19.286m) has recorded growth of 26.67 and 60.72%, respectively, in last seventeen years (FAO, 1997).

The word camel is said to be derived from the Greek word "Kremal". The camel belongs to the order *Artiodactyla* with Suborder *Tylopoda.* There are two types of camels, the one humped or dromedary camel (*Camelus dromedarius*) and the two humped or bactrian camel (*Camelus bactrianus*). They are also referred to as 'Old world camels' as opposed to the New world camels which include the domesticated

llama (*Lama glama*) and alpaca (*Lama alpacas*) as well as the wild guanaco (*Lama guanaco*) and vicugna (*Vicugna vicugna*). The fossils indicate that the family Camelidae (camel, llamas, alpacas, guanaco and vicuna) originated in North America about five million years ago and they migrated through Bering Straits to North Africa, western and central Asia. Some members of the family (llamoids) migrated to South America. The vicuna is now very rare and found in the high lands of Peru and Bolivia whereas the Guanaco has discontinuous distribution from Bolivia to Tierra del Fuego.

Genetic diversity is essential for long term genetic improvement and conservation programme of specific breed. Genetic diversity in different species is decreasing due to some of the breeds considered to be uneconomical, and erosion of the important indigenous germplasms. Maintenance of the genetic diversity is important for long term improvement and conservation programmes in different species to meet the requirements of growing population and the unforeseen challenges arising through changing production systems and agroclimatic conditions. This can be conserved by conserving the subsets of all breeds in a species showing most genetic differentiation among them.

Genetic characterisation of breed is first step in the conservation programme, as it will help the policy maker to identify genetically unique breeds so that they may be prioritised for breed conservation programme. Genetic variability is the most suitable criteria for identifying the breeds for genetic uniqueness, which is an important criterion that can be used when breeds are identified for conservation in order to conserve widest range of genetic diversity. Also, in the present era of patent rights, the genomic pictures of different livestock species

and breeds need documentation. Hence, it is very essential to look for and document the genetic variation for conserving the wealth of the nation and safe guarding interest of the countrymen.

Breed characterisation requires complete description of the genetic differences within and between populations (Hatzel and Drink Water, 1992). Most of the improvements made so far in different livestock species have been on the basis of the evaluation of the variability at gross phenotypic level by conventional biometrical methods using the principles of quantitative genetics. Breed characterisation at the phenotypic and molecular genetic level has become essential to know the present status of different species and their breeds available in different agro-climatic zones of the country. Morphological characters are often influenced by environment. Special breeding programmes and experimental designs are needed to distinguish genetic from phenotypic variation. Moreover, some of the traits are sex limited and age dependent so attention has been focused to the ultimate variability- the DNA molecule because nucleic acids are either the genetic material itself or the products of gene transcription (Prescott *et al.,* 1993 a).

Several researchers have evaluated production potential of different Indian camel breeds. There exists significant effect of breed on the production of hair (Sahani *et al.,* 1996), milk (Sahani *et al.,* 1998), draught potential, speed and stride (Rai *et al.,* 1992) etc. However, no significant differences could be detected by cytogenetic (Sahai and Vijh, 1993) and biochemical studies. The studies in biochemical polymorphism using 447 blood / serum samples in 15 systems viz. haemoglobin, albumin, amylase, transferin, alkaline phosphatase, acid phosphatase, lactase dehydrogenase (LDH), malate dehydrogenase

(MDH), phosphohexoseisomerase, glucose-6 phosphate dehydrogenase (G-6-PD), phosphoglucomutase (PCM), carbonic anhyderase, serum esterases, hexokinase and iso-citrate revealed no polymorphism. Milk protein polymorphism was also studied in 90 samples for α- casein, β - casein, γ -casein and β-lactoglobin but no polymorphism could be detected (Tandon *et al.,* 1997a, 1997b; Tandon, 1998). So looking for the genetic polymorphism under such circumstances appears very challenging.

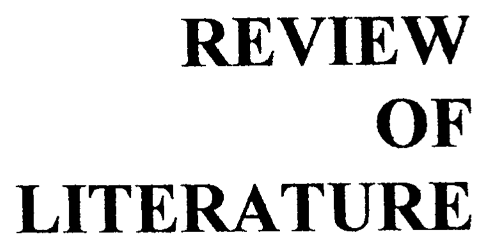
Various techniques have been developed in molecular genetics for molecular genotyping, such as Restriction Fragment Length Polymorphism (RFLP: Botstein *et al.,* 1980), DNA finger printing (Jeffreys *et al.,* 1985), Polymerase Chain Reaction (PCR: Saiki *et al.,* 1988), Arbitrary Primed-PCR (AP-PCR) or PCR-Random Amplified Polymorphic DNA (PCR- RAPD: Williams *et al.,* 1990), PCR-Single-Stranded Conformational Polymorphism (PCR-SSCP: Orita *et al.,* 1989) and Double-Strand Conformational Polymorphism-PCR (PCR-DSCP: Kirkpatric *et al.,* 1993), which have the potential to readout variations across individuals, genetic groups, strains, breeds or species. The advantages of analyzing genetic polymorphisms at the level of DNA are manifold. There is more information in DNA than in protein sequences. A large number of DNA markers have been developed for the study of variability in both coding as well as non-coding sequences of DNA.

The camel of Jaisalmeri breed is of medium size and lightly built. The colour of coat are mostly light brown. They have thin skin and short hairs on the body. Though, some work has been done on their characterisation at gross morphological and biochemical levels, a little information is available on the molecular characterisation of camels.

Microsatellite markers that contribute most for molecular genetic characterisation of livestock breeds due to their highly informative polymorphic nature. It is fast, requires less DNA and easier to automate. Owing to their high heterozygosity, Mendelian co-dominant inheritance, ubiquity throughout the genome and ease of scoring by PCR, microsatellite are now considered the most powerful genetic marker for characterisation of different breeds (Goldstein and Pullock, 1997). So there is a need to study the diversity within and among breeds and also to characterise them at the molecular level using microsatellite markers.

The present study was conducted with the following objectives

1. To screen microsatellite markers for DNA profiling in Jaisalmeri camel.
2. To study the DNA polymorphism in Jaisalmeri camel revealed by microsatellite primers.



REVIEW OF LITERATURE

CHAPTER - II

REVIEW OF LITERATURE

Camel is a unique species and very little is known about this species in terms of molecular genetics. However, available literature on different molecular techniques along with their applications on camel have been presented in the following sections.

2.1 Genetic characterisation

Genetic characterisation of an organism by its genetic information represent a precise nucleic acid sequence. The sum total of this sequence information present in its cell is called genome. Eukaryotic genome comprises of both the coding as well as non-coding sequences, the coding sequences that control the essential functions and do not vary much between individuals. However, the function of non-coding sequence is still obscure.

The total complement of the hereditary material in haploid genome is known as C–value. It is fairly constant within a species. It varies greatly among species in both prokaryotes and eukaryotes. The variation in C–value in eukaryotes is much larger than in bacteria, i.e. from 9 MB to 690,000 MB approximately, about 80,000 fold. This lack of correspondence between C–value and number of genes of different eukaryotes has been termed as C-value paradox. This has led to the studies on composition and organisation of the DNA in different species.

Density gradient separation and renaturation studies on DNA laid the foundation of satellite and repetitive DNA research.

Different sequence classes of heterochromatin DNA are as follows:

1. Unique sequences are present only once per haploid genome.

2. Fold back DNA consists of palindrome DNA sequences that can form hairpin double stranded structure as soon as denatured DNA is allowed to renature.

3. Moderately repetitive sequences are present at a frequency of 10^3 to 10^8 copies per haploid genome. They occur in segments of 100bp to several thousand bp that are interspersed with larger blocks of unique DNA.

4. Highly repetitive DNA consisting of short sequence from a few to hundreds of nucleotide long, which are repeated thousand or even millions of times. It forms a separate satellite band on density gradient centrifugation in $CsCl_2$. The tandem repetitive DNA sequences are further classified according to the size of repeat units.

The repetitive sequences are mostly in non-coding regions of DNA. These non-coding sequences included intragenic DNA consisting of pseudogenes, introns, leaders, trailer and extragenic DNA. The extragenic DNA consists of unique low number DNA, moderate copy number DNA and high copy number DNA. The high copy numbers are further classified into satellite, minisatellite and microsatellite depending the size of the nucleotide repeat unit. These are dispersed in the genome in various forms or arranged in tandem repeats. The tandemly repeated sequences may be especially due to misalignments during chromosome

pairing and thus the size of tandem clusters tend to be highly polymorphic with wide variation between individuals. The difference in the size of the tandem cluster causes the allelic variation.

Satellite DNAs represent a unique class of genomic DNA with high repetitive index and occur as tandem repeats in the heterochromatin region of the genome. Satellite DNA is named so because it forms a satellite band on buoyant density gradient centrifugation. The satellite DNA sequences consist of high repetitions (10^3 to $>10^5$ copies) of a basic motif of 2-100 bp and form very long heterochrometic stretches of DNA. In some species, satellite DNAs have been studied for their physical properties, structural organisation and sequence homologies among individuals, within as well as between breeds and species. Minisatellite (Jeffreys *et al.,* 1985) consist of shorter motifs of 10-60bp and show a lower degree of repetition at a given locus. Microsatellites (Litt and Luty, 1989) also named as simple sequences repeats (Tautz and Renz, 1984) consist of very short motifs of 1-5 bp with higher degree of repetition.

2.2 Molecular markers and their different types

Various types of markers are used for the genetic characterisation. Morphological and chromosomal markers usually show low degree of polymorphism and hence are not very useful for studying variation within and between species. Biochemical markers have been used for the study of genetic diversity. However, there are some drawbacks associated with this kind of marker system. These markers underestimate the variation because they reflect the variability in the coding sequences

only, which constitute around three percent of the total genome.

The molecular markers are capable of detecting the variation at the DNA sequence level. These markers have several advantages over classical markers. The limitations of classical markers include low degree of polymorphism, sex and age dependence, influence of environment and dominance effect. The advantages of molecular markers are many as they reveal almost large amount of polymorphism at the DNA sequence level that can potentially be exploited as genetic markers. These markers are numerous and distributed throughout the genome. These markers follow mendalian inheritance, they are unaffected by environmental factors and do not have pleiotrophic effect. These markers reveal variation in both the coding and non-coding sequence regions. DNA markers also have some methodological advantages that make them more attractive and amenable to genetic analysis.

Molecular techniques can be classified into two major categories

1. Hybridization based technique
2. PCR based techniques

1. Hybridization based technique

It includes RFLP analysis in which DNA is digested with restriction enzyme (Meselson and Yaun, 1968) and the resulting fragments are separated by gel electrophoresis, transferred to a filter by southern blotting (Southern, 1975), probes are hybridized to the filter

and sequences in genome bound with hybridized probe are then visualized as hybridization bands. Hybridization can be carried out with probes for structural genes and with genome derived cloned probes of different families of hypervariable repetitive DNA sequences (minisatellite, microsatellite). Molecular variability detected by cloned gene probes mainly produces diallelic. Hybridisation with hypervariable regions composed of tandem repeats and synthetic oligonucleotides give unilocus / multilocus multiallelic patterns.

2. PCR Based Techniques

The advent of polymerase chain reaction (PCR) has greatly simplified the manner in which genetic analyses were carried out. With the development of PCR the necessity for probe hybridisation steps could be avoided. The technique has greatly facilitated population level screening of genetic variation by saving time and reducing expenses.

PCR based methods can further be subdivided into three categories depending on the type of primers used.

(1) Arbitrary PCR assays
(2) Semi-specific PCR assays
(3) Sequence specific PCR assays

(1) Arbitrary PCR assays involve the amplification of anonymous polymorphic DNA using arbitrary primers. A common feature of these techniques is the non-requirement of sequence information from the genome under investigation.

The Random Amplified Polymorphic DNA (RAPD) (Williams *et al.,* 1990) is technically the simplest variation of the arbitrarily primed PCR methods. Short 10 mer primers with at least 50% GC content are used. The amplification products are separated in agarose and stained with ethidium bromide.

DNA Amplified Fingerprinting (DAF) involves use of short primers (5 to 8 nucleotides). In this only two steps temperature cycle programme are used. The fragments are separated on polyacrylamide gels and visualised by silver staining.

Arbitrarily Primed PCR (AP-PCR) (Welsh and Mc Clelland, 1990) involves the use of primers of 20 or more bases. The PCR products are radiolabelled with $\propto^{32}$ PdCTP and separated on polyacrylamide gel electrophoresis and viewed by autoradiography.

(2) Semi-specific PCR assays:- Semi-specific primers complementary to repetitive DNA elements interspersed in the genome are used in a variety of PCR assays like Alu PCR (Ledbetter *et al.*, 1991). Microsatellite Primed PCR (mP- PCR) is carried out using microsatellite as primers.

(3) Sequence Specific PCR assays: In sequence specific PCR assays a particular target sequence of the DNA is amplified using a pair of primers. Depending upon the type of the sequence variation in the target DNA, this category has three variants:-

(a) Simple repeat length polymorphism in variable number of tandem repeats is revealed by using a pair of primers that flank the simple sequence repeats and minisatellite (Jeffreys *et al.*, 1988) or microsatellite (Litt and Lutty, 1989).

(b) Cleaved Amplified Polymorphic Sequence (CAPS or PCR–RFLP) involves amplification of a specific region of DNA encompassing the polymorphic restriction enzyme site using a pair of sequence specific primers, digestion of amplified DNA fragment with the respective restrictive enzyme and visualization of products on an agarose gel (Saperstein and Nickerson, 1991). Variations arising due to changes in the restrictive enzyme sites mainly in the unique coding sequences of the DNA are studied by PCR-RFLP technique.

