

Cytogenetic analysis detects presence of Riverine, Swamp and their hybrid buffaloes in North-east India

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ABSTRACT

There is still not a settled view about the centre of domestication of buffaloes. Different workers have proposed different centres of domestication including western India, south-east China or South China. North eastern part of India constitutes the dividing line of these proposed centres of domestication of buffaloes. We, thus, presumed that this region should form the dividing boundary between domesticated riverine buffaloes and semi-domesticated swamp buffaloes. A total of 158 representative buffaloes belonging to six different regions of North East India i.e. Assamese-upper (Brahmaputra valley, 46), Assamese-Lower (Silchar, 18), Assamese-North (Dibrugarh, 26) Nagaland (12), Manipur (40) and Mizoram (16) were evaluated cytogenetically to ascertain their riverine/swamp status. The results revealed the existence of domesticated forms of all varieties of water buffalo i.e. river, swamp and river X swamp hybrid types in close geographic proximity. Of the six buffalo populations investigated in the study, one was predominantly pure riverine (upper-Assamese), four were pure swamp (Assamese-North, Manipuri, Nagaland and Mizo) and one was significantly hybrid (44.4%) of river X swamp (Lower-Assamese). The north-east region of India, thus, constitutes the demarcation line between swamp (inhabitants of south-eastern countries) and riverine (Major buffalo populations of India, Pakistan, Sri-Lanka, etc.) buffaloes.

Key words: Centre of domestication, domesticated, riverine, swamp, geographic proximity

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INTRODUCTION

Since their domestication about 5000 years ago, water buffalo has assumed a significant position amongst domestic animals due to their remarkable contribution in terms of milk, meat and draught power in several countries. They provide more than 5% of the world's milk supply. Their milk is remarkably affluent, containing less water and more fat, lactose, and protein as compared to cow milk. It is used to make butter, butter oil (ghee), better quality cheeses, and a variety of other products. Buffalo meat is especially tender and appetizing and is hard to discriminate from beef. Their hides also are of substantial significance as they make outstanding leather products. They are amazing creatures of burden throughout much of their range. Water buffalo are considered comparable to tractors in Southeast Asia, providing around 20% to 30% of farm power. Additionally, they also serve as means of transportation and can pull heavier loads than cattle, and their dung is collected and used as fire cakes or manure. Water buffalo eat less digestible feeds than cattle making them easier to maintain using locally accessible roughages. In addition, water buffalo are used as cash--to be sold whenever the need arises; thus securing the financial standing of many families. Lastly, water buffalo are also important for some hunting

business (Nowak, 1999; Singh, et al., 2000; Shackleton and Harestad, 2003).

Water buffalo are naturally found in tropical and subtropical forests, marshes, wet grasslands and swamps. The natural habitats of water buffaloes classically include rivers, streams, mud holes, tall grasses and trees which provide sufficient drinking and wallowing water, food and coverage. Asian water buffalo (*Bubalus bubalis*) has been chiefly domesticated and thus is now prevalent in many parts of the world. The purported native domain of *B. bubalis* was believed to extend from Central India to southern Nepal in the west to Vietnam and Malaysia in the east. It is believed that representative wild populations still exist in parts of India, Nepal, Bhutan, and Thailand. Domesticated and/or feral populations are very widespread. River buffalo, a domesticated form, is found more in the west and populate in Indochina, the Mediterranean, and parts of South and Central America. Swamp buffalo, another variety under domestication, is more easterly in distribution and is found in Indochina and Southeast Asia as well as Australia (Gurung and Singh, 1996; Nowak, 1999; Singh et al., 2000).

The world buffalo population is approximately 177 million spread in some 41 countries. Preponderance of these, 171 million, approximately 96.6%, exists in Asia,

and only 3.4% are distributed in rest of the world (FAO 2008). India alone harbours 98.7 million buffaloes constituting about 55.7% of the total world buffalo population. In South America there is a large population of swamp buffalo/river buffalo hybrids, because many of the buffaloes were imported from India where river buffalo are predominant, and Australia, which has a high population of swamp buffalo. These 177 million water buffalo represent only 11.3% of the world's total bovid population, but more people depend on the water buffalo than on any other domesticated species in the world (Scherf 2000). As such, unlike other domesticated bovids, the water buffalo population has enlarged by about 1.13% per year world-wide during the last 10 years.

