

Full Length Research Paper

Biochemical and genetic identification of Senegalese cattle breeds (Artiodactyla: Bovidae)

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Accepted 23 November, 2012

In Senegal, the genetic improvement of cattle herd by crossbreeding between local cattle (Gobra, N'dama or Djakoré) and exotic breeds originated from Europe or Brasilia, has led to an uncontrolled dissemination of crossbreds throughout the country. And this fact may lead to a failure in the management of the local cattle genetic resources. Therefore, purebred and crossbred can no longer be distinguished one to another. The aim of this work was to analyze the variations of some serum biochemical markers and the partial Cytochrome B gene sequences in order to identify Senegalese cattle breeds. The biochemical analysis showed variations in total protein, albumin, cholesterol and magnesium concentration in the different breed's sera. The sequencing of partial Cytochrome B gene revealed 22 haplotypes of supposed purebreds and crossbreds. A high haplotype diversity ($H_d = 1$) was observed in each breed population. The intra – breed genetic distance among supposed pure N'dama from Santamba village, showed that 29% of the total genetic variability was due to sequence divergence between haplotypes of the same breed. A low genetic distance ($D = 0.15$) was distinguished between the assumed pure Gobra from Birkelane and Dahra localities. Different phylogenetic trees were constructed and revealed that only the local breed Gobra was pure and the other N'dama and Djakoré were not pure. Moreover Senegalese cattle breeds had no direct relationship with European or Asian or American *Bos taurus*, Asian *Bos indicus*, *Bos javanicus* and *Bos grunniens*.

Key words: Mitochondrial DNA, cytochrome B, biochemical markers, cattle breeds, phylogeny, Senegal.

INTRODUCTION

The West Africa, area of an important bovine genetic diversity, gives a unique blend of the main bovine species worldwide (Bradley, 1992). The introduction of cattle in this part of the continent dated to 2,000 years before J-C (Payne and Hodges, 1997). The clear origins and distribution of livestock diversity are essential for their current use and their long term conservation (Hanotte et al., 2006). Thus, cattle domestication has been well documented in detail. These works indicated distinctively the initial domestications of three subspecies of different

aurochs (*Bos primigenius*). The *Bos primigenius primigenius*, domesticated in the Fertile Crescent about 8,000 years ago, and the *Bos primigenius opisthomous*, at least 9,000 years ago, in the North Eastern region of Africa (Wendorf and Schild, 1994), respectively, are the ancestors of humpless cattle (*Bos taurus*) in the Near East and Africa. The humpback zebu (*Bos indicus*) were domesticated afterwards; there are about 7,000 or 8,000 years ago in the valley of Hindu in the present Pakistan (Loftus et al., 1994; Bradley et al., 1996; Bradley and Magee, 2006). Under the influence of environmental factors and the actions of human, several genetic types or breeds have appeared which the specific character see uniqueness (Hanotte et al., 2002), is at the core of livestock production systems in West Africa and

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even in Central Africa (Shaw and Hoste, 1991).

Senegal, hosts 3.5 million cattle (Gueye, 2011). Cattle population is composed into three local purebreds. Among of which, the Peulh zebu or Gobra zebu breed in the northern and central part of the country. The Gobra zebu was described by several authors, as one of the finest beef breeds in West Africa (Doutressoule, 1947; Ndiaye and Balam, 1977). The second genetic type encountered is the N'dama humpless cattle. This local breed has long horns and is a trypanotolerant cattle in West Africa (Gueye et al., 1981). Owing to its trypanotolerance, this breed is essentially encountered in the region of Casamance, the southern part of Senegal (from Kolda to Velingara) (Touré, 1977). The third intermediate cattle breed so-called Djakoré is reared in the "Bassin arachidier" area and the region of Haute Casamance. This breed is derived from a natural crossbreeding between the Gobra zebu and N'dama taurin. Because of the low dairy potential, 1 to 4 liters per day (Kouamo et al., 2009), these local breeds are ever more crossed with exotic improved breeds to generate an alternative genetic types through bovine artificial insemination. The crossbreds are widespread throughout Senegal. This uncontrolled crossbreeding by bovine artificial insemination, has reached a level so much that one can no longer make a distinction between purebreds and crossbreds. This fact can cause difficulties on the management of bovine genetic resources.