(c) Allele Specific PCR (AS-PCR) (Nichlos *et al.*, 1989), PCR Amplification of Specific Alleles (PASA) (Sarkar *et al.*, 1990) involve selective amplification of specific alleles sequences using primers that match the nucleotide sequence of one, but mismatch the sequence of the other allele.

Various techniques to visualise DNA sequence polymorphism have been developed in the last two decades (Botstein *et al.*, 1980; Jeffreys *et al.*, 1985; Nakamura *et al.*, 1987; Saiki *et al.*, 1988 and William *et al.*, 1990). Most commonly used techniques for studying DNA polymorphism include:

1. Restriction Fragment Length Polymorphism
2. Random Amplified Polymorphic DNA
3. Minisatellite
4. Microsatellite

1. Restriction fragment length polymorphism

Variation at the DNA sequence level is often detected as a variation in length of fragments produced by cutting DNA with restriction enzymes (Botstein *et al.,* 1980) referred such polymorphism as RFLP. In this method a DNA molecule digested with a particulars restriction endonuclease results in a set of reproducible DNA fragments of well defined lengths. These fragments are then separated by gel electrophoresis and subsequently transferred on to solid support like nylon membrane (Southern, 1975). The fragments are visualized by hybridisation with an appropriate labeled probe.

The individual carrying different allele variation for a locus will show different banding pattern (Mitra, 1994). Recently, alternate methods (PCR-RFLP) for generating RFLPs have been developed that take the advantage of PCR (Saperstein and Nickerson, 1991)

The amplified DNA is digested by restrictive endonuclease, electrophoresed and detected by Ethidium Bromide staining. PCR-RFLP has several advantages over southern blotting approach (Karl and Avise, 1993) being less time consuming, more sensitive and requiring very little amount (nanogram) of DNA.

The studies on RFLPs have some limitations:

The mean heterozygosity of eukaryotic DNA is low, approximately 0.0001 per base pair (Cooper and Schmidtke, 1984) and if any RE could detect a few RFLPs at a given locus the detected RFLPs are usually dimorphic. RFLPs will be uninformative in pedigree analysis whenever, the critical individuals are homozygous. RFLPs are not much

useful in population genetic studies at intraspecific level due to their di-allelic nature and low heterozygosity.

2. Random Amplified Polymorphic DNA (RAPD)

It involves *in vitro* amplification of DNA using Polymerase Chain Reaction (PCR) as given by Kary B. Mullis (1987). This technique has made possible the study of any region of genome. In this technique a very small amount of genomic DNA is subjected to PCR using short synthetic oligonucleotides of random sequence. The principle behind RAPD is that the genome contains several priming sites close to one another (i.e. with in amplification distance) that are in an inverted orientation. This technique scans a genome for these small inverted repeats and amplifies DNA segments of variable length. The amplification products are then resolved on agarose gel by electrophoresis resulting in DNA fingerprinting type of banding pattern. Since the primers used are arbitrary nucleotide sequences those markers are named as RAPD (Williams *et al.*, 1990)

Advantages of RAPD

(1) Easy to carry out.

(2) Requires a very small amount of DNA.

(3) It does not require any radiolabelled nucleotide.

(4) It does not require any prior knowledge of nucleotides.

(5) Any organism can be mapped with same set of primers.

(6) Primers can be detected against a repeated motif sequence (Welsh and McClelland, 1990).

There are some limitations of RAPD

(1) The size of primer determines the degree of specificity. Amplification of few numbers of bands with shorter primer has been observed (Mac. Pherson *et al.,* 1993).

(2) The main limitations of RAPD fingerprinting are the lack of specificity as well as its sensitive to reaction conditions therefore the reproducibility of the results is not always very high (Panner *et al.,* 1993)

3. Minisatellite marker

Minisatellite (Jaffreys *et al.,* 1985) also known as Variable Number of Tandem Repeat (VNTR) (Nakamura *et al.,* 1987) comprises arrays of short sequence units (11-60 bp), which are tandemly repeated in the genome at different loci. The resulting length variation is detected with restriction enzymes, which does not cleave the repeat unit and a multilocus probe under reduced stringency conditions.

The probe being used for DNA polymorphism studies are available from atleast three different sources viz. cloned cDNA and genomic DNA, synthetic oligonucleotides and PCR generated probes. Hypervariability in minisatellites results from changes in number of repeats. Hypervariability occurs due to unequal crossing over between homologous tandem repeats or due to gain or loss of repeat unit at particular locus during replication.

Nakamura *et al.* (1987) used synthetic oligonucleotides corresponding to consensus sequences of tandem repeats present in the

introns of several genes for screening and isolation of VNTRs. Jeffrey's probes were isolated from intron adjoining to human myoglobin (Jeffreys *et al.,* 1988). These probes can cross hybridise to produce DNA fingerprints in many species like mammals (Jeffrey's and Mortion, 1987), birds (Burke *et al.,* 1987). Minisatellite based DNA Fingerprinting (DFP) has a wide range of applications viz. individual identification (Jeffreys *et al.,* 1985) and parentage determination (Jeffreys *et al.,* 1985; Bovenhus and Van Arendock *et al.,* 1991). Population studies involving estimation of level of individual and population homozygosity, relatedness between individuals, estimation of within and between population variation (Kuhnlein *et al.,* 1989; Dolf *et al.,* 1992); linkage analysis. Main limitation of this technique is that probes are sometimes species specific, which makes it very laborious and costly to develop the probes for each species.

4. Microsatellite markers

The microsatellite markers have revolutionised genetic analysis. In the early 1980's a sub-class of repetitive loci was described with a repeat unit of only two base pairs, named microsatellites (Tautz *et al.,* 1984). It was not however, until 1989 that the polymorphic nature of microsatellites was recognised (Weber and May, 1989; Litt and Luty, 1989). As with larger VNTRs, microsatellite vary between individuals in the number of repeats in the array. Their nomenclature is informal and such loci are referred as Short Tandem Repeats (STR), Variable Small Sequence Markers (VSSMs), Short Sequence Repeats (SSR), Dinucleotide Repeats, CA blocks etc. The repeat unit may be from 1 to 6 bp and the most common microsatellite repeat motifs are A, AC, AAAN,

AAN, AG and AT (Beckmann *et al.,* 1992). The best characterised are dinucleotide, dC-dA/dC-dT repeats. Microsatellites are abundant, occurring with an estimated frequency of one short tandem repeats every 6 kb of human genomic sequences (Beckmann *et al.,* 1992). Simple sequence repeats amplified by PCR as a new kind of polymorphic marker. These are tandemly repeated motifs of 1-5 nucleotides and are evenly distributed at large number of loci in the genome (Weber and May, 1989). They occur at a frequency of one simple sequence repeats /10 kb DNA (Tautz, 1989) and numbering to a total of 50,000 – 1,000,00 in the mammalian genome (Moazami-Goudarzi *et al.,* 1997). These sequence motifs were first recognised in early 1970's, e.g. $(TAGG)_n$ in satellite DNA of hermit crab (Skinner *et al.,* 1974). Dinucleotide repeats, particularly CA/GT repeats are very abundant (Stallings *et al.,* 1991) and have great potential as genetic markers (Todd *et al.,* 1991). Many of the microsatellites that have been reported are polymorphic, the variation in length of different alleles being shown to arise from the variation in the number of repeat units (Oudet *et al.,* 1991). Their short length (< 300) makes them amenable to amplification by PCR (Rassmann *et al.,* 1991) and subsequent separation by polyacrylamide gels with the resolution of alleles differing by as little as single base pair (Weber and May, 1989). Many microsatellite markers have been found to be highly polymorphic and this variability is derived from variation in the number of repeat units in different alleles. Microsatellite are easy to isolate and PCR based typing of alleles can be automated. They permit a highly precise detection of the genetic structure of population or breed (Buchanan *et al.,* 1994; Blovin *et al.,* 1996).

This technique is fast, requires less DNA and is easier to automate. Owing to their high heterozygosity, Mendelian co-dominant

inheritance, ubiquity throughout the genome and ease of scoring by PCR, microsatellites are now considered the most powerful genetic markers.

Microsatellite variation results due to the difference in number of repeat units. These differences are thought to be caused by errors in DNA replication. The DNA polymerase slips while copying the repeats and hence changes the number of repeats. Larger changes in repeat number are thought to be due to the processes such as unequal crossing over and the differences are detected on polyacrylamide gel electrophoresis, where repeats migrate at distances according to their size.

4.1 Applications of Microsatellite Marker

Microsatellite markers have got much wider application as compared to conventional dimorphic RFLPs and minisatellite markers. These include individual identification, parentage determination, breed, line or strain characterisation and population genetic studies. These markers can also be used for linkage analysis of economically important traits and gene mapping.

4.1.1 Individual identification and parentage determination

Individual identification and parentage determination are very important in livestock species. Identity testing is useful in artificial insemination programme particularly for checking the semen of prized male. Parentage determination is helpful in progeny testing programmes and paternity related disputes.

Ron *et al.* (1996) used 12 microsatellite loci to test the paternity of 113 cows and 102 bulls and found out that 2-5% of cows and 2-9% of bulls had misidentified paternity.

Heyen *et al.* (1997) undertook parentage study by using 22 microsatellite primer pairs on 17 bovine autosomes in 1022 Holstein cattle and 311 beef cattle belonging to five breeds.

4.1.2 Population studies

Genetic structure of population holds the key to the selection strategies which in turn are very important for the genetic improvement of the livestock species. The population studies involve individual and population homozygosity, estimation of within and between population variations etc.

Arranz et *al.* (1996) compared 14 protein markers and 5 microsatellite loci in differentiation of 5 populations of cattle. The genetic variation at microsatellite loci was greater than protein loci. Genetic distance estimates based on microsatellite variation were 4-12 fold greater than those from protein marker.

Moazami and Gouderzi, 1997 conducted studies to determine the genetic variation between 10 cattle breeds by using 17 microsatellite loci and 13 biochemical markers. A total of 210 alleles of the 17 microsatellites were detected in this study and average heterozygosities ranged from 0.53 in the Jersey breed to 0.66 in the Parthenalis breed.

Poelman *et al.* (1998) analysed genetic variability of 23 bovine microsatellite markers in five main cattle breeds in Belgium (Holstein

friesian, Belgian blue, Belgian red pied and East flenish). Estimates of genetic distances between these breeds confirmed the widely accepted proposition that the Belgian blue is the most genetically distinct of these breeds.

4.1.3 Linkage analysis

The findings of many workers have shown linkage of molecular markers to economically important traits and offered the possibility of Marker Assisted Selection (MAS) in animal breeding, which is expected to improve genetic response by influencing both accuracy and time of selection (Soller and Beckmann, 1982).

Ashwell *et al.* (1997) typed 16 microsatellite markers in 7 large half-sib US Holstein cattle families. Potential Quantitative Trait Loci (QTL) were identified for somatic cell score, fat percentage and protein percentage. These markers (BM-203, BM-4805, BM-2078) reported to be associated with significant effects for more than one trait. The results further suggested the presence of a QTL for milk yield and protein yield on chromosome.

2.3 Microsatellite marker studies in different species

Kemp *et al.* (1995) studied a panel of 97 bovine microsatellite markers in cattle, sheep and goat. Of these, 39 markers were polymorphic in sheep and 32 in goat. The workers identified a set of 18 robust markers that were polymorphic in all three species. Cross-species sequence conservation of microsatellites was also studied between *Bos*

taurus (cattle), *Capra hircus* (goat) and related species (pepin *et al.*, 1995). Their study show that about 40 percent of the microsatellites isolated from cattle proved useful to study the caprine genome and to characterise economically important genetic loci in these species.

deGortari *et al.* (1997) studied the conservation of cattle microsatellite in sheep and reported 58 percent (605/1036) bovine primer pairs amplified a locus in sheep, of which 67 percent were detected as polymorphic. It was further reported that the marker heterozygosity, allele number and range of allele sizes were significantly lower in sheep than cattle.

Slate *et al.* (1998) evaluated the use of 174 bovine microsatellite markers in Soapy sheep, Red deer and Sika deer. They found that 73.4 percent (127/173) gave a product in Soapy sheep of which 54 (42.5%) were polymorphic. In Red deer 74.1% (129/174) markers gave a product, of which 55.8% were polymorphic. In Sika deer 37.7% (126/171) markers amplified a product, of which 37.8 % were polymorphic.

Yadav *et al.* (1998) reported the use of bovine microsatellite primer pairs in 36 animals of Murrah breed of buffalo. All the 22 primer pairs amplified a product and 14 of which were polymorphic.

Ganai *et al.* (1999) used 16 bovine primers in 3 breeds of goats (*Capra hircus*). All the primers amplified products. However, marker heterozygosity, allele number and range of allele size of all the loci were significantly lower in goats than the cattle sampled in study.

Lindgren G. (2000) observed that ten microsatellite screened for polymorphism in five Gotland ponies, five Shetland ponies, five

Swedish Ardennes and five Swedish coldblood horses. The numbers of allele were microsatellite locus ranged from 4 to 7 and the heterozygosity ranged from 0.35 to 1.00.

Maddox *et al.* (2000) studied the dinucleotide repeat polymorphism at the ovine MCAM1 MCAM2 MCAM5 MCAM8 MCAM9 MCAM11 MCAM14 MCAM20 MCAM24 MCAM26 loci. Ten microsatellite were identified and PCR primers were designed to amplify these loci. The distribution of alleles were examined in sheep from nine breeds (Merino, Border, Leicster, Poll dorset, Dorest horn Suffolk, Rommy, Karakul, Finnish Lanrace and Carpet master) the number of alleles of the ten microsatellite ranged from 5 to 17, the heterozygosity ranged from 0.49 to 0.90.

