

Vol. 11 No. 1

January – June 2014

Mithun Digest

The bi-annual panorama.....

National Research Centre on Mithun



INSTITUTIONAL ACTIVITIES

Republic day celebration

The 67th Republic Day of the Nation was celebrated in the Institute on 26 January, 2014 in a befitting manner with a pledge to work for the benefit of the motherland. The Director unfurled the tricolor in the morning with the singing of National Anthem and encouraged all the staff to work with a purpose and remain ever vigilant for safeguarding the reputation of the Institute in particular and the nation in general. The children and family members also took active part in this national celebration.



Institute Research Council Meeting

The Institute Research Council (IRC) Meeting was held at the meeting hall of the Institute on 22nd February, 2014 under the chairmanship of the Director, Dr. Chandan Rajkhowa. Dr. R. Roychoudhury, Professor & Head, LPM; Dr. Arnab Sen, Head & Pr. Scientist, Animal Health; Dr. P.C. Sarmah Professor & Head, Dept. of Parasitology; Dr. K.K. Baruah, OSD & I/C Associates, DEAN, College of Fisheries; Dr. K.C. Nath, Professor, Dept. of Gynaecology also attended the meeting as external experts and gave valuable inputs for refinement of the ongoing research work of the Scientists.

Mega Event – Silver Jubilee Celebration and Mithun Festival



All the staff members of the Institute led by the Director, Dr. Chandan Rajkhowa celebrated the Institute Silver Jubilee on 27th March, 2014 followed by Mithun Festival on 28th March, 2014 at Porba, Phek District with pomp and gaiety. A galaxy of dignitaries including Sri Yitachu, Parliamentary Secretary, Vety & A.H., Govt of Nagaland; Dr. Benjongliba Aier, Parliamentary Secretary, Dept of Agriculture, Govt of Nagaland; Dr. S. Ayyappan, Hon'ble Secretary, DARE & DG, ICAR; Prof. K. M. L.Pathak, DDG

(AS); Dr. B. S. Prakash, ADG (AN & P). A Technology Exhibition showcasing various technologies developed by the Institute and mithun value added products was also organized on this occasion participated by many self-help groups and NGOs. An interface meeting was held with the scientists and various stake-holders of mithun husbandry. It was a matter of great achievement as the Institute completed 25 years of existence with a number of great scientific achievements in the field of mithun research.

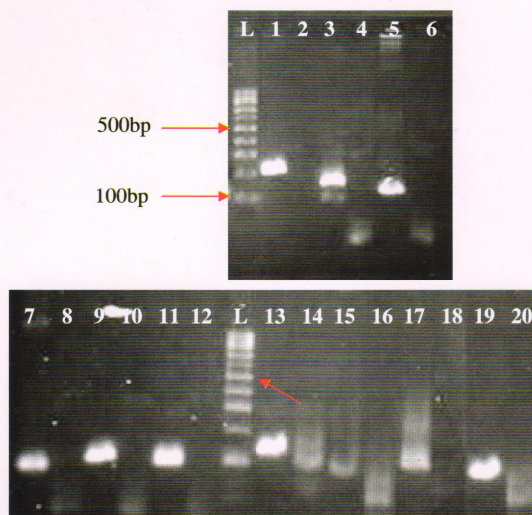


RESEARCH HIGHLIGHTS

Mithun specific Microsatellites - New Beginning in Mithun Genetics

Mithun, considered as the living wealth of the tribals of North Eastern Hill Region of India, needs immediate attention towards development of genetic markers for genetic diversity estimation in various mithun populations and implement genetic improvement programmes. Microsatellites as markers are very useful as they are highly polymorphic, co-dominant and multi-allelic in nature. In the absence of mithun specific microsatellite markers, the genetic characterization of mithuns was being done using cattle microsatellite markers. A breakthrough has been made by isolating and characterizing Mithun-specific microsatellites markers for use in characterization and biodiversity analysis. For

this purpose, genomic DNA was digested with a tetra-cutter restriction enzyme, ligated with 20bp adapters, denatured and hybridized to biotinylated microsatellite probes. Streptavidin coated Magnetic beads were added in this mixture for enrichment. This procedure separated fragments containing microsatellites. Having cloned the amplified products from these selected DNA using primers designed for the linkers, in suitable vector, products were sequenced. Primers for each microsatellite loci were designed using sequences. Out of the 50 microsatellites generated for mithun so far, eight were found to be Mithun-specific (Figure). Two of these new microsatellite sequences were submitted in GenBank (KJ458991- KJ458992). Further genotyping work was in progress (Sabyasachi Mukherjee, Anupama Mukherjee, Sanjeev Kumar, Sosang Longkumer, Moonmoon Mech, Kezhavituo, Kobu Khate, Chandan Rajkhowa)