For ruminants most of markers of protein - energy and hydro - electrolytes derived from feed. The serum concentration of these components are likely to vary from one animal to another, from one breed to another, and this through the intervention of specific animals factors (breed, pregnancy, lactation) and environmental factors (food, season, temperature) (Labouche, 1964). Meanwhile, few studies have been done on these markers to discriminate local cattle breeds according to their biochemical profile.

The Cytochrome B (Cyt B) gene contains abundant phylogenetic information, given its mode of maternal transmission and its high rate of variability. The Cyt B is also lacking introns. Furthermore it is considered to be a good marker to study the genetic differentiation and phylogenetic relationships among species within the same genus or the same family (Browers et al., 1994; Zardoya and Meyer, 1996). Cyt B gene is widely used in studies on origin, taxonomy and phylogeny of the subfamily Bovinae (Kikkawa et al., 1997; Birungi and Arctander, 2001; Hassanin and Ropiquet, 2004).

In the present study, we analyzed the variations of some serum biochemical markers and the partial Cyt B gene sequences in order to identify Senegalese cattle breeds. These data, combined with Cyt B sequences of other bovine species from GenBank, were used to perform phylogenetic analysis.

The results of this study would let to differentiate local breeds and crossbreds, to estimate genetic diversity of

our breeds in order to develop strategies for genetic conservation and to better orient genetic crossing for improvement of milk production.

MATERIALS AND METHODS

Study site

Samples were collected in several localities of the "Bassin arachidier" localized at the north-sudanian zone (situated between the isohyets 500 and 1000 mm): Kahone, Birkelane, Mbadakhoun, and Santamba. Five samples were collected in the Centre de Recherches Zootechniques (CRZ) of Dahra which is localized in the sahelian zone of the sylvo-pastoral, zone situated between the isohyets 350 and 600 mm (Figure 1).

Sampling

The study focused on three supposed purebreds (Gobra zebu, N'dama taurin and Djakoré cattle) and two crossbreds. A total of 54 animals were sampled of which 49 in traditional cattle farms. The sampling concerned with 1 to 10 cattle/farmer/locality/breed, except the five subjects from the Centre de Recherches Zootechniques of Dahra (Table 1). The subjects of Gobra and Djakoré crossbreds are products of crossing between a local cattle and an exotic breed originate from Europe or Brazilia. The interest of using these crossbreds in this study, is the fact that they were subjects (F1) and the other having a maternal origin assumed to be a local purebred, needed to better identify local cattle and to emphasize the purity of cattle.

Biological samples are reflected in blood samples taken at the jugular vein. Forty-nine blood samples were collected in dry tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) to obtain serum for biochemical analysis. Thirty blood samples were collected in EDTA (ethylene diamine-tetra-acetic acid) tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) for DNA extraction.