Ritz *et al.,* (2000) studied the phylogenetic analysis of the tribe bovine using 20 microsatellite loci. They concluded that Boss, Poephagus and Bibos each emerged as a subgenus within the genus Boss, on the other hand, Bison, Bubalus and Syncercus, each formed a separate discrete genus. A suitable large panel of polymorphic markers for each species especially those that are less common and less studied. Cross species utilization of microsatellite loci not only save time but also enables the construction of comparative maps between related species.

Tozaki *et al.* (2000) evaluated the isolation and characterisation of 34 equine microsatellite loci. They designed the 34 microsatellite and PCR primers and used to amplify from the DNA of 35 to 40 thoroughbred horses. The number of alleles per locus ranged from 2 to 8. The heterozygosity ranged from 0.056 to 0.805. The polymorphic information content ranged from 0.054 to 0.776 and the probability exclusion values ranged from 0.027 to 0.621.

Bjornstad and Roed (2001) demarcated 8 horse breeds including indigenous Norweigun breed and Icelandic horse using 26 microsatellite loci. The phylogenetic analysis utilizing simple allele sharing statistics revealed clear demarcation among these breeds.

Lubieniecka *et al.* (2001) studied microsatellite based genetic diversity in the Polish red cattle population kept under the national preservation programme. Genetic variation at 26 microsatellite loci was investigated in the population of polish red cattle (southern and northern group). The overall heterozygosity of the whole population was estimated at 0.702. The average expected heterozygosity did not differ significantly between groups. Differences in allele frequency distribution were observed at 7 loci and group specific alleles were found in 15 out of the 26 loci analysed.

Wu Wei *et al.* (2001) reported the genetic structure of five Chinese and foreign cattle breeds using microsatellite DNA markers. Genetic structure including gene frequency, heterozygosity, Polymorphism information content, numbers of effective alleles and genetic distance were studied in five cattle types (Nanyang, Yanbian, Hanwoo, Simmental and Piemontese) using four microsatellite markers. Cluster analysis was conducted based on microsatellite polymorphism.

Ivankovic *et al.* (2002) reported genetic variation in 3 Crotian donkey populations, Istrian (IS), north Adriatic (NA) and Littoral Dinaric (LD) was analysed using eight microsatellite loci and by sequence. The analysis of microsatellite loci revealed observed heterozygosities in the range of 0.37 (MPZ 002 in LD) to 0.85 (AHT 21 in LD) and PIC values in the range of 0.36 (MPZ002 in NA) to 0.78 (AHT 21 in LD). The overall probability of exclusion was 0.991. Two

populations (IS and NA) were closely related (Fst =0.0034), whereas genetic distance between IS and LD (Fst=0.021) and NA and LD (Fst=0.027) were higher.

Kim *et al.* (2002) evaluated the genetic diversity of north-east Asian cattle based on microsatellite data. Thirteen microsatellite loci were analysed for a total of 200 individuals including Korean, Chinese, Japanese Black and European Holstein cattle. Observed and expected heterozygosities, two estimators (Fst and Gst) of gene differentiation and Nei's DA distance were evaluated. The lowest genetic diversity was exhibited in Japanese Black cattle (H_E=0.471) and the highest in Chinese cattle (HE=0.744), Korean Cattle revealed relatively high degree of genetic diversity (H_E=0.728.

Navani *et al.*, (2002) studied a set of cattle microsatellite DNA markers for genome analysis of reverine buffalo (*Bubalus bubalis*). The 108 microsatellite primer pairs, originally identified from cattle were evaluated for their applicability in buffalo. Eighty one primer pairs (75%) amplified discrete products and of these 61 pairs (56%) gave polymorphic band patterns on a panel of 25 buffaloes. The mean number of alleles per polymorphic marker was 4.56±0.20 and the mean heterozygosity per polymorphic marker was 0.66±0.02.

Van Hooft *et al.* (2002) reported applicability of bovine microsatellite markers for population genetic studies in African buffalo (*Syncerus caffor*). A total of 168 microsatellite markers were tested for PCR amplification on a test panel of seven African buffalo. Amplification was observed for 139 markers (83%) and 101 markers were studied further with 91 (90%) being polymorphic. The mean number of alleles per marker was 5.0 (SE=0.2) and the mean

heterozygosity per marker was 0.61 (SE=0.03). It was concluded that most bovine microsatellite markers are applicable in African buffalo.

Behl *et al.* (2002) studied genetic identify of two Indian pig types using microsatellite markers. A total 50 samples, 25 samples per population North Indian (NR) and North East Indian (NE) were used. The total number of alleles ranged between 4 (SO 178 in NE) and 12 (SO 355 in NR). The effective number of alleles ranged from 2.8 (SO 178) to 7.9 (S0005) in NR and from 2.5 (SO 178) to 8.7 (CGA) in NE. The mean effective number of alleles for all 23 loci was 5.0 in NR and 5.3 in NE. The mean observed heterozygosities was 0.71±0.14 and 0.68 ±0.12 in NR and NE, respectively. It was concluded that close identity of North and North-East Indian pig types.

2.4 Molecular markers studies in camelids

Mishra *et al.* (1998a) used eight random 10-mer primers to assay genetic polymorphism in three Indian camel breeds viz. Bikaneri, Jaisalmeri and Kachchhi. Overall 5 to 9 randomly amplified polymorphic DNA fragments per bands were observed on agarose gel with different oligos and described the technique of immense importance in the study of genetic variation in camels.

Mishra *et al.* (1998b) studied the possible application of DNA finger printing technique in precise identification of individuals and study of genetic variation in camel breeds.

Obreque *et al.* (1998) studied the genetic characterisation of alpaca using 10 dinucleotide microsatellite markers. Reaction were made

in 25 μl volume in buffer and PCR condition were denaturation at 95 °C for 4 minutes followed by 30 cycles of denaturation step at 94 °C for 1 min, annealing step at 55 °C for 1 min and final extension at 72 °C for 1 min. They reported allele number and size range as well as the expected heterozygosity and Polymorphic information content values. The allele number ranged from 2 to 12 and maximum heterozygosity was found in the microsatellite primer, VOLP-03.

Penedo *et al.* (1999) studied 8 microsatellite markers to evaluate polymorphism in South American Camelidae and reported allele frequency and heterozygosity. Alleles ranged from 4 to 18 and maximum heterozygosity was found in microsatellite primer, LCA 65.

Jianlin *et al.* (2000) studied the amplification of polymorphic loci in Old world Camelidae using 20 microsatellite markers. Only one primer pair failed to amplify DNA from both the dromedary and the bactrian camel, three primer pairs were monomorphic in both species. Of the remaining 16 primer pairs, 11 amplified polymorphic loci in the dromedary and 16 in the Bactrian camel. The mean observed heterozygosity was similar between the two species 0.52 and 0.55 for dromedary and bactrian, respectively. The mean expected heterozygosity was slightly higher in the bactrian compared with the dromedary 0.63 versus 0.55.

Lang *et al.* (1996) studied the polymorphic information content (PIC) and probability of exclusion using 15 polymorphic dinucleotide microsatellites. Polymorphic information content and probability of exclusion were determined from the typing of 50 unrelated animals. The total probability of exclusion for all 15 microsatellites was calculated to be 0.99996. They reported that these markers are useful for

parentage testing in both Llama and Alpacas.

Mehta *et al.* (2000) studied the PCR random amplification of polymorphic DNA using six oligonucleotide primer in Bikaneri, Jaisalmeri and Kachchhi breeds of camel. Breed specific markers were identified and between and within breed similarity was estimated. Genetic distance and population subdivision was also studied.

At National Bureau of Animal Genetic Resources 12 microsatellite primers were used in Indian camel and they reported number of allele ranging from 2 to 6 and expected heterozygosity ranged from 0.066 to 0.785. Lowest heterozygosity found in LCA-05 and highest heterozygosity found in LCA-63. PIC value ranged from 0.064 to 0.785.

Rieder *et al.* (2000) studied the characterization of Swiss new–world-camelidae breeds by microsatellites. The ninety one New–world-camelids (llamas, guanacos, alpaca and vicugnas) from Switzerland were genetically typed for six microsatellite markers and their parentage was determined. Calculation of alleles frequencies, heterozygosity, polymorphic information content, deviation from Hardy-Weinberg equilibrium and exclusion probability of markers showed high genetic variability with in the chosen population. It is concluded that this will allow selection for certain traits. The estimated genetic distance between different Camelidae species and breeds was in accordance with their expected phylogenetic origins.

MATERIALS
AND
METHODS

CHAPTER - III

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Experimental animals

The blood samples were collected from unrelated camels of Jaisalmeri breed maintained at the National Research Center on Camel, Bikaner and from Jaisalmer, Barmer district of Rajasthan. The camels of Jaisalmeri breed are of medium size and lightly built. It has a small head well carried on a thin neck, a small mouth closely set and small ears, prominent eyes and a narrow muzzle but no distinct 'stop'. The colour of the coat are mostly light brown. It has thin skin and short hair on the body. Luxuriant growth of black hair on their eyebrows, eyelids and ears, they are known as "Jheepras" are not present.

3.1.2 Equipments

DNA Thermal Cycler 480 (Eppendrof master cycle gradient), UVP-GDS 7600 Gel Documentation System (UVP International), Refrigerated Centrifuge (Jounan), Submarine Gel Electrophoresis System (Genei), UV Spectrometer (Milton Roi), Deep freeze (Blue star), Electrophoresis power supply (Atto Crop.), Sequential Gel

Electrophoresis (BioRad), Microcentrifuge (Genei). Microwave (Kanstar), Digital balance (Adier Dutt, Vortex mixture (Genei), Autoclave, Hot air oven, Incubator, Digital pH meter (Indigenous make) and Rapid dry system (Atto). Micro pipettes, p5, p10, p20, p50, and p1000 (Gilson/Finni Pipette/Nichipet) were used.

3.1.3 Chemicals

Agarose (Genei), Iso-amyl alcohol (Qualigenes), Phenol (SISCO), Ethidium Bromide (SISCO), 6X Loading dye (Genei), Triton X-100 (Qualigenes). Sodium chloride (Qualigenes), Magnesium chloride (Qualigenes), sucrose (qualigenes), Tris base (SISCO), Sodium acetate (Qualigenes), Ethylene dichloride tetra acetic acid (EDTA) (SISCO), Xylene cyanol (S.D. fine chem. Ltd.), Bromophenolblue (SISCO), Acrylamide (SISCO), Bis-Acrylamide (SISCO), Urea (SISCO), Sigmacote (Sigma), ϒ- methacryloxypropyl- Trimethoxysilane (Bind Silane) (Sigma), 37% Formaldehyde (S.D. fine chem. Ltd.), Sodium thiosulphate (SRL), Sodium carbonate (Glaxo), Silver nitrate (SISCO), Ammonium per sulphate (SRL), Acetic acid (Qualigens), Formamide (SRL) were used.

3.1.4 Kit and Other chemicals

PCR Kit (Genei), dNTP (Genei), Synthetic oligonucleotide primers (Genei / Genetix), Taq DNA buffer (Genei), Taq DNA Polymerase (Genei).

2. Resuspended the pellet in 800 µl of solution II (10mM Tris pH 7.5, 10mM KCl, 10mM $MgCl_2$, 0.5M NaCl, 0.5% SDS, 2mM EDTA) and 400 µl of distilled phenol was added with 1M Tris pH 8.0 and mixed well. The mixture was centrifuged at 10,000 rpm for 1 minute. The upper aqueous phase was gently aspirated and transferred into a fresh tube.

3. An equal volume of phenol (200 µl) and chlorophorm : isoamyle alcohol (24:1) was added and mixed by inverting. The tube was centrifuged at 10,000 rpm for 1 minute and upper phase was transferred to a clean tube.

4. 700 µl of chloroform : isoamyl alcohol (24:1) was added and followed by centrifugation at 10,000 rpm for 1 minute. Upper aqueous phase was collected in a fresh tube. DNA was precipitated by adding double volume of ice-cold ethanol. Transferred the DNA fibers to a microcentrifuge tube containing 1 ml. of 70% ethanol and air dried and sufficient amount of distil water was then added. The DNA samples were stored at -20°c for further use.

3.2.2 Quantitative and qualitative estimation of genomic DNA

The quality of genomic DNA was determined by standard method of Sambrook *et al.* (1980). Optical Density (OD) was determined at 260 and 280 nm wavelength by standard method of ultraviolet spectrophotometeric against distilled water as blank sample and the ratio of optical density at 260nm/280nm was calculated. The concentration of DNA was calculated using a following formula.

Quantity of DNA (μg / ml.) = OD at 260nm X Dilution factor X 50

The quality of genomic DNA was also checked by agarose gel electrophoresis.

3.2.3 Optimisation of PCR for specific microsatellite amplification

3.2.3.1 Camel Microsatellite Primer Pairs

Eleven camel microsatellite primer pairs were used (Obreque *et al.*, 1998, Jianlin *et al.*, 2000 and Lang *et al.*, 1996) for present study. Each primer was tested on 12 random DNA samples. The primers were screened for their conservation (amplification of product) and also polymorphism in camel were further studied using large number of DNA samples. The details of each marker in camel such as number of allele, allele size range (bp), annealing temperature and primer sequence are given in Table A

Table A Information of eleven microsatellite markers used in the present study.