Microsatellite Marker Validation versus Mithun and Thotho

Validation of 10 Microsatellite markers against Mithun and Thotho; loaded on 2% Agarose gel

Lane Description:

L – 100bp DNA Ladder

Arrow indicates 500bp band

1 - Marker1 with Mithun	11 – Marker63 with Mithun
2 – Marker1with Thotho	12 – Marker63 with Thotho
3 – Marker17 with Mithun	13 – Marker55 with Mithun
4 – Marker17 with Thotho	14 – Marker55 with Thotho
5 – Marker29 with Mithun	15 – Marker64 with Mithun
6 – Marker29 with Thotho	16 – Marker64with Thotho
7 – Marker13 with Mithun	17 – Marker69 with Mithun
8 – Marker13 with Thotho	18 – Marke69 with Thotho
9 – Marker18 with Mithun	19 – Marker 70with Mithun
10 – Marker18 with Thotho	20 – Marker 70 with Thotho

Application of FISH to find out unique features of Mithun Chromosomes

The eukaryotic chromosome is a conserved structure, with the DNA double-helix wrapping around octamers of histone proteins to form the chromatin which is further packaged into chromosomes. The centromere defines the kinetochore, the region of spindle microtubule attachment that pulls the two replicated chromatids of each chromosome apart during cell division. As with other bovidae, chromosome studies indicate that the gaur evolved from a wild ancestor, differentiating the domestic taurine and zebu cattle. In the present study the centromere specific probe were used for studying the chromosomal rearrangements in the sub metacentric autosomes. The clear fluorescent signal was observed on the centromeric region of all the metaphase chromosome. The signals were compared with the Cattle (*Bos indicus*), where also clear signals were captured on the centromeric region of the metaphase chromosome. The attempt is made to develop a set of molecular markers for mithun/ gaur assigned by FISH, and that can be applied as a tool in mithun/ gaur clinical cytogenetics.

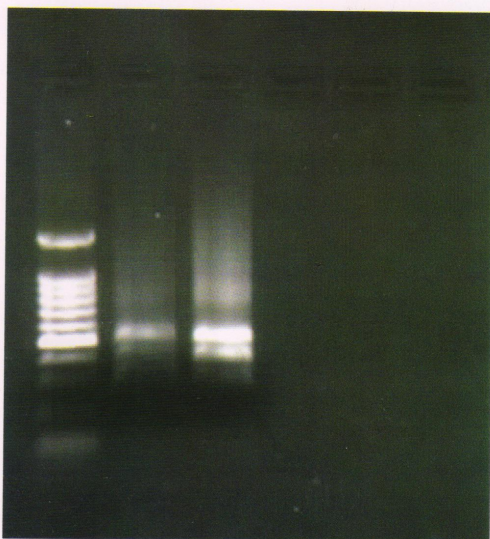


Figure. PCR Amplification of Centromeric region of Gaur as revealed under gel electrophoresis with ethidium bromide



Figure. Images taken for gel electrophoresis showing Labeled probe with fluorescent dyes

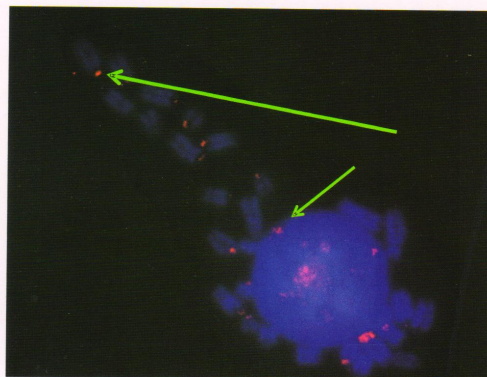


Figure. Fluorescent image of metaphase spread with interphase nuclei indicating bunches of centromeric signals all around the centromeric region in Indian Gaur.

Computer-assisted sperm analysis of mithun semen

The motility and velocity parameters of mithun sperm of good and poor quality ejaculates was assessed by computer assisted sperm analyser (CASA, Hamilton-Thorne Semen Analyser IVOS 11, USA). Fifty ejaculates (twenty five ejaculates each for good and poor quality) were collected from matured mithun bulls. Good and poor quality semen was selected based on the mass activity and individual motility of fresh semen. CASA parameter such as forward progressive motility (FPM) (%), total motility (TM) (%), curvilinear velocity (VCL) ($\mu\text{m}/\text{sec}$), straight line velocity (VSL) ($\mu\text{m}/\text{sec}$), average path velocity (VAP) ($\mu\text{m}/\text{sec}$), linearity (LIN) (%), straightness (STR) (%), wobble (WOB) (%), amplitude of

lateral head displacement (ALH) (μm) and beat/cross frequency (BCF) (Hz) were measured by CASA analyser. The result revealed that these parameters varied significantly ($P < 0.05$) between the good and poor quality semen and good quality semen has significantly ($P < 0.05$) higher value than poor quality ejaculates. This indicated that good quality sperm has structural stability than the poor quality sperm and that good quality sperm has higher functional sperm structures to move faster and forward direction. Moreover CASA system proved its usefulness in routine evaluation of mithun semen especially in frozen semen bank.