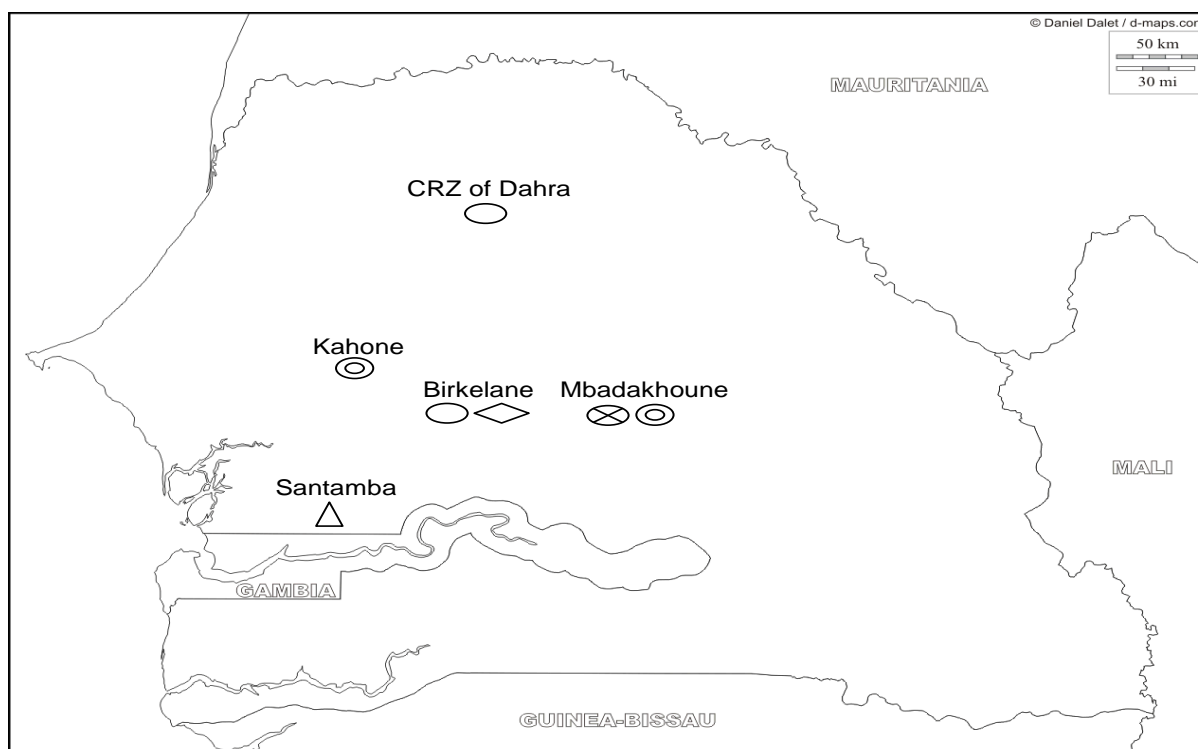
Biochemical parameters analysis

Biochemical analysis was made from serum samples that were frozen at -20°C in hemolytic tubes (Bibby Scientific®, France). The biochemical assays were focused on markers of protein - energy such as total protein, albumin and cholesterol, and hydro - electrolytes such as calcium, magnesium and phosphorus. The biochemical assays were performed using commercial kits (BIOSYSTEMS SA®, Barcelona, Spain) and the experimental protocols used were provided by the manufacturer. The dosages were based on colorimetric and absorbance readings were made using a spectrophotometer (BIOSYSTEMS® BTS-310, Barcelona, Spain).

Genetic study

DNA extraction

The extraction of genomic DNA was done using the whole blood collected in EDTA tubes (BD Vacutainer® Systems,



Legend :

- ◊ Gobra crossbred
- ◎ Djakoré crossbred
- Supposed pure Gobra
- ⊗ Supposed pure Djakoré
- △ Supposed pure N'dama

Figure 1. Map showing the geographical origins for three supposed purebreds (Gobra, N'dama and Djakoré) and two crossbreds. Sites of sampling: Kahone (16°25'W, 14°41'N), Birkelane (15°45'W, 14°09'N), Mbadakhouné (14°11'W, 14°09'N), Santamba (16°25'W, 13°43'N) and the Centre de Recherches Zootechniques (CRZ) of Dahra (15°25'S, 15°21'E).

Plymouth, United Kingdom). QIAGEN DNEASY[®] tissue kit for animal blood was used according the manufacturer's instructions.

Amplification and Sequencing

The partial Cytochrome B gene was amplified by using forward primer H15915 (5'-TCT CCA TTT CTG GTT TAC AAG AC-3') and reverse primer L14723 (5'-ACC AAT GAC ATG AAA AAT CAT CGT T-3'), described in Leconte et al. (2002). These primers were specific to rodents and amplify sequences up to 1140 base pairs. PCR was performed in a 25 µl reaction mixture containing 10.8 µl of water MilliQ, 2.5 µl PCR buffer, 1.5 µl of additional MgCl₂, 1 µl of dNTPs, 2.5 µl of each primer, 0.2

Units of Taq polymerase and 4 µl of DNA template. The standard PCR conditions were as follow: initial denaturation at 94°C for 3 min then, 40 cycles of denaturation at 94°C for 45sec, annealing at 48°C for 1min and extension at 72°C for 30 sec and after that a final extension at 72°C for 10 min. After DNA amplification, PCR products were subsequently sent to company Macrogen in South Korea for sequencing.

Statistical analysis

The processing of data from the biochemical assays was performed using the Stata SE version 9.2. Comparisons were made between breeds by analysis of variance of differences between means using the Fischer test.

Table 1. Cattle breed populations according to: localities and breeds type.