Locus	Primer 5'→ 3'	Alleles (n)	Size (bp)	Temp (°C)	Reference
VOLP-10	CTTTCTCCTTTCCTCCCTACT CGTCCACTTCCTTCATTTC	7	250-268	55	Obreque *et al.*, 1998 Jianlin *et al.*, 2000
VOLP-08	CCATTCACCCCATCTCTC TCGCCAGTGACCTTATTTAGA	3	146-150	55	Obreque *et al.*, 1998 Jianlin *et al.*, 2000
YWLL-09	AAGTCTAGGAACCGGAATGC ACTCAATCTACACTCCTTGC	9	154-180	58	Lang *et al.*, 1996 Jianlin *et al.*, 2000
YWLL-44	CTCAACAATGCTAGACCTTGG GAGAACACAGGCTGGTGAATA	11	86-120	60	Lang *et al.*, 1996 Jianlin *et al.*, 2000
YWLL-58	GGCATCTCTTCCTCATCAAT GACATCTCCAACTTGGAATC	6	175-194	58	Lang *et al.*, 1996
YWLL-59	TGTGCAGGAGTTAGGTCTA CCATGTCTCTGAAGCTCTGGA	10	96-136	58	Lang *et al.*, 1996 Jianlin *et al.*, 2000
YWLL-08	ATCAAGTTTGAGGTGCTTTCC CCATGGCATTGTGTTGAAGAC	13	135-177	60	Lang *et al.*, 1996 Jianlin *et al.*, 2000
YWLL-19	CTCACAGACCACAGTTCCAGT AAGGTCCATTCATGTTGTCAC	6	137-161	58	Lang *et al.*, 1996
YWLL-43	ATACCTCTCTTGCTCTCTCTC CCTCTACAACCATGTTAGCCA	10	131-157	60	Lang *et al.*, 1996
VOLP-03	AGACGGTTGGGAAGGTGGTA CGACAGCAAGGCACAGGA	5	145-172	60	Obreque *et al.*, 1998 Jianlin *et al.*, 2000
VOLP-67	TTAGAGGGTCTATCCAGTTTC TGGACCTAAAAGAGTGGAG	18	150-196	55	Obreque *et al.*, 1998 Jianlin *et al.*, 2000

3.2.3.2 In Vitro amplification of microsatellite loci using PCR

In vitro Amplification of the microsatellite loci in Jaisalmeri camel was carried out by using the PCR, protocol reported by Jianlin *et al.* (2000) with minor modifications.

3.2.3.2 Optimisation of PCR Parameters

The PCR parameter, viz. annealing temperature (T_A OP^+) and cycling conditions were optimized to obtain a specific amplified product in sufficient quantity. The reaction volume was kept constant at 25 µl.

3.2.3.4 Preparation for PCR amplification

The master mix for PCR was prepared as follows

Genomic DNA (template)	100ng
Primer	5 pmol
Each dNTP	2.5 mM each
Taq DNA Polymerase	1.5 U
Taq Polymerase buffer 10X	2.5 µl.

[10 mM Tris HCl (pH 9.0), 1.5 mM $MgCl_2$ 50 mM KCl and 0.01% gelatin]

3.2.3.5 Protocol for PCR Amplification

1. In a 500 µl GeneAmp tube (Perkin Elmer Cat No. N-801-0737) 24 µl of PCR mix was taken and subsequently 1µl of genomic DNA was added.

2. PCR amplification was carried out in a thermal cycler using the following optimised conditions as given in Table B.

Table B General PCR programme

Step	Temperature	Time	No. of cycle
1. Initial Denaturation	95°C	5 min.	One
II. Cycle			
Denaturation	94°C	45 Sec.	30
Annealing	T_A^{opt}	1 min.	
Synthesis	72°C	1 min.	
III.Final extension	72°C	15 min.	One
IV. Hold	22°C		

3.2.3.6 Agarose gel electrophoresis

For agarose gel electrophoresis 1 % agarose gel was used in 1X TBE buffer system at 40 – 60 constant volt. Electrophoresis was carried out for 4-6 hrs. depending upon the band size. DNA bands were visualized

by ethidium bromide staining under UV Transluminator and documented. 50bp ladder (Gene Ruler^{TM}) was used as DNA marker.

3.2.4 Preparation and running of denaturing PAGE

The PCR amplified products were separated by denaturing sequencing gel electrophoresis using Sequ.Gel-GT electrophoresis apparatus manufactured by BioRad. The detail procedure is described below.

3.2.4.1 Cleaning and Siliconizing Plates

1. Both glass plates and spacer were cleaned thoroughly with soapy water. Both the glass plates were rinsed with distilled water and allowed to dry at room temperature. The clean and dry glass plates were wiped with ethanol to remove any traces of grease.

2. 12 µl of Bind silane was applied on edges of smaller plate. The glass plate was wiped with tissue paper pad, which is dipped in ethanol and allowed it to dry.

3. The bounded glass plate was siliconized with 200 µl of Sigmacote. The Sigmacote was wiped over the surface of the plate with a pad of tissue paper. The plate was rinsed with distilled water to remove the excess solution. Prior to assembling the plates, each glass plate was finely wiped to dryness with clean tissue paper.

3.2.4.2 Assembling the glass plates

The spacers were cleaned thoroughly and coated with high vacuum grease to avoid leaking. One spacer along each long side of the plate was placed over the smaller plate. Large plate was placed on the spacer which carefully aligned with bottom edges of the smaller plate. Each side of the sandwich was secured by binder clip.

3.2.4.3 Preparation of the Gel

Following constituents were mixed to prepare the working solution

Acrylamide	11.4 gm
Bis-Acrylamide	0.6 gm
10X TBE	10 ml.
Urea	84 gm
Distilled Water	up to 200 ml

This mixture was filtered by 3mm Whatman paper to remove undissolved particles. Freshly prepared 10% ammonium sulphate 500 µl was dissolved and 40 µl TEMED was added. The solution was mixed by swirling. The gel was sucked up into barrel of 120 µl syringe, inverted the syringe and expelled any trapped air bubble that has entered the barrel. The nozzle of syringe was introduced in the notched region on the plates. The flat edge of the comb (shark tooth comb) was inserted properly. Care was taken to prevent trapping of air bubbles. The comb was held at this position with bulldog metal clamp. The gel was left for 60 minutes at room temperature for polymerization.

3.2.4.4 Setting up for operation

1. Comb was removed gently and top of the gel was rinsed with distilled water for dissolving any crystallized urea around the comb.

2. The comb with teeth facing the gel was inserted till the teeth of the comb just enter the gel surface.

3. The integrated plate chamber assembly containing the polyacrylamide gel was placed into the universal base.

4. The lower and upper buffer tanks of the electrophoresis apparatus were filled with 1X TBE.

3.2.4.5 Pre-Run

The safety covers on top of the buffer chamber were fixed to prevent evaporation of buffer. The gel was then pre-run at constant temperature 70°C for 30 minutes.

3.2.4.6 Loading of sample

1. The power supply was turned off and then groove was rinsed with the help of syringe to remove any precipitated salt.

2. 1.5 to 3 µl PCR product was mixed with equal amount of 2X formamide dye. The samples were heated to 95°C for 5 minutes for denaturation of the DNA and immediately quenched on ice. The product was loaded slowly in each wells (between teeth of the comb). The wells were numbered for identification of each sample or animal.

3. DNA ladder was loaded along the side of samples as size marker.

3.2.4.7 Running the gel

1. After making sure that both safety covers were in place, the voltage was turned on and the gel was allowed to run at constant wattage (75w) for 1.5 to 3 hrs depending upon the allele size range.

2. On completion of electrophoresis power was turned off, the glass plate assembly was taken out and upper buffer was discarded. The gel assembly was dismantled by removing sidebars and was placed on the table with bounded plate upper side, combs were removed and the bounded plate was slowly lifted leaving the gel on the other glass plate.

3.2.5 Silver staining

The gel was stained with silver nitrate procedure using following protocol:

1. The gel was fixed in Fixer solution (100ml glacial acetic acid solution in 900ml. distilled water) for 20 minutes. The gel was rinsed three times with distilled water using shaking.

2. Gel was impregnated with staining solution (1g silver nitrate and 1.5ml of 37% formaldehyde and 1 liter of distilled water) for 30 minutes. The tray was kept in dark. The tray was regularly agitated. The gel was then washed with distilled water for 10 seconds.

3. The gel was incubated in chilled developer solution (Na_2CO_3 30 gm, 37% formaldehyde 1.5 ml, 20µl of sodium thiosulphate in 1 liter

distilled water). After appearance of bands the reaction was stopped with 10% acetic acid solution. Then gel was washed with distilled water for 2 minutes.

4. Whatman filter paper (3mm) sheet was laid on the gel and gently pressed with a tissue paper flat so that the gel stuck to the paper. The gel was picked up carefully by lifting the filter paper from one end. The gel was covered with saran wrap. The extra edges of the gel were trimmed of so that the gel could be fit into the gel dryer.

5. The gel was then dried under vacuum at 80°C for 45 minutes to 1 hr.

3.2.6 Analysis

During electrophoresis, PCR products were size marked by running the 50 bp DNA ladder. An analysis was done after scoring of alleles and genotyping. Scoring was done manually by comparing the band size with the standard 50 bp ladder.

3.2.6.1 Scoring of microsatellite loci

The conservation of the camel microsatellite loci was observed and recorded. The conserved primer pairs were further observed for polymorphism. Scoring of the alleles was done for each primer revealing polymorphism.

3.2.6.2 Estimation of the allele frequency

The allelic frequency was calculated using the formula.

$$P_i = Ki/N$$

Where,

P_i = Allele frequency

K_i = Number of observation of ith allele.

N = Total number of observation

3.2.6.3 Estimation of heterozygosity

The observed heterozygosity was calculated by taking the ratio of heterozygotes to total number of observations. The expected heterozygosity was calculated using the following formula (Nei *et al.*, 1974)

$$H = 1 - \sum P_i^2$$

Where,

H = Heterozygosity

P_i = frequency of ith allele

3.2.6.4 Estimation of Polymorphic Information Content

The PIC was estimated by using the following formula (Botstein *et al.*, 1980)

$$PIC = \sum P_{ij} (1 - 0.5 P_{ij})$$

$$P_{ij} = 2 P_i P_j$$

Where,

P_i = Frequency of ith allele.

P_j = Frequency of jth allele.

RESULTS

CHAPTER - IV

RESULTS

The present investigation was carried out to study the DNA polymorphism in Jaisalmeri camel using microsatellite primers. The results obtained in the study are presented under different sections

4.1 Qualitative and quantitative estimation

The quality and quantity of genomic DNA was measured by UV Spectrophotometer. The ratio of optical density at 260 to 280nm was calculated. The DNA samples having the ratio between 1.7 to 1.9 were selected for present investigation. The quality of the DNA samples was also checked by 1% agarose gel electrophoresis. The intact genomic DNA was observed. Samples showing no smearing were used for further studies.

4.2 Optimisation of PCR parameters

A total of 11 microsatellite primers were tried for optimisation of polymerase chain reaction for microsatellite study. The variation in PCR cycling conditions and annealing temperature were used for this purpose. The PCR conditions for 6 out of 11 microsatellite primers (54%) were optimised and they were used in subsequent study. Primers VOLP-08, VOLP-10, YWLL-09, YWLL-44, YWLL-58 and YWLL-59 amplified the specific bands. These primers were further used on thirty Jaisalmeri camel DNA samples for microsatellite studies. The annealing

temperatures for the microsatellite primers are given in Table-1.

4.3 Number of alleles, allele size and their frequencies

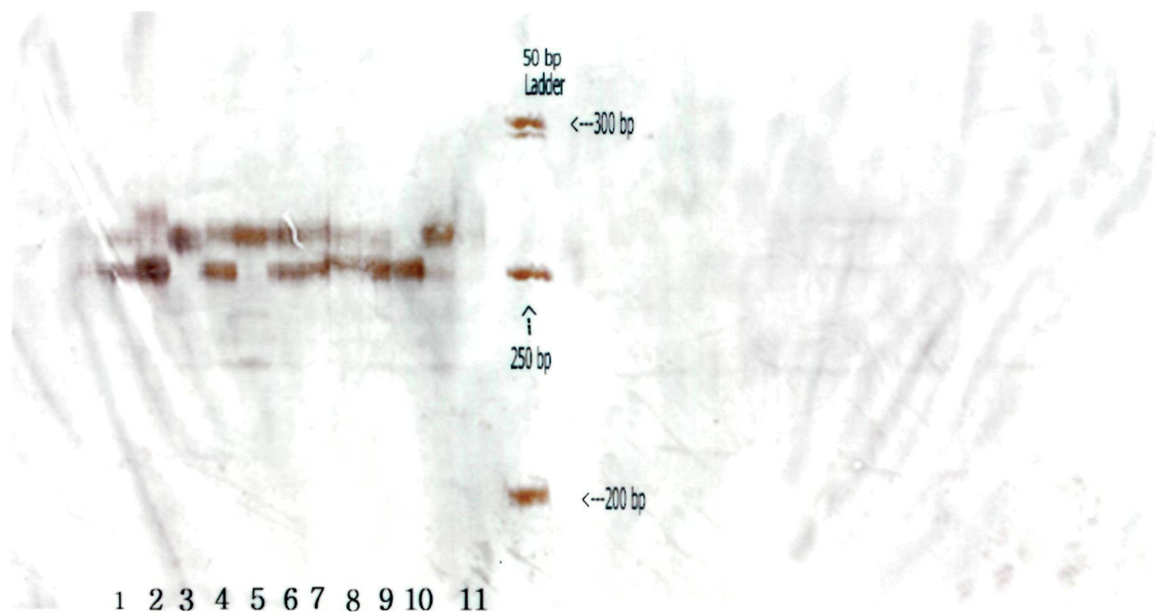
All the six microsatellite primers used in the present investigation were found to be polymorphic in Jaisalmeri camel. Figure 1 to 6 presents the number of allele and their size as resolved in 6 % urea polyacrylamide gel electrophoresis (Urea-PAGE) in microsatellite primers VOLP-08, VOLP-10, YWLL-09, YWLL-44, YWLL-58, and YWLL-59, respectively. The number of alleles and their frequencies at microsatellite loci were scored manually. Allele size was calculated by comparing the band size with the standard 50 bp ladder. The number of observed alleles ranged from two to five in Jaisalmeri camel for different microsatellite markers. The most polymorphic primers were VOLP-10 and YWLL-44, in which a total of 5 alleles were observed at each locus. The number of alleles, allele size and their frequencies for the six microsatellite markers are presented in Table-2.