Effect of Low density lipoprotein on the liquid storage (5oC) of mithun (*Bos frontalis*) semen

The effect of low density lipoprotein (LDL) on replacement of whole egg yolk was assessed on sperm motility, viability, total sperm abnormality, acrosomal and plasma membrane integrity, nuclear abnormality, vanguard distance travelled by sperm in the bovine cervical mucus (BCMPT), enzymatic profiles such as aspartate amino transaminase (AST), alanine amino transaminase (ALT), lactate dehydrogenase (LDH) and biochemical profiles such as total antioxidant capacity (TAC), malonaldehyde (MDA) production and cholesterol efflux. These seminal parameters, enzymatic and biochemical profiles were assessed at 5oC for 10 hr of incubation. Inclusion of LDL into diluent resulted in a significant ($p < 0.05$) decrease in percentages of dead, abnormal spermatozoa, acrosomal and nuclear abnormalities as compared with control group. Additionally, LDL at 8% has significant ($p < 0.05$) improvement in quality of mithun semen than LDL at 10% and control stored in in- vitro for 10 hr. The possible protective effects of LDL on mithun sperm parameters was revealed as it protects sperm cells, prevents MDA production and preserve the intracellular enzymes and antioxidants during preservation.

Molecular characterization of *Homalogaster paloniae* from buffaloes of north eastern region based on spacer region of ITS-2

The second internal transcribed spacer (ITS-2) region of *Homalogaster paloniae* sequence analysis was carried out to perceive the occurrence of intra-specific variations within the species. The adult flukes collected from rectum of buffaloes were identified as *H. paloniae* based on

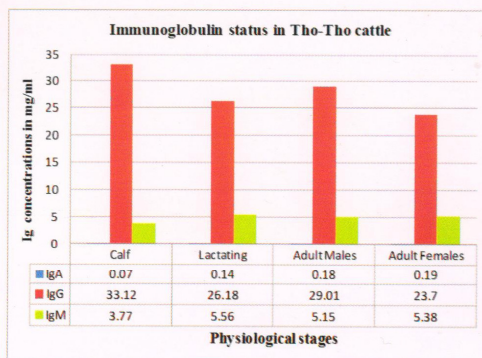
morphological criteria. ITS-2 was used as a genetic marker for identification and genotypic analysis of the parasite. PCR targeting conserved regions of ITS-2 yielded a 516 bp fragment which on sequencing showed 100% similarity with that of *H. paloniae*. Phylogenetic studies by neighbour joining method revealed close relationship with *Gigantocotyle explanatum*, *Paramphistomum epiclitum* and *Calicophoron calicophorum*



Figure: PCR amplification of ITS-2 region of *H. paloniae*. (Lane 1-4: PCR amplification showing 516 bp amplicons; Lane M: 100bp plus DNA ladder)

Estimation of plasma immunoglobulin concentration under different physiological stage in Tho-tho cattle

The highest concentration of Immunoglobulin A was found in adult female and lowest in calves under 1 years of age. The immunoglobulin G concentration was recorded highest in calves while lowest in adult females. The plasma concentration of immunoglobulin M was found highest in lactating females while lowest in calves.



PERSONALIA**Transfer/Joining/Promotion**

- Dr. Sabyasachi Mukherjee, promoted from Sr. Scientist to Principal Scientist (AGB) w.e.f. 16.01.2014.

Visiting Dignitaries

- Dr. Tuminiu Adebambo – C.V.Raman International Scholar delivering the research work conducted at NRCM on 2 January 2014.



- Students from Pranab Jubilee College, Dimapur visited the Institute on 28 June, 2014 on an educational tour.

Regular farm practices in NRCM farm, Medziphema

The Institute farm is maintaining mithuns of four different strains viz. Nagaland, Manipur, Mizoram and Arunachal. Basically, mithun is a forest loving animal and prefer to browse on tree leaves rather than grazing. Mithun in farm condition however, live in different way as they are fed with mixture of concentrate feed ingredients and other highly nutritive feedstuff. All the green roughages and hay are offered to mithun after chaffing only which has been found more economic as wastage becomes very less and intake also increased. Recently a new grass, Congo grass which has its origin from Congo of Africa has been sown as mithun forage. The merits of this grass is that, it is very easy to cultivate without much maintenance requirement as it can dominate other grasses. The grass can be harvested thrice in a year and

good for hay preparation. More emphasis is given for raising of calves by keeping similar age group together in shed with standard feeding regime which has improved growth and lessened calf mortality.