Sites of sampling	Supposed pure Gobra	Supposed pure N'dama	Supposed pure Djakoré	Gobra crossbred	Djakoré crossbred
Kahone	-	-	-	-	DjK (05)
Mbadakhouné	-	-	DjMP (10)	-	DjM (04)
Birkelane	GoBP (10)	-	-	GoB (10)	-
Santamba	-	NdSP (10)	-	-	-
CRZ of Dahra	GoDP (05)	-	-	-	-

Identifying of our samples was done in three ways, geographical origin, type of breeds (Gobra, N'dama or Djakoré) and type of generations (F0 or F1). Each sample is encoded using the two first letters of breed, followed by the first letter of the locality. For discrimination between F0 and F1, a P is added to supposed purebreds (F0) to distinguish those crossbreds (F1).

Table 2. Mean serum concentrations of biochemical parameters observed in supposed purebreds and crossbreds.

Paramètres	DjP (10)	NdP (10)	GoP (10)	Dj (09)	Go (10)
PT (g/l)	87,7 ± 5,1	88,5 ± 8,9	94,9 ± 6,7	78,03 ± 4,3	80,09 ± 10,2
Alb (g/l)	28,6 ± 4,02	25,9 ± 3,3	30,4 ± 2,4	30,5 ± 2,5	30,06 ± 7,8
Cholest (mmol/l)	4,45 ± 1,12	3,13 ± 0,47	3,64 ± 0,93	3,47 ± 1,44	2,99 ± 0,83
Ca (mmol/l)	2,52 ± 0,20	2,64 ± 0,36	2,49 ± 0,09	2,58 ± 0,15	2,54 ± 0,17
Mg (mmol/l)	1,46 ± 0,11	1,22 ± 0,12	1,52 ± 0,22	1,38 ± 0,16	1,47 ± 0,17
P (mmol/l)	1,73 ± 0,33	1,79 ± 0,28	2,01 ± 0,63	2,00 ± 0,27	2,03 ± 0,49

Breeds: DjP (pure Djakoré), NdP (pure N'dama), GoP (pure Gobra), Dj: (Djakoré crossbred) and Go: (Gobra crossbred), Biochemical parameters: PT (total protein), Alb (Albumin), Mg (Magnesium), Cholest (Cholesterol), Ca (Calcium) and P (Phosphorus).

For comparison with our data, additional mtDNA Cytochrome B sequences of six bovine species including *Bos taurus*, *Bos indicus*, *Bos grunniens*, *Bos javanicus*, *Bison bonasus* and *Bison bison* were collected from GeneBank. The *Bos taurus* sequences were obtained from: Asian cattle (GenBank Accession No. AY526085, DQ124389, AY885283 and DQ186203), American cattle (GenBank Accession No. AY676860, AY676861 and AY676866), European cattle (GenBank Accession No. V00654, AF492351, EU177834 and DQ124413). The cited sequences of *Bos indicus* were obtained from GenBank Accession No. AF492350, AF531473, AY126697 and AY689190. The cited sequences of *Bos grunniens* were obtained from China and other regions (GenBank Accession No. AF091631, AY684273, AY955225 and EF494177). The cited sequences of *Bos javanicus* were obtained from GenBank Accession No. AY689188, EF197952, D34636 and two bison species that are *Bison bonasus* (Y15005) and *Bison bison* (AF036273). We used an outgroup *Bubalus bubalus* partially nucleotide sequence removed from GenBank accession No. D88635. Alignment of sequences was carried out using the software BiotEdit[®] 7.0.9.0 (Hall, 1999). The software DnaSP[®] 5.10 (Librado and Rozas, 2009) was utilized to estimate intra-breed genetic variability by the determination of the number of polymorphic sites (variables) SNPs (Psites), the number of haplotypes (Hn), the number of mutations (Eta), the average number of nucleotide differences between haplotypes in a population (k), haplotype diversity (Hd) and nucleotide diversity (Pi). The standard genetic distance of Nei (Nei, 1972) was calculated with the method of Kimura 2-

parameter and phylogenetic reconstructions was performed with the Neighbor-Joining (NJ) method of Saitou and Nei (1987), the method of maximum parsimony (MP) (Farris, 1970) and the maximum likelihood (ML) (Felsenstein, 1981), by using the software MEGA[®] 5.5 (Tamura et al., 2011). The statistical confidence of each node in the trees was also estimated by 1000 random bootstrap resamplings (Felsenstein, 1985).