Divergence of river buffalo and the swamp buffalo occurred approximately 10,000 to 1.7 million years ago, long before domestication (Kierstein et al., 2004; Kumar et al., 2007). Therefore, it is likely that there were distinct domestication events for river buffalo in India and for swamp buffalo in China. The genetic diversity of Indian buffaloes is characterized by 12 well recognized breeds besides many lesser known, hitherto unexplored populations of significant local importance. Most of these breeds/populations are distributed in the mainland of India covering all regions (North, South, East, West and Central parts). Apart from these, there are several buffalo populations in North Eastern states with unique morphological and behavioural characteristics. Most of these buffaloes of North East India resemble swamp type like Toda and Chilika buffaloes in their appearance, although no genetic evidence is available except for some limited karyological analysis based on limited samples (Yadav et al., 1998). Hence the present study was undertaken with the objectives of cytogenetic screening of buffaloes from different regions of North East India to ascertain their river/swamp/hybrid types and delineate the boundary of river-swamp divide, if any.

MATERIALS AND METHODS

Animals studied: Blood samples in heparinized tubes of 158 buffaloes belonging to Assamese-upper (Brahmaputra valley, 46), Assamese-Lower (Silchar, 18), Assamese-North (Dibrugarh, 26), Nagaland (12), Manipur (40) and Mizoram (16) buffalo populations were used for leukocyte culture. Samples were collected from different villages covering the entire native tract of

each population. Samples from upper Assamese buffaloes were collected from various locations in Brahmaputra valley (in and around Guwahati and neighbouring areas) while North Assamese samples were collected from Dibrugarh district and Lower Assamese samples were collected from Silchar and neighbouring areas. Manipuri buffalo samples were collected from different areas of Manipur state comprising the districts of Senapati, Chandel and Thoubal. Nagaland buffalo samples were collected from different villages of Dimapur and Peren districts and those of Mizo buffaloes from North Van-Lai-Phai, Thenzawl and Champhai districts of Mizoram. The different states of north-east region from where blood samples were collected are shown in the map (Figure 1).

Cytogenetic analysis : Blood samples were collected in heparinised tubes for leukocyte culture. Peripheral blood cells were cultured for 72 h at 37.5°C, using standard technique. Whole blood (0.5 mL) was added to 4.5 mL of RPMI 1640 culture medium (Gibco-BRL) enriched with 20% fetal bovine serum, pokeweed mitogen, 1% glutamine (Gibco-BRL) and antibiotics. Colchicine was added 60 minutes before harvesting. The cells were treated with hypotonic solution (potassium chloride solution 0.075 M) for 15 minutes at 37.5°C and fixed at least 3 times with 3:1 methanol-acetic acid. Air-dried slides were prepared. At least 25 metaphase spreads were analyzed per animal to determine its chromosomal constitution. Representative karyotypes were prepared using the automatic karyotyping software Genus (Applied Imaging Corporation, USA).

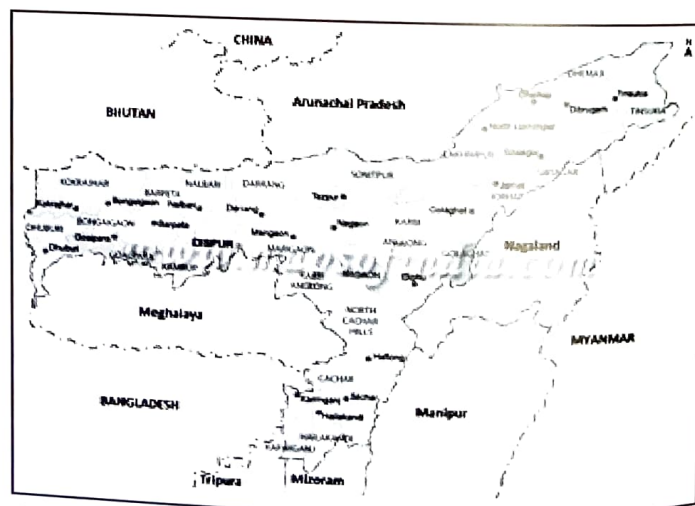


Figure 1. Map of north-eastern states from where blood samples were collected

RESULTS AND DISCUSSION

Riverine and swamp buffaloes can be distinguished based on their karyotypic features. Riverine buffaloes have a somatic chromosomal count of 50, comprised of 24 pairs of autosomes and a pair of sex chromosomes. Of the 24 pairs of autosomes, 5 pairs are biarmed (meta/submetacentric) and the remaining 19 pairs are acrocentric. The X chromosome is the largest acrocentric chromosome while the Y is a small acrocentric chromosome not distinguishable from the smaller autosomal pairs. Typical male and female riverine buffalo karyotypes are provided in Figure 2.