4.4 Heterozygosity and polymorphic information content

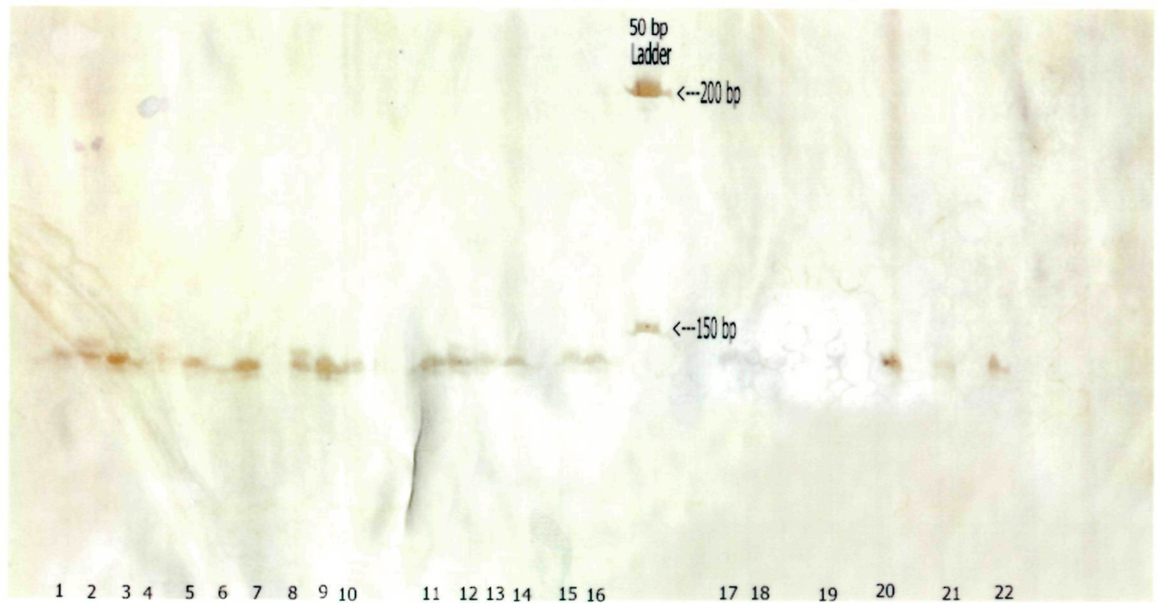
The observed and expected heterozygosity along with the polymorphic information content for the six microsatellite loci are presented in Table-3. The expected heterozygosity was calculated from allele frequencies, considering the population in Hardy-Weinberg equilibrium. The expected heterozygosity in Jaisalmeri camel ranged from 0.32 (YWLL-09) to 0.651 (VOLP-10) and the Polymorphic information content values in Jaisalmeri camel ranged from0.268 (YWLL-09) to 0.588 (VOLP-10).

FIGURE –1

FIGURE -2



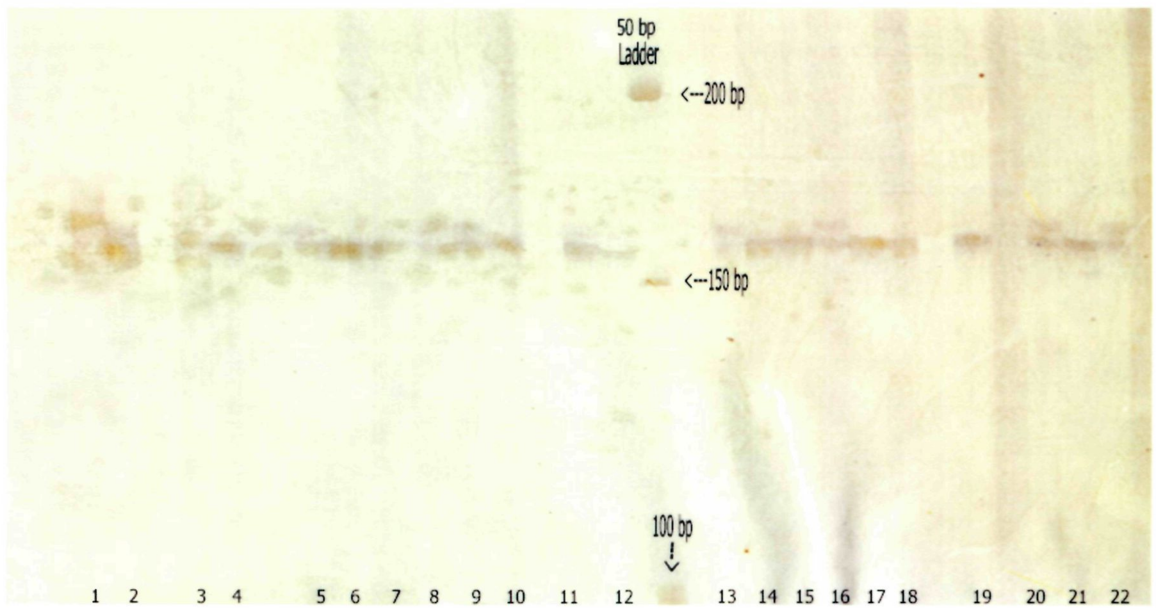
Photograph showing alleles at microsatellite locus (VOLP-10) in Jaisalmeri camels



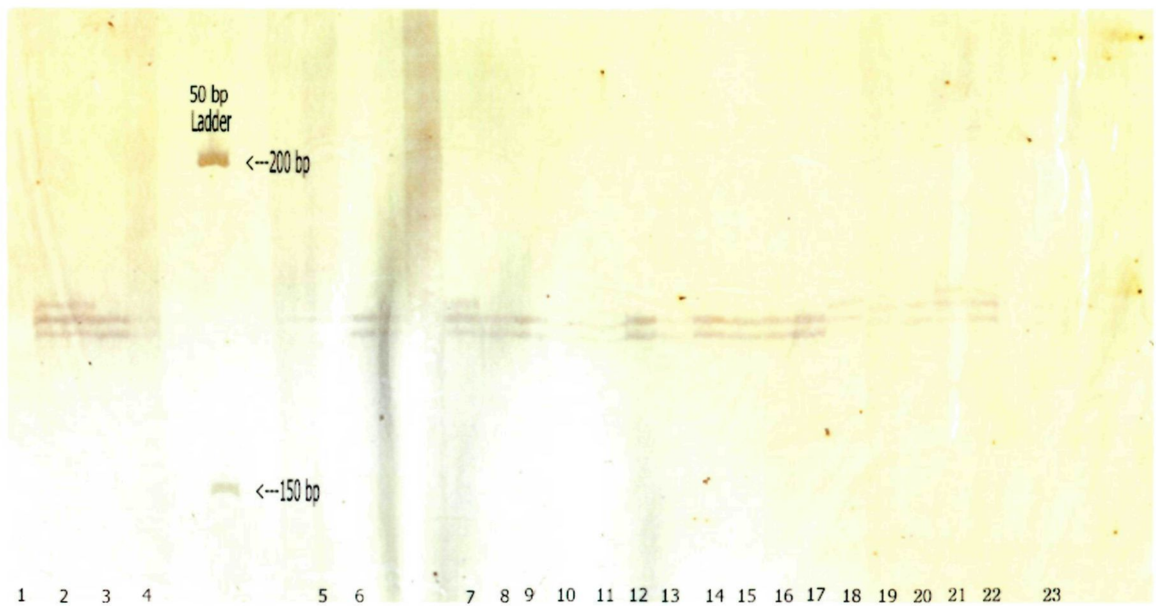
Photograph showing alleles at microsatellite locus (VOLP-08) in Jaisalmeri camels

FIGURE –3

FIGURE -4



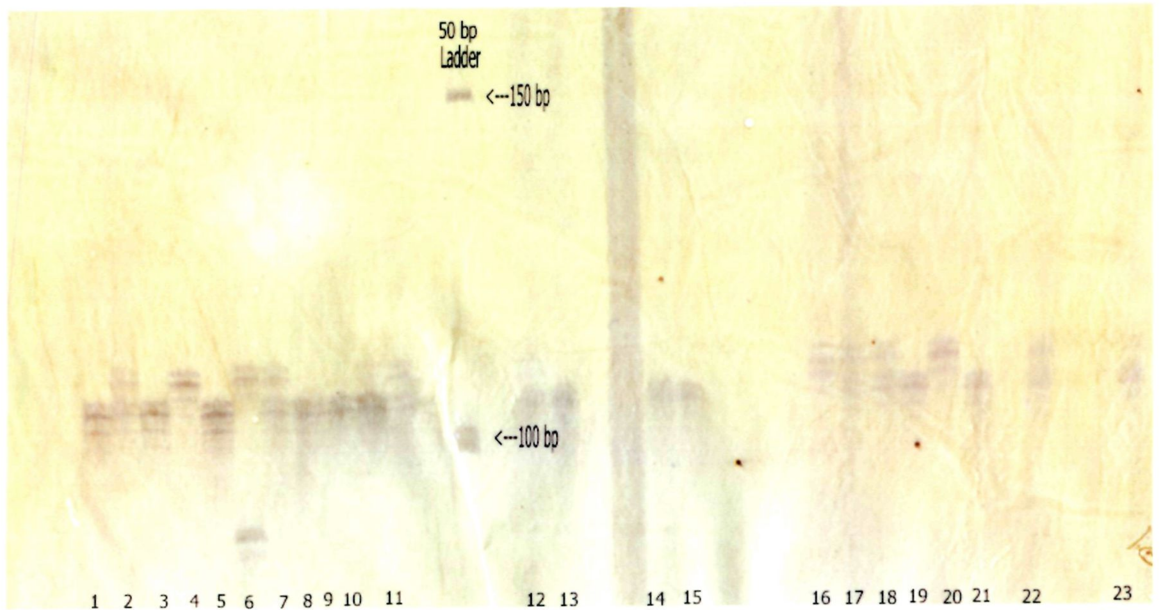
Photograph showing alleles at microsatellite locus (YWLL- 09) in Jaisalmeri camels



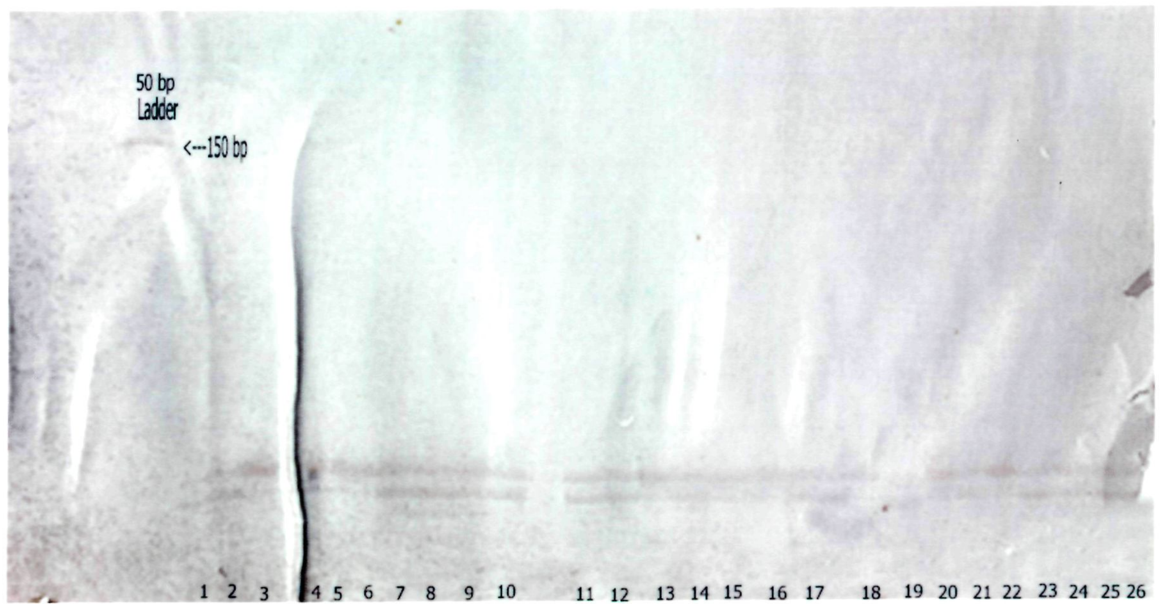
Photograph showing alleles at microsatellite locus (YWLL- 58) in Jaisalmeri camels

FIGURE -5

FIGURE -6



Photograph showing alleles at microsatellite locus (YWLL-44) in Jaisalmeri camels



Photograph showing alleles at microsatellite locus (YWLL-59) in Jaisalmeri camels

Table-1 Annealing temperatures for different microsatellite primers

Locus	Primer 5'→ 3'	Annealing Temp (°C)
VOLP-08	CCATTCACCCCATCTCTC TCGCCAGTGACCTTATTTAGA	50
VOLP–10	CTTTCTCCTTTCCTCCCTACT CGTCCACTTCCTTCATTTC	55
YWLL-09	AAGTCTAGGAACCGGAATGC ACTCAATCTACACTCCTTGC	53
YWLL-44	CTCAACAATGCTAGACCTTGG GAGAACACAGGCTGGTGAATA	55
YWLL-58	GGCATCTCTTCCTCATCAAT GACATCTCCAACTTGGAATC	51
YWLL-59	TGTGCAGGAGTTAGGTCTA CCATGTCTCTGAAGCTCTGGA	53
YWLL-08	ATCAAGTTTGAGGTGCTTTCC CCATGGCATTGTGTTGAAGAC	---
YWLL-19	CTCACAGACCACAGTTCCAGT AAGGTCCATCCATGTTGTCAC	---
YWLL-43	ATACCTCTCTTGCTCTCTCTC CCTCTACAACCATGTTAGCCA	---
VOLP-03	AGACGGTTGGGAAGGTGGTA CGACAGCAAGGCACAGGA	---
VOLP-67	TTAGAGGGTCTATCCAGTTTC TGGACCTAAAAGAGTGGAG	---

Table-2 Allele number, allele size and allele frequency at six Microsatellite loci in Jaisalmeri camel.