RESULTS

Biochemical parameters

The biochemical assays allowed determining the averages serum concentrations in different parameters according to each breed (Table 2). However, these averages ranged in the reference values. Analysis of variance of these average values allowed observing significant differences between breeds for some biochemical markers (Table 3).

The significant variations showed that the difference between the alleged pure Djakoré and Djakoré crossbred from Kahone and Mbadakhouné localities, between the presumed pure Gobra and Gobra crossbred of Birkelane, and between the supposed pure Gobra and the presumed pure Djakoré from Mbadakhouné, was due to variations of plasma protein concentration. Also the

Table 3. Differences between breeds in terms of some biochemical markers.

Breeds	Biochemical makers	P-value
DjP vs Dj	PT	0.0004*
GoP vs Go	PT	0.001*
GoP vs DjP	PT	0.01*
GoP vs NdP	Alb	0.003*
	Mg	0.002*
NdP vs DjP	Cholest	0.003*
	Mg	0.0004*

Breeds: DjP (pure Djakoré), NdP (pure N'dama), GoP (pure Gobra), Dj: (Djakoré crossbred) and Go: (Gobra crossbred), Biochemical parameters: PT (total protein), Alb (Albumin), Mg (Magnesium), Cholest (Cholesterol), * P <0.05 (significant), vs: versus.

Table 4. Summary statistics of mtDNA for supposed purebreds and crossbreds.

Breeds	Dj	DjP	Go	GoP	NdP
n	5	3	4	7	3
Hn	5	3	4	7	3
N	287	287	287	287	287
Psites (SNPs)	98	93	97	94	101
Eta	107	96	105	105	102
k	49,50	63	56,33	40,42	67,66
Hd	1	1	1	1	1
Pi	0,17	0,21	0,19	0,14	0,23

Breeds: DjP (pure Djakoré), NdP (pure N'dama), GoP (pure Gobra), Dj (Djakoré crossbred) and Go (Gobra crossbred). Statistical parameters: n (number of sequences), Hn (number of haplotypes), N (total number of sites), Psites (number of polymorphic sites SNPs, single nucleotide polymorphic sites), Eta (number of mutations), k (average number of nucleotide differences between haplotypes in a population), Hd (haplotype diversity), Pi (nucleotide diversity).

difference between the supposed pure Gobra from Birkelane and the presumed pure N'dama of Santamba village was caused to fluctuations in serum albumin and magnesium. Conversely the difference between the assumed pure N'dama of Santamba and the alleged pure Djakoré from Mbadakhouné is related to variations in serum cholesterol and magnesium.

DNA sequences

After sequencing and alignment, a 287 bp fragment of Cyt B gene was obtained from supposed pure Gobra, N'dama, Djakoré and Gobra, Djakoré crossbreds. A 307 bp portion of Cyt B neighboring was obtained in many vertebrates (Kocher et al., 1989) and in sheep polandais (Karpinski et al., 2006). Among these 26 sequences, 22 (DjK01, DjK02, DjK03, DjM06, DjM07, DjMP02, DjMP03, DjMP04, GoB01, GoB03, GoB04, GoB05, GoBP01, GoBP02, GoBP03, GoBP04, GoBP05, GoDP08, GoDP09, NdSP01, NdSP02 and NdSP03) were analyzed and the remaining 4 (DjMP01, DjMP05,

NdSP04 and NdSP05) were eliminated because they showed a very large difference in their nucleotide composition. Multiple alignments were performed with the 22 nucleotide sequences to obtain equity sequences without deletions.

Analysis of genetic diversity intra and inter – breeds

Analyses of bovine haplotype sequences in DnaSP® 5.10 highlighted the genetic variability at intra and inter – breeds (Table 4).

A total of 22 haplotypes were distinguished among five breed populations analyzed. Therefore a high haplotype diversity ($H_d = 1$) observed in all supposed purebreds including those crossbreds, showed that each individual constitutes itself a single haplotype. The characteristic parameters of genetic polymorphism (Psites, k, and Pi) have revealed a high variability between individuals in all cattle populations. However, this inter-individual variability was more pronounced among the population of the alleged pure N'dama from Santamba village (Psites =

Table 5. The intra and inter – breeds genetic distances.