The somatic chromosome number of Swamp buffalo is 48, comprising 5 pairs of meta/submetacentric autosomal pairs, 18 pairs of acrocentric autosomes and a pair of sex chromosomes. Sex chromosomes are identical to that of riverine buffaloes. Chromosome pair 1 of swamp is much larger than any of the biarmed chromosome pairs of riverine buffalo. Representative male and female swamp buffalo karyotypes are provided in Figure 3 and that of river x swamp hybrid in Figure 4. The largest chromosome pair of Swamp buffalo is the result of tandem fusion of chromosome 4 and 9 of river buffaloes (Bongso et al., 1982; Chaowdhary et al., 1989). This chromosome pair is morphologically distinguishable owing to its larger size and is thus a marker chromosome for distinguishing between River and Swamp buffalo based on karyotypic characteristics.

Based on the distinctive karyotypic characteristics of the two types of buffaloes, riverine, swamp as well as

their hybrid type buffaloes were identified in the North-eastern states of India. The findings are summarised in Table 1.

Out of 46 animals investigated from upper Assam inhabiting majority of buffalo population of Assam, 41 were riverine type and the rest 5 were River X Swamp hybrids. None of the 46 animals investigated was a pure swamp type characterized by 48, XX or 48, XY. Animals were included from diverse regions i.e. Nalbari District bordering Bhutan and Kamrup district bordering the state of Meghalaya. Thus majority of the buffaloes from upper Assam are riverine type like majority of the Indian buffaloes from different parts of the country, except north-east (Balakrishnan and Yadav, 1984; Kumar and Yadav 1991; Joshi and Govindaiah, 1997; Patel and Meadow, 1998). Yadav et al. (1998), also obtained almost identical results while screening 15 Assamese buffaloes and found that 14 were riverine type ($2n=50$) and only one male was a riverine $\times$ swamp hybrid ($2n = 49$). The source of swamp inheritance in the 5 animals could not be ascertained. The buffaloes in this region are taken for grazing and released in the forests. The wild buffalo is native to these forests and mating takes place between the wild buffalo bull and domestic female buffaloes resulting in hybrid progenies. Probably it is the wild buffalo of Assam which is genetically swamp ($2n=48$) and might have contributed the swamp type inheritance to the 5 animals. Similar observations regarding Assamese buffaloes have also been obtained based on cytogenetic and microsatellite analysis (Mishra et al., 2010).

Table 1. Riverine, swamp or their hybrid status of buffaloes from various states established from karyotypic characteristics

State	Region/Districts	No of animals screened	Status		
			Riverine	Swamp	Hybrid
Assam	Upper Assam (Brahmaputra Valley, Nalbari and Kamrup)	46	41	0	5
	Lower Assam (Silchar)	18	0	10	8
	North Assam (Dibrugarh)	26	0	26	0
Mizoram	North Vanlaiphai , Thenzawl and Serchhip	16	0	16	0
Nagaland	Dimapur and Peren	12	0	12	0
Manipur	Senapati, Chandel and Thoubal	40	0	40	0
Total		158	41	104	13

However, it needs to be ascertained the riverine/swamp status of wild buffaloes of Assam. Despite our best efforts we could not obtain samples from these buffaloes as these are legally protected. It thus becomes essential not to classify swamp and riverine buffaloes simply based on their morphological characteristics. It should be ascertained by cytological studies. Toda buffaloes of Nilgiri Hills in Tamil Nadu and Chilika buffaloes of Odisha were considered as swamp type based on their morphological features. However, cytogenetic evaluation confirmed that these buffaloes are riverine type characterised by $2n=50$ and not swamp as believed (Nair et al., 1986, Murali et al., 2009, Mishra et al., 2009).