Locus	Allele No.	Allele size and Allele frequency				
		A	B	C	D	E
VOLP-08	3	143bp 0.350	144 bp 0.500	146 bp 0.150	---	---
VOLP-10	5	250 bp 0.366	254 bp 0.050	262 bp 0.450	263 bp 0.083	264 bp 0.050
YWLL-09	2	160 bp 0.200	162 bp 0.800	---	---	---
YWLL-44	5	96 bp 0.016	104 bp 0.0833	106 bp 0.583	107 bp 0.066	108 bp 0.25
YWLL-58	3	173 bp 0.366	175 bp 0.500	176 bp 0.133	---	---
YWLL-59	2	115 bp 0.333	117 bp 0.666	---	---	---

Table-3 Heterozygosity and Polymorphic information content at six microsatellite loci in Jaisalmeri camel.

Locus	Observed Heterozygosity	Nei's Expected Heterozygosity	PIC Value
VOLP-08	0.300	0.605	0.526
VOLP-10	0.630	0.651	0.588
YWLL-09	0.388	0.32	0.268
YWLL-44	0.630	0.585	0.533
YWLL-58	1.00	0.597	0.516
YWLL-59	0.660	0.444	0.345

DISCUSSION

CHAPTER - V

DISCUSSION

PCR is a method for amplifying specific DNA sequences in *in vitro* conditions. PCR makes possible the genetic analysis of tiny samples of DNA by a relatively simple process. Short length and ubiquitous distribution makes microsatellite typing very useful and easy through PCR. The microsatellite primers were used to amplify the specific bands by making little variation in the annealing temperature and cycling conditions in PCR. Annealing temperature in all the six primers was observed to be little less than the reported (Lang *et al.*, 1996; Jianlin *et al.*, 2000; Obreque *et al.*, 1998 and Penedo *et al.*, 1998). Such variations in annealing temperatures are often encountered due to the differences in the environmental and laboratory conditions. Lang *et al.* (1996) reported polymorphism at 15 dinucleotide microsatellite loci in Llamas and Alpacas. Obreque *et al.* (1998) reported 10 polymorphic microsatellite loci in Alpaca and Penedo *et al.* (1999) reported polymorphism at 8 microsatellite loci in South American Camelids consisting of Alpacas, Llamas and Guanacos. Jianlin *et al.*, in the year 2000 used 8, 9 and 2 microsatellite primers reported by Lang *et al.*, 1996, Obreque *et al.* (1998) and Penedo *et al.* (1999), respectively in dromedary and bactrian camel. In present investigation four primers used by Lang *et al.* (1998) and Jianlin *et al.* (2000) were successfully used in amplifying the polymorphic bands in dromedary camel. The other two primers VOLP-08 and VOLP-10, reported to be polymorphic by Obreque *et al.* (1998) and Jianlin *et al.* (2000) were also successfully

utilized in amplifying the polymorphic bands in dromedary camel. The present results also suggest that a single panel of microsatellite primers can be used for genetic studies and characterisation of closely related breeds.

The number of alleles at different marker loci and their frequencies are simple indicators of the genetic variability. Many workers have reported similar results on the use of heterologous microsatellite markers. In the microsatellite primer VOLP-08 three alleles with allele size ranging from 143 to 146 bp were observed. The present results are in close agreement with the findings of Jianlin *et al.* (2000) who reported 3 alleles in the size range of 146-150bp in 34 samples of dromedary. Obreque *et al.* (1998) also reported 3 alleles in the size range of 148-152bp in 36 samples of Alpacas. However, Mishra (2002) reported five alleles in Indian camel. At VOLP-10 microsatellite locus, five numbers of alleles with the allele size ranging from 250-264bp were observed. The allele size range reported by Obreque *et al.* (1998) and Jianlin *et al.* (2000) are in close agreement with present findings. However, Jianlin *et al.* (2000) reported 7 alleles in dromedary camel whereas Obreque *et al.* (1998) reported 3 alleles in Alpacas. At YWLL-09 microsatellite locus, two number of allele with allele size ranging from 160-162bp were observed. The allele size range reported by Lang *et al.* (1996) and Jianlin *et al.* (2000) are in close agreement with present findings. However, Jianlin *et al.* (2000) reported three number of alleles in dromedary camel whereas Lang *et al.* (1996) reported nine alleles in Alpacas and Llamas. At YWLL-44 microsatellite locus, five number of alleles with allele size ranging from 96-108bp were observed. The allele size range reported by Jianlin *et al.* (2000) are in close agreement with the present findings. However, Jianlin *et al.*

(2000) reported three number of alleles in dromedary camel and Lang *et al.* (1996) reported eleven number of alleles in the size range of 86-120bp in Alpacas and Llamas. At YWLL-58 microsatellite locus, three number of alleles with allele size ranging from 173-176bp were observed However, Lang *et al.* (1996) reported six number of alleles in the size range of 175-194bp in Alpacas and Llamas. At YWLL-59 microsatellite locus, two number of alleles with allele size ranging from 115—117bp were observed. The number of alleles reported by Jianlin *et al.* (2000) is in agreement with the present study. However, Lang *et al.* (1996) reported ten number of alleles in the size range of 96-136bp in Alpacas and Llamas. Number of alleles observed at microsatellite loci in indigenous camel population by National Bureau of Animal Genetic Resources (Annual Report 2001-2002) are less than those reported in the literature (Jianlin *et al.*, 2000; Obreque *et al.*, 1998; Lang *et al.*, 1996). This indicates that the genetic variation is relatively less in Indian population. Present results further confirms this finding. Variation in the number of alleles and their frequencies, reported in this study and by many other workers, within breeds over different markers indicated existence of genetic variation within and among species. The number of alleles and allele frequency could be of tremendous use in calculating the genetic distance between different strains or breeds of livestock species.

Heterozygosity is an appropriate measure of genetic variability within a population. In the microsatellite primer VOLP-08, expected heterozygosity and polymorphic Information Content were observed to be 0.605 and 0.526, respectively. However, Obreque *et al.* (1998) reported 0.226 expected heterozygosity and 0.214 PIC in 36 samples of alpacas whereas Jianlin *et al.*, 2000 reported 0.32 expected heterozygosity in 34 samples of dromedary camels. At VOLP-10

microsatellite locus, expected heterozygosity and PIC were observed to be 0.651 and 0.588, respectively. The PIC reported by Obreque *et al.* (1998) are in agreement with present study. However, Jianlin *et al.*, (2000) reported 0.75 expected heterozygosity in dromedary camels whereas Obreque *et al.* (1998) reported 0.596 expected heterozygosity in Alpaca. At YWLL-09 microsatellite, locus, expected heterozygosity and PIC were observed to be 0.32 and 0.268, respectively. However, Lang *et al.* (1996) reported 0.797 PIC in Alpacas and Llamas and Jianlin *et al.* (2000) reported 0.29 expected heterozygosity in dromedary camel. At YWLL-44 microsatellite locus, expected heterozygosity and PIC were observed to be 0.585 and 0.533, respectively. However, Lang *et al.* (1996) reported 0.845 PIC in Alpacas and Llamas whereas Jianlin *et al.* (2000) reported 0.66 expected heterozygosity in dromedary camel. At YWLL-58 microsatellite locus, expected heterozygosity and PIC were observed to be 0.597 and 0.516, respectively. However, Lang *et al.* (1996) reported 0.600 PIC in Alpacas and Llamas. At YWLL-59 microsatellite locus, expected heterozygosity and PIC were observed to be 0.444 and 0.345, respectively. Jianlin *et al.* (2000) who reported the 0.36 expected heterozygosity in dromedary camel. The present results are in close agreement with the findings of Jianlin *et al.* (2000). However, Lang *et al.* (1996) reported 0.769 PIC in Alpacas and Llamas. In the present investigation in Jaisalmeri camel, the expected heterozygosity was lowest (0.32) at the locus YWLL-09 followed by YWLL-59 with the expected heterozygosity of 0.44. Rest of the four microsatellite locus viz. VOLP-08, VOLP-10, YWLL-44 and YWLL-58, expected heterozygosity was more than 58%. This indicates that the about six primers can very well be utilized for further genetic studies which may include characterisation, conservation and production

enhancement. The polymorphism information content is another important measure of DNA polymorphism. Besides being a measure of genetic variation, it is also used in the context of gene mapping. The values of PIC are lower than heterozygosity for the corresponding marker because in PIC, a quantity is subtracted from heterozygosity that corresponds to the probability of offspring being uninformative.

SUMMARY

CHAPTER-VI

SUMMARY

The genetic diversity of most livestock species is reducing and it is not possible to preserve all livestock breeds. In order to preserve as much of the genetic diversity as possible we must first have a robust method of measuring the genetic differences between breeds. Genetic characterisation of Jaisalmeri camel has long been a challenging issue due to lack of observable differences in cytogenetic and biochemical studies, despite existence of phenotypic distinctions among breeds. The microsatellite have the potential to read out variations across breeds. Microsatellites are considered the most powerful genetic markers for genetic characterisation of different breeds.

The blood samples for isolation of genomic DNA were collected from NRCC, Bikaner and from Field. The genomic DNA was isolated by phenol-chloroform extraction method with minor modification. The qualitative and quantitative estimation of genomic DNA was determined by spectrophotometer and agarose gel electrophoresis.

The polymerase chain reaction using eleven microsatellite primer pairs was done for amplification of microsatellite loci. The PCR was performed in 25μl reaction mixture in a 500 μl GeneAmp tube. PCR amplification was carried out in a thermal cycler. The PCR products were electrophoresed in 1 % agarose gel in 1X TBE buffer system. DNA bands were visualised by ethidium bromide staining under UV

Transluminator and documented. The PCR amplified products were separated by denaturing sequencing gel electrophoresis. The gel was stained with silver nitrate procedure and then dried under vacuum at 80°c for 45 minute to 1 hr. The conservation of camel microsatellite primer pairs were observed and recorded. Scoring of the allele was done manually. The allele frequency, expected heterozygosity and polymorphic information content were observed using the standard formulas.

The six out of eleven microsatellite primer pairs were optimised. Primers VOLP-08, VOLP-10, YWLL-09, YWLL-44, YWLL-58 and YWLL-59 amplified the specific bands. Number of alleles ranged from two to five was observed in Jaisalmeri camel at six microsatellite loci. The most polymorphic primers were VOLP-10 and YWLL-44 in which a total of 5 alleles were observed at locus. The expected heterozygosity in Jaisalmeri camel ranged from 0.32 to 0.651. The expected heterozygosity was lowest at the locus YWLL-09 followed by YWLL-59 with the expectation of 0.444. Rest of the four microsatellite loci viz. VOLP-08, VOLP-10, YWLL-44 and YWLL-58 revealed the expected heterozygosity was more than 58%. This indicate that the above six primers can very well be utilized for further genetic studies which may include characterisation, conservation and production enhancement.

The results obtained in the present study can be of immense help in the further studies mainly in the fields mentioned below:

1. A single panel of microsatellite primers can be used for genetic studies and characterisation of closely related species e.g. Alpacas, Llamas, Dromedary and Bacterian camel.

2. The microsatellite primers used in this study were found to be polymorphic with good Polymorphic Information Content. Hence they can be used for further genetic studies.

3. Genetic variation is less in Indian camel population as compared to South American Camelids.

4. The present information with six microsatellite primers meets the partial requirement for breed documentation as per international standards. This information can also very well be used for determining the genetic distance between breeds and construction of phylogenetic tree.

5. Characterisation of individual breed using the microsatellite primers may be of tremendous use to the planners and scientists in formulating the projects for increasing the production and utilize of the breed. Screening by microsatellite markers may help in identification of sires of high genetic merit, which are true to their breed.

6. The information derived from microsatellite marker study for individual breed may help the conservation scientists in deciding the purity of the breed and to take necessary steps for *in vitro* and *in vivo* conservation of the breed.

LITERATURE
CITED

CHAPTER - VII

LITERATURE CITED

Arranz, J. J.; Bayon, Y. and San Primitivo, F. (1996) : Comparison of markers and microsatellites in differentiation of cattle populations. *Anim.Genet.* 27 (6): 415-417.