Breeds	Dj	DjP	Go	GoP	NdP
Dj	0,20				
DjP	0,20	0,26			
Go	0,20	0,20	0,23		
GoP	0,19	0,25	0,24	0,15	
NdP	0,20	0,22	0,21	0,23	0,29

Breeds: DjP (pure Djakoré), NdP (pure N'dama), GoP (pure Gobra), Dj (Djakoré crossbred), and Go (Gobra crossbred). Bold values correspond to intra – breeds genetic distances.

101, $k = 67.66$ and $Pi = 0.23$) and less pronounced among supposed pure Gobra from localities of Birkelane and Dahra ($Psites = 94$, $k = 40.42$ and $Pi = 0.14$).

Using the Kimura 2-parameter model made possible to estimate Nei's genetic distance intra and inter – breeds (Table 5). Considering the intra and inter-breeds evolutionary distances, the highest values were observed in a same breed (example in the alleged pure N'dama, $D = 0.29$); this means that 29% of the total genetic variability was due to sequence divergence between haplotypes from the same breed. However, the genetic distance between haplotypes supposed pure Gobra ($D = 0.15$) was rather low. The alleged pure Djakoré from Mbadakhoun displayed lower evolutionary distance from the supposed pure N'dama of Santamba village ($D = 0.22$) and higher against supposed pure Gobra from Birkelane and Dahra localities ($D = 0.25$). In the majority of cases, the genetic distances between supposed purebreds were more significant than those between them and the crossbreds.

Phylogenetic analysis

In this study, phylogenetic analysis was based on 22 Cyt B partial sequences (287 bp) of three supposed purebreds, two crossbreds and 24 homologous fragments of Cyt B sequences cited in GenBank (above). The NJ, MP and ML phylogenetic trees of the Bovinae (Figures 2, 3 and 4) were constructed with *Bubalus bubalis* (Accession No. D88635) as outgroup. The topologies obtained from the different reconstruction algorithms highlighted the phylogenetic relationships between taxa. The distribution of haplotypes bovine in different phylogenetic trees, showed eight different mtDNA lineages of which six were represented by the following species (*Bos taurus*, *Bos indicus*, *Bos javanicus*, *Bison bonanus*, *Bison bison* and *Bos grunniens*) and the two others by supposed purebreds and crossbreds. These eight different major lineages could be the result from eight separate maternal origins. Nevertheless, similar study conducted by Xuan et al. (2010), had demonstrated the existence of these six different mtDNA lineages corresponding to the six

species of cattle mentioned above. The two major maternal lineages from which were derived Senegalese cattle breeds, gave rise two clades: A and B (Figures 2, 3 and 4). The clade A was composed of a sub-clade A, individuals of supposed pure N'dama and Djakoré, of Gobra and Djakoré crossbreds, and of supposed pure Gobra (GoBP02 and GoDP09). The sub-clade A was composed essentially individuals of supposed pure Gobra especially those from Birkelane. However the two individuals of assumed pure Gobra in this case GoBP02 (Birkelane) and GoDP09 (Dahra) partnered with a strong bootstrap value (50% in MP). This showed that in spite of being original different geographical, both supposed pure Gobra could be from a maternal sub-lineage of pure Gobra. Thus all individuals of supposed pure Gobra had close maternal links.

The dispersion among individuals of presumed pure N'dama from Santamba village one hand and those of supposed pure Djakoré of Mbadakhoun other hand, showed that these two supposed purebreds were composed of individuals from several different native sub-lineages thus having distant genetic relationships.

The clade B brought together individuals of crossbred Gobra (GoB03 and GoB04), of crossbred Djakoré (DjK01), supposed purebred Djakoré (DjMP02) and purebred N'dama (NdSP01), which together formed a monophyletic group.

DISCUSSION

The data analysis of serum concentrations of biochemical markers showed differences between cattle breeds and these were due mainly to variations in total protein, sera albumin, cholesterol and magnesium. The variability of these makers from one breed to another was tied to the effect of factors specific to animals (breed, pregnancy, lactation) or environmental factors (temperature, feeding or season) (Labouche, 1964). Hence, the fodder and agricultural byproducts were the staple food of ruminants, which were feed shared by all these breeds.

Fluctuations in serum protein concentration observed between