All along, Assamese buffaloes have been described as swamp type, perhaps exclusively based on their phenotypic similarity with swamp buffaloes (Annon,

1998; Kalita et al., 2010). This study thus reveals that Assamese buffaloes are not only swamp type but pure riverine and riverine x swamp crosses. Majority of the buffaloes in Assam are riverine type. Assamese buffaloes in the areas adjoining the states of Nagaland, Manipur or Mizoram are either pure swamp like Naga, Manipuri and Mizoram buffaloes or swamp x riverine hybrids.

All the 18 buffaloes from North Assam (Dibrugarh region) were pure swamp type, characterised by $2n=48$ (Figure 2). This part of Assam is contiguous to Nagaland and Naga buffaloes have been identified to be pure swamp type (present study). Similarly, of the 18 buffaloes investigated from Silchar region of Assam, 10 were pure swamp and 8 were river x swamp hybrids. Silchar region of Assam lies in between Manipur and Bangladesh. Manipuri buffaloes have been identified as

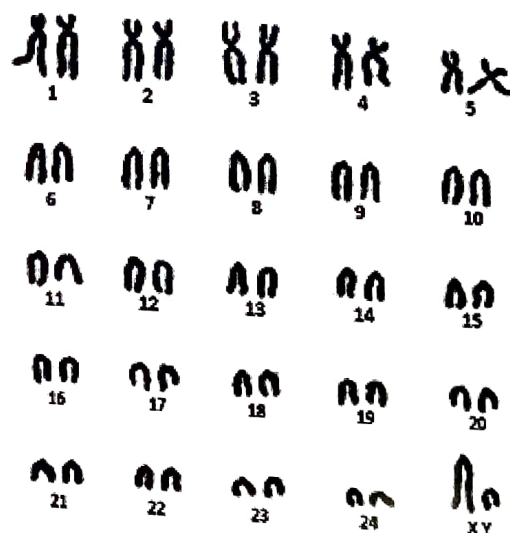


Figure 2. A metaphase spread (left) and karyotype (right) of a male riverine buffalo from Assam



Figure 3. A metaphase spread (left) and karyotype (right) of a female swamp buffalo from Manipur

pure swamp type. The riverine blood might have come either from Bangladesh or from other parts of Assam inhabiting riverine buffaloes.

All the 16 buffaloes from Mizoram, 12 from Nagaland and 40 from Manipur analysed in this study were pure swamp type. The representative karyotypes have been provided in Figure 3. Thus cytogenetic screening of different buffalo populations of North-East India revealed the existence of all three types of buffaloes typical of river, swamp and hybrid types. North-Eastern region of India thus can be considered as a virtual goldmine of buffalo genetic diversity with diverse kind of germplasm (riverine, swamp and their hybrids). The population of buffaloes in seven political states of this region account for about 0.5 million in Assam, 62,167 in Manipur, 35,022 in Nagaland, 5,832 in Mizoram, 22,627 in Meghalaya, 14,257 in Tripura, 3,208 in Arunachal Pradesh and 243 in Sikkim (Livestock Census, 2007). This region is also the habitat of Asiatic wild buffalo (*Bubalus arnee*) which is regarded as the progenitor of domesticated water buffalo (Mathur et al., 1995; Lei et al., 2007).

The present study provides the first comprehensive report on systematic characterization of North-Eastern buffalo germplasm based on cytogenetic analysis. This paper also reveals the existence of domesticated forms of all varieties of water buffalo i.e. river, swamp and river x swamp hybrid types in close geographic proximity. Of the six buffalo populations investigated in the study, one was predominantly pure riverine (upper-Assamese), four were pure swamp (Assamese-North, Manipuri, Nagaland and Mizoram) and one was significantly hybrid (44.4%) of river x swamp (Lower-Assamese). The existence of domesticated hybrid type buffaloes in the region is more interesting with questions on the type of their maternal and paternal founders and their relationship with river and swamp buffaloes in the region. Additional genetic studies involving local buffaloes from hitherto uninvestigated states of North-east India (Meghalaya, Sikkim, Arunachal Pradesh and Tripura) need to be undertaken to clarify the status of buffaloes of entire north-east region vis-a-vis other regions of India.

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