Ashwell, M. S.; Pekroad, C. E.; Jr.; Miller, R. H.; VanRaden, P. M. and Da, Y. (1997) : Detection of loci affecting milk production and health traits in an elite US Holstein population using microsatellite markers. *Anim. Genet.* 28: 216-222.

Beckmann, J. S. and Weber, J. L. (1992) : Survey of human and rat microsatellites. *Genomics,* 12: 627-631.

Behl, R. Kaul, R. Sheoran, N. Behl, J. Tantia, M. S. and Vizh, R. K. (2002) : Genetic identify of two Indian pig types using microsatellite markers. *Anim. Genet.* 33 (2) 158-167.

Bjornstad, G. and Roed, K. H. (2001) : Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers *Anim Genet.* 32 (2) 59-65.

Blovin, M. S.; Parrons, M.; Lacaille, Y. and Lotz, S. (1996) : *Mol. Ecology.* 5: 393-401.

Botstein, D.; White, R. L.; Skolinick, M. and Davis, R. W. (1980) : Construction of a genetic linkage map in man using restriction

fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-31.

Bovenhus, H. and van Arendock, J. A. M. (1991) : Estimation of milk protein gene frequencies in crossbred cattle by maximum likelihood. *J. Dairy Sci.* 74: 2728.

Buchanan, F. C.; Adams, L. J.; Littlejohn, R. P. (1994) : Determination of evolutionary relationships among sheep breeds using microsatellites. *Genomics,* 22: 397-403.

Burke, T. and Bruford, M. W. (1987) : DNA fingerprinting in birds. *Nature* (London). 327:149-152.

Cooper, D. N. and Schmidtke (1984) : DNA restriction fragment length polymorphisms and heterozygosity in the human genome. *J. Human Genet.* 66: 1-16.

deGortari, M. J.; Freking, B. A.; Kappes, S. N. (1997) : Extensive genome conservation of cattle microsatellite heterozygosity in sheep. *Anim. Genet.* 28: 274-290.

Dolf, G.; Glowatzke, M. L. and Gaillard, C. (1992) : DNA fingerprinting in cattle using the probe PV97. *Anim. Genet.* 23: 63-69.

Ganai, N. A., Yadav, B. R. and Mitra, A. (1999) : Genomic conservation of cattle microsatellite loci in goats. Abst. P.11. In a symposium ' Emerging DNA technologies for the next millennium' Held at CCMB/CDFD, Hyderabad, 23rd Feb. 1999.

Goldstein, D. B. and Pollock, D. D. (1997) : Launching microsatellite: A review of mutation processes and method of phylogenetic inferences. *J. Heredity*, 88: 335-342.

Hatzel, D.J.S. and Drink Water, R.D. (1992) : The use of DNA technologies for conservation and improvement of animal genetic resources. FAO Expert Consultation on the management of Global Genetic Resources, Rome April 1992.

Heyen, D. W.; Beever, J. E.; Da, Y.; Evert, R.E.; Green, C.; Bates, S. R. E.; Ziegle, J. S. and Lewin, H. A. (1997) : Exclusion probabilities of 22 bovine microsatellite markers in fluorescent multiplexes for semiautomated parentage testing. *Anim. Genet.* 28: 21-27.

Ivankovic, A.; Kavas, T.; Caput, T.; Mioc, B.; Panic, V. and Dorc, V. (2002) : Genetic diversity of three donkey populations in the Croation coastal region. *Anim. Genet.* 33, 169-177.

Jefferys, A. L.; Wilson, V. and Thesin, S. L. (1985) : Hypervariable " minisatellite" region in human DNA. *Nature*. 314: 67-73.

Jeffreys, A. J. and Morton, D. B. (1987) : DNA fingerprinting of dogs and cats. *Anim. Genet.,* 18: 1-15.

Jeffreys, A. J.; Royle, N. J.; Wilson, V. and Wrong, Z. (1988) : Spontaneous mutation rates to new length alleles at random-repetitive hypervariable loci in human DNA. *Nature* (London), 332: 278-281.

Jianlin, H.; Mburu, D.; Oching, J.; Kaufmann, B.; Rega, J. E. O. and Hanotte, O. (2000) : Application of New Word Microsatellite primer for amplification of polymorphic loci in Old Word Camelids. *Anim. Genet.* 31, 404-419.

Karl, S. A. and Avise, J. C. (1993) : PCR based assays of Mendalian

polymorphisms from anonymous single-copy nuclear DNA: Techniques and application for population genetics. *Mol. Biol. Evol.,* 10: 342-361.

Kemp, S. J.; Hishida, O.; Wamburger, J. (1995) : A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Anim. Genet.* 24: 363-365.

Kim, K. S.; Yeo, J. S. and Choi, C. B. (2002) Genetic diversity of north-east Asian cattle based on microsatellite data. *Anim. Genet.* 33, 201-204.

Krikpatrick, B. W.; Huff, B. M. and Cases-Carrillo, E. (1993) : Double strand DNA conformation polymorphism as a source of highly polymorphic genetic marker. *Anim. Genet.* 24: 155-61.

Kuhnlein, U.; Danee, Y.; Zadworny, D. and Ganora, J. S. (1989) : DNA fingerprint a tool for determining genetic distances between strains of poultry. *Theor. Appl. Genet.* 71: 669-672.

Lang, K. D. M.; Wang, Y.; and Plante, Y. (1996) : Fifteen polymorphic dinucleotide microsatellites in Llamas and Alpacas. *Anim. Genet.* 27, 293.

Ledbetter, S. A.; Nelson, D. L. (1991) : Genome amplification using primers directed to interspersed sequences (IPR-PCR). In: PCR: A Practical Approach, pp. 107-119.

Lewin, B. (1997) : In: Genes *VI*. Oxford University Press.

Lindgren, G. (2000) : Ten equine dinucleotide microsatellite repeats HTG 18-19, HTG 22-24, HTG 26-27, HGT 29-30 and HTG 32.

Anim. Genet. 31 (2) 145-146.

Litt, M. and Luty, J. A. (1989) : A hypervariable microsatellite reveled by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44: 397-401.

Lubieniecka, J.; Grzybobowski, G.; Lubieniecki, K. and Zurkowski, M. (2000) : Microsatellite based genetic diversity in the Pollish Red cattle population kept under the national prevention programme. *Animal Science Paper and Reports* 18 (4) 227-236.

Mac. Pherson, J. M.; Eckstein, P. E.; Soles, G. J. and Gajadhar, A. A. (1993) : Molecular and cellular probes. 7: 293-299.

Maddox,J. F.; Reffkin, C. D.; Beh,K. J. (2000) : Dinucleotide repeat polymorphism at Ovine MCMA-1, MCMA-2, MCMA-5, MCMA-8, MCMA-9, MCMA-11, MCMA14, MCMA20, MCMA-24, MCMA-26 loci. *Anim. Genet.* 31 (2) 148-149.

Mehta, S. C. (2000) : Molecular Markers for Genetic Characterisation of *Camelus dromedarius*. Ph.D. Thesis submitted to the Rajasthan Agriculture University, Bikaner.

Meselson, M. and Yuan, R. (1968) : DNA restrictive enzyme from E. coli. *Nature*, 217 : 1110-1114.

Mishra, B. P.; Tandon, S. N. and Khanna, N. D. (1998a) : Genetic variation in Indian dromedary using random amplified polymorphic DNA (RAPD) technique. *The International Meeting on camel production and future perspective.* May 2-3,1998. Al Ain, UAE.

Anim. Genet. 31 (2) 145-146.

Litt, M. and Luty, J. A. (1989) : A hypervariable microsatellite reveled by bin vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44: 397-401.

Lubieniecka, J.; Grzybobowski, G.; Lubieniecki, K. and Zurkowski, M. (2000) : Microsatellite based genetic diversity in the Pollish Red cattle population kept under the national prevention programme. *Animal Science Paper and Reports* 18 (4) 227-236.

Mac. Pherson, J. M.; Eckstein, P. E.; Soles, G. J. and Gajadhar, A. A. (1993) : Molecular and cellular probes. 7: 293-299.

Maddox,J. F.; Reffkin, C. D.; Beh,K. J. (2000) : Dinucleotide repeat polymorphism at Ovine MCMA-1, MCMA-2, MCMA-5, MCMA-8, MCMA-9, MCMA-11, MCMA14, MCMA20, MCMA-24, MCMA-26 loci. *Anim. Genet.* 31 (2) 148-149.

Mehta, S. C. (2000) : Molecular Markers for Genetic Characterisation of *Camelus dromedarius*. Ph.D. Thesis submitted to the Rajasthan Agriculture University, Bikaner.

Meselson, M. and Yuan, R. (1968) : DNA restrictive enzyme from E. coli. *Nature,* 217 : 1110-1114.

Mishra, B. P.; Tandon, S. N. and Khanna, N. D. (1998a) : Genetic variation in Indian dromedary using random amplified polymorphic DNA (RAPD) technique. *The International Meeting on camel production and future perspective.* May 2-3,1998. Al Ain, UAE.

Mishra, B. P.; Tandon, S. N. and Khanna, N. D. (1998b) : DNA fingerprinting in camels (Camelus dromedarius) using microsatellite oligo probes. *The International Meeting on camel production and future perspective.* May 2-3,1998. Al Ain,UAE.

Mishra, B. P. (2002) : Genetic variation in Indian camels using Microsatellite markers. *National Bureau of Animal Genetic Resources, Annual Report,* 2001-2002.

Mitra, A. (1994) : Studies on restriction fragment length polymorphisms in cattle and buffalo. Ph. D. Thesis, NDRI Deemed University, Karnal.

Moazami-Goudarzi, K.; Laloe, D.; Furet, J. P. and Grosclande, F. (1997) : Analysis of genetic relationship between 10 cattle breeds with 17 microsatellites. Anim. Genet. 23: 338-345.

Nakamura, Y.; Leppert, M.; O'Connell, P.; Wloff, R.; Holm, T.; Culver, M.; Martin, C.; Fujimoto, E.; Hoff, M.; Kumlin, E. and White, R. (1987) : Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science*, 235: 516-522.

Navani, N.; Jain, P. K.; Gupta, S.; Sisodia, B. S. and Kumal, S. (2002) : A set of cattle microsatellite DNA markers for genome analysis of rivirne buffalos (Bubalis bubalis) *Anim. Genet.* 33, 149-154.

Nei, M and Roychoudhary, A. K. (1974) : Sampling variance of heterozygisity and genetic distance. *Anim. Genet.* 76: 379-390.

Nichlos, W. C.; Liepnicks, J. J.; Mckusick, V. A. and Benson, M. D. (1989) : Direct sequencing of the gene for Maryland/German

familial amylodotic polyneuropathy type II and genotyping of allele-specific enzymatic amplification. *Genomics*, 5: 535-540.

Obreque, V; Coogle, L; Henney, P. J; Bailey, E; Mancilla, R; Garcia-Hinrichsen, P and Cothran, E. G (1998) : Characterization of 10 polymorphic alpaca dinucleotide microsatellite. *Anim. Genet.* 29, 461.

Orita, M.; Suzuki, Y.; Seika, T. and Hayashi, K. (1989) : Rapid and sensitive detection of point mutation and DNA polymorphisms using the polymerase chain reaction. *Genetics*. 5: 874-9.

Oudet, C. ; Heilig, R.; Hanaher, A. and Mandel, J. L. (1991) : Non radioactive assay for new microsatellite polymorphism at the 5' end of the dystrophin gene and estimation of intragenic recombination. *Am. J. of Hum. Genet.* 49: 311-319.

Panner, G. A.; Bush, A. and Wise, R. (1993) : Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. In: PCR Methods and Application Cold Spring *Harbor Laboratory Press, pp.* 341-345.

Penedo, M. C. T; Caetano, A. R. and Crdova, K. (1998) : Eight microsatellite markers for South American camelids. *Anim. Genet.* 30, 161-168.

Pepin, L.; Amigues, Y.; Lepingle, A. (1995) : Sequence conservation of microsatellites between cattle, goat and related species. Examples of use in parentage testing and phylogeny analysis. *Heredity*, 74: 53-61.

Poelman, L. J.; Mortiaux, F.; Zeveren, A. V.; Danscrcoci, A.; Mommens, G.; Coopman, F.; Bouquet, Y.; Burny, A.; Renaville, R. and Portetelle, D. (1998) : Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breeds. *Anim. Genet.* 29: 161-167.

Prescott, L. M.; Harley, J. P. and Klein, D.A. (1993a) : *Microbial Taxonomy*. In: Microbiology (2nd edn.) pp. 404-425. WCB Publisher.

Rai, A. K.; Roy, A. K. and Khanna, N. D. (1992) : Speed and strides of different breeds of camel. *Indian Journal of Animal Science*, 62 (1) 91-92.

Rassmann, K.; Schlotterer, C. and Tautz, D. (1991) : Isolation of simple sequence loci for use in PCR based DNA fingerprinting. *Electrophoresis*, 12: 113-118.

Rathore, G. S. (1986) : Camels and their management. ICAR publication, New Delhi. Report of the camel Development Committee (1988) Goven. of India Ministry of Agriculture, Department of agriculture and cooperation, Animal Husbandry Division.

Rieder, S.; Barlocher, H. U.; Jorg, H.; Hagger, C.; and Stranzinger, G. (2000) : Characterisation of Swiss new-world-camelidae breeds by microsatellites. *Schweizer Archiv fiir Tierheilkunde*. 142 (3) 120-125.

Ritz, L. R. Glowatzki-mullis, M. L.; Machogh, D.E. and Gaillard, C. (2000) : Phylogenetic analisis tribe Bovini using microsatellites.

Anim. Genet. 31 (3) 178-185.

Ron, M., Blanc, Y, Band, M., Erza, E. and Weller, J. I. (1996). : Misidentification rate in the Israeli dairy Cattle population and its implications for genetic improvement. *J. Dairy Sci.* 79: 678-681.

Rosbash, M.; Ford, P. T. and Bishop, J. O. (1974) : Analysis of the C-value paradox by molecular hybridization. Proc. *Natl. Acad. Sci. USA*. 71: 3746-3750.

Sahai, R. and Vijh, R. K. (1993) : *Cytogenetics of camel*: a monograph. Published by National Institute of Animal Genetics, Karnal and National Research center on camel, Bikaner.

Sahani, M. S.; Rathinasabapathy, M.; Gorakhmal and Khanna, N. D. (1998) : Milking techniques and other factors affecting milk production potential in different breeds of camels under farm condition. *Indian Journal of Animal Science*. 68 (3) : 54-56.

Sahani, M. S.; Sharama, N. and Khanna, N. D. (1996) : Hair production in Indian camels (*Camelus dromedarius*) managed under farm condition. *Indian Veterinary Journal*. 73 (5): 531-33.

Saiki, R. K.; Gelfand, D. H.; Stoffel , S.; Scharf, S. J.; Higuchi, R.; Horn, G. T.; Mullis, K. B. and Erlich, H. A. (1988) : Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Nature* (London), 239: 487-497.

Sambrook, J.; Fritsh, E. F. and Manities, T. (1989) : Molecular cloning: A Laboratory Manual. Cold spring Harbour Laboratory Press (2nd edn.). New York.

Saperstein, D. A. and Nickerson, J. M. (1991) : Restriction fragment length polymorphism analysis using PCR coupled to restriction digests. *Biotechniques*, 10: 488-489.

Sarkar, G.; Cassady, J.; Bottema, C. D. K. and Sommer, S. S. (1990) Characterisation of polymerase chain reaction amplification of specific alleles. *Anal. Biochem.*, 186: 64.

Skinner, D. M.; Beattic, W. G.; and Blattner, F. R.; (1974) : The repeat sequence of an Hermit crab satellite DNA is $(T\text{-}A\text{-}G\text{-}C)_n$ X $(\text{-}A\text{-}T\text{-}C\text{-}C\text{-})_n$, Biochemistry, 13: 3930-3937.

Slate, J.; Coltman, D. W.; Goodman, S. J. (1998) : Bovine microsatellite loci are highly conserved in red deer (*Cervus elaphus*), sika deer (*Cervus Nippon*) and Soya sheep (*Ovis aries*). *Anim. Genet.* 39: 307-315.

Soller, M. and Beckmann, J. S. (1982) : RFLP and genetic improvement in: Proc. 2nd world Cong. Genetics Applied to livestock production, Madrid 6: 396-404

Southern, E. M. (1975) : Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, 98: 503-517.

Stallings, R. L.; Ford, A. F.; Nelson, D.; Torney, D. C.; Hildebrand, C. E. and Moyzir, R. K. (1991) : Evaluation and distribution of $(GT)_n$ repetitive sequences in mammalian genomes. *Genomics*. 10 : 807-815.

Tandon, S. N. (1998) : Studies on blood group and biochemical

polymorphism in Indian camels. *The International Meeting on camel production and future perspective.* May 2-3,1998. Al Ain,UAE.

Tandon, S. N.; Kasturi, M.; Raisinghani, G. and Khanna. N. D. (1997a) : Protein polymorphism in Indian camel. *Indian Veterinary Journal.* 74 (6): 533-34.

Tandon, S. N.; Raisinghani, G.; Kasturi, M. and Khanna. N. D. (1997b) : Electrophoretic studies of certain red cell enzymes in dromedarius. *Indian Veterinary Journal.* 74 (6): 535.

Tautz, D. (1989) : Hypervariable of simple sequences as a general source of polymorphic DNA markers. *Nucl. Acids Res.*, 17: 6463-6471.

Tautz, D. and Renz, M. (1984) : Simple sequences are ubiquitous repetitive compounds of eukaryotic genomes. *Nucl. Acids Res.*, 12: 4127-4138.

Todd, J. A.; Altman, T. J.; Cornall, R. J.; Ghosh, S.; Halh, J. R. S.; Hearne, C. M.; Knight, A. M.; Lovein, J. M.; McAleer, M. A.; Prins, Pressy, A.; Delarato, N. H.; Peterson, L. B. and Wicker, L. S. (1991) : Genetic analysis of auto immune type I dibetes mellitus in mice. *Nature,* 357: 542-547.

Tozaki, T.; Kakoi, H.; Mashima, S.; Hirota, K.; Hasegawa, T.; Ishida, N.; Miura, N.; Tomita, M. (2000) : The isolation and characterisation of 34 equine microsatellite loci, TKY 290- TKY 323. *Anim. Genet.* 31 (3) 234-236.

Van Hooft, W. R.; Hanotte, O.; Wenink, P. W.; and Greon, A. R. and

Sugimoli, Y. (1999) : Applicability of bovini microsatellite markers for population genetic studies on African buffalo (Syncerus caffer). *Anim. Genet.* 30, 214-220.

Weber, J. L. and May, P. E. (1989) : Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44: 388-396.

Welsh, J. and McClelland, M. (1990) : Fingerprinting genomes using PCR with arbitrarily primers. *Nucleic Acids Res.* 20: 303-306.

Williams, J. G. K.; Kubbelik, A. R.; Livak, K. J.; Rafalski, J. A. and Tingey, S. V. (1990) : DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-35.

Wu wei; Wang Dong and Cao Hanhe (2000) : Genetic structure of five Chinese and foreign cattle breeds using microsatellite DNA markers. Journal of Jilin Agricultural University. 22 (4) 5-10.

Yadav, B. R.; De, S. and Mitra, A. (1998) : Bovine microsatellite primers reveal DNA polymorphisms in river buffalo. In the Proc. Indo-Brazilian Workshop on Agricultural Biotechnology. Held at NDRI, Karnal, April 27-29, 1998, P.26.

Genetic Characterisation of Jaisalmeri Camel Using Microsatellite Markers

M. V. Sc. Thesis,

Department of Animal Breeding and Genetics,
College of Veterinary and Animal Science,
Rajasthan Agricultural University,
Bikaner – 334001.

Submitted by : **Lokesh Gautam**

Major Advisor : **Dr. R. S. Gahlot**

ABSTRACT

Eleven microsatellite primers were employed to investigate the genetic characterisation in Jaisalmeri camel. Eleven microsatellite primers were tried for amplification on 30 unrelated camels.

Polymerase chain reaction was carried out using eleven microsatellite primers for amplification of microsatellite loci. Denaturing sequencing gel electrophoresis was used to separate the PCR amplified products. The gel was stained with silver nitrate procedure. The allele frequency, expected heterozygosity and Polymorphic information content were scored.

The six microsatellite primers amplified polymorphic DNA bands in Jaisalmeri camel. The number of alleles ranged 2 to 5. The expected heterozygosity ranged from 0.32 (YWLL–09) to 0.651 (VOLP–10). The polymorphic information content ranged from 0.268 (YWLL–09) to 0.588 (VOLP–10) on the basis of our observation, it was revealed that these microsatellite primers will be of great use in characterisation and documenting the Jaisalmeri camel.

जैसलमेरी उष्ट्र में आनुवंशिक चरित्रण हेतु माइक्रोसेटेलाइट चिन्ह का अध्ययन

स्नातकोत्तर शोध ग्रंथ
पशु प्रजनन एवं आनुवंशिक विभाग
पशु चिकित्सा एवं पशुविज्ञान महाविद्यालय
राजस्थान कृषि विश्वविद्यालय
बीकानेर ।

अग्रेषक : **लोकेश गौतम**
मुख्य उपदेष्टा : **डॉ0 आर0 एस0 गहलोत**

सारांश

जैसलमेरी उष्ट्र में आनुवंशिक चरित्रण के अध्ययन के लिए माइक्रोसेटेलाइट डी. एन. ए. का विश्लेषण किया गया । तीस असम्बंधित उष्ट्र पर 11 माइक्रोसेटेलाइट डी. एन. ए. का परिक्षण किया ।

ग्यारह माइक्रोसेटेलाइट बिन्दुपथों का विस्तार पोलीमरेज चैन रिएक्शन द्वारा किया गया । पोलीमरेज चैन रिएक्शन विस्तारित उत्पाद के पृथ्थकरण के लिए विघटित सिक्वेन्सिंग जेल इलेक्ट्रोफोरेसिस का उपयोग किया गया । जेल को सिल्वर नाइट्रेट प्रक्रिया द्वारा स्टेन किया । एलिल फिक्वेन्सि, अनुमानित विषमताएँ, पोलिमोरफिक इन्फोरमेशन कन्टेन्ट का अध्ययन किया ।

जैसलमेरी उष्ट्र में छः माइक्रोसेटेलाइट डी. एन. ए. द्वारा विस्तारित बहुप्रकारीय बैंड पाये गये । एलिल की संख्या 2 से 5 पायी गयी । अनुमानित विषमताएँ 0.32 (वाइ डब्ल्यू एल एल – 09) से 0.651 (वी ओ एल पी–10) पायी गयी । पोलिमोर्फिक इन्फोमेशन कन्टेट 0.268 (वाई डब्ल्यू एल एल – 09) से 0. 588 (वी ओ एल पी–10) पायी गयी ।

सारे निरिक्षण के आधार पर पाया गया कि यह माइक्रोसेटेलाइट डी. एन. ए. जैसलमेरी उष्ट्र के आनुवंशिक चरित्रण में उपयोगी सिद्ध हुए ।

COMPOSITION OF BUFFERS AND REAGENTS

GENOMIC DNA ISOLATION

1M Tris (pH 7.5)

Tris base	121.1 g
H_2O	800 ml

5M NaCl

NaCl	292.2 g
Sterile dH_2O	800 ml

1M $MgCl_2$

$MgCl_2.6H_2O$	203.3 g
Sterile dH_2O	800 ml

10% SDS Solution

Sodium dodesyl sulphate	5g
Sterile distilled water	50 ml

0.5 M EDTA (pH 8.0)

Disodium ethyle tetra acetic acid 0.2 H_2O	186.1 g
Sterile dH_2O	800 ml

Stir on magnetic stirrer, adjust pH to 8.0 with NaOH

Solution-I

Tris pH 7.6	10 mM
KCl	10 mM
$MgCl_2$	10 mM

Solution –2

Tris pH 7.5	10 mM
KCl	10 mM
$MgCl_2$	10 mM
NaCl	0.5 M
SDS	0.5%
EDTA	2 mM

Tris Saturated phenol

Crystalline phenol was redistilled twice at 160°C and cooled at room temperature. 8 hydroxyquinoline (0.1% w/v) was added and extracted several times with an equal volume of Tris buffer (1M Tris-HCl, pH 8.0 followed by 0.1 M Tris-HCl, pH 8.0 and 0.2% β-mercaptoethnol). The tris-saturated phenol was stored at 4 °C layering with 0.1 M Tris-HCl, pH 8.00.

Phenol : Chloroform (1:1)

Tris saturated phenol mixed with equal volumes of chloroform (Chloroform : Iso-amyl alcohol :: 24 :1)

AGAROSE GEL ELECTROPHORESIS BUFFERS/DYE

5 X TRIS BORATE EDTA (TBE) BUFFER

Tris Base	54 g
Boric Acid	27.5 g
0.5 EDTA pH 8.0	20 ml
Sterile distilled water	up to 1000 ml

ETHIDIUM BROMIDE SOLUTION (10MG/ML)

Ethidium bromide	0.2 g
Sterile dH_2O	20 ml

Mix well, store 4°C in dark, handle with gloves and avoid inhalation.

POLY ACRYLAMIDE GEL ELECTROPHORESIS

6 % DENATURING PAGE SOLUTION

Urea	84 g
Acrylamide	11.4 g
Bis-Acrylamide	0.6 g
10 X TBE	20 ml
Sterile dH_2O	up to 200 ml

AMMONIUM PER SULPHATE (10%)

Dissolve in 1 g in 10 ml of dH_2O. Make fresh and store at 4°C.

TEMED

40 µl in 100ml of PAGE solution

2X FORMAMIDE DYE

Formamide	4.75 ml
1M NaOH	50 µl
0.5 M EDTA	200 µl
Bromophenol dye	1 mg
Xylene Cynol	1 mg

Bind Silane

Glacial Acetic Acid	5 µl
γ-metha cryloxypropyl Trimethoxy silane	3 µl
Absolute Alcohol	up to 1000 µl

MOLECULAR WEIGHT MARKER

50 bp Ladder

Number of fragments : 13

Size of the fragments : 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, and 50 bp.

ABBREVIATIONS

OD	Optical Density
EDTA	Ethylene Diamine Tetra Acetic acid
TBE	Tris-Borate EDTA
TE	Tris EDTA
WBC	White Blood Cell
RBC	Red Blood Cell
DNA	Deoxyribo Nucleic Acid
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
PAGE	Poly Acrylamide Gel Electrophoresis
w/v	Weight / Volume
V	Volt
cm	centimeter
nm	nanometer
ml	milliliter
µl	micro liter
kb	kilo base
bp	base pair
pmol	picomole
mM	millimole
U	Unit
A	Absorbance
°C	Degree centigrade
Fig	Figure
g	gram
mg	milligram
rpm	rotation per minutes