Technology Injection Programme

Technology Injection Programme under TSP at Mizoram

A Technology Injection cum input distribution programme was organized by Dr. Sabyasachi Mukherjee, PS in association with Dept. of Vety & A.H., Govt. of Mizoram at distant Chhualung village under Saiha district of Mizoram on 24 June, 2014. A total of 56 mithun farmers have participated in this programme. The farmers were given awareness and scientific information related to

profitable mithun husbandry practices through distribution of published literature covering various aspects of mithun husbandry. The importance of age determination through dentition of mithun, AI, prevention of disease through vaccination and deworming were explained to the mithun owners. Inputs viz. stationary items, salt, mineral mixture and ropes for handling mithun were also distributed.

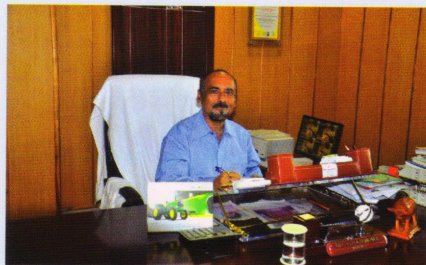


From the Director's Desk

It gives me immense pleasure to present this document of Newsletter of our Institute for the period January to June 2014. We have already published ten volumes of this document with wide circulation. Due to this wider circulation, in Mithun inhabited areas; we are receiving lots of queries in regard to various aspects

in Mithun husbandry from the people who are engaged in Mithun rearing. I must congratulate the editor for making this document attractive and popular.

During January to June 2013, the Scientist could generate many valuable information in regard to various aspects of Mithun husbandry. The development of mithun specific microsatellite markers is one of the most important advances in mithun genetics which will be very useful towards genetic diversity estimation and genetic improvement programme of this unique species. Other studies mainly application of FISH to find out unique features of mithun chromosomes, computer assisted mithun semen analysis work and immunoglobulin estimation in mithun are quite notable and sure to find out new information




on this species.

During this period, we have celebrated the Mega Events - Silver Jubilee of the Institute on 27 March 2014 at Jharnapani followed by Mithun Festival at Porba on 28 March 2014. Both the programme were attended by a host of VIP dignitaries including Dr. Ayyappan, Secretary DARE and Director

General, ICAR; Prof. K.M.L. Pathak, Deputy Director General (AS); Dr. B. S. Prakash, Asstt. Director General (AN&P); Directors of ICAR Institutes of NEH Region; Officers from State Vety & AH Depts. etc. We have also successfully conducted IRC meetings where experts of different field of studies visited our Institute. The proceedings of those meetings helped us to refine our research programmes.

Lastly, I pray to Lord Almighty to bless us all with knowledge, wisdom and skill so that we can deliver maximum for the benefits of tribal Mithun rearers.


(C. Rajkhowa)
Director

The editorial escapade



While the world was celebrating the beginning of a New Year 2014, we the lesser mortals were busy in this mithun country in the labs with new exploration of Mithuns and the scientists all over the world in their new discoveries and inventions! We could develop a new set of mithun specific microsats, analysed mithun semen with CASA and estimated the immunoglobulin level in the cattle.

The United Nations has declared 2014 the International Year of Family Farming and Crystallography. A study published in Nature in 1st January shows that the role of cloud formation in climate change has been underestimated. As a result, global temperatures could increase by 4 °C by 2100 and possibly 8 °C by 2200, thereby affecting terribly one-fifth of 720 UNESCO Heritage Sites world over due to rising sea level.

While Illumina, Inc., the noted sequencing pioneer company has demonstrated the first \$1,000 genome, a new gene therapy technique has restored the sight of six patients who would otherwise have gone blind. The leech *Ozobranchus jantseanus* has been shown to survive for 24 hours at -196 °C and for nine months at -90 °C, a finding that could yield insights into

cryopreservation for humans.

British agriculture scientists have developed genetically modified potatoes capable of resisting blight, while a new method to obtain human-induced pluripotent stem cells (hiPSCs) from a single drop of finger-pricked blood is achieved.

In genetics field, geneticists have created the first synthetic chromosome for integration into a yeast cell and demonstrated in its first evidence that CRISPR can reverse disease symptoms in living animals. Using this new gene-editing technique, MIT researchers cured mice of a rare liver disorder. While Cell Biologists created new human embryonic stem cells by fusing DNA from an adult with an enucleated egg cell, a form of human cloning. The sequencing of the tsetse fly genome, which causes the deadly sleeping sickness in Africa, is completed after a 10-year multimillion-dollar effort.

This Newsletter highlights the activities of the Institute as well as other interesting science bits in abridged manner and every efforts are made to include the information about the Institute and the services done for the mithun and their owners. If this proves to be helpful and serves the purpose, then our editorial efforts would be worthy to be cited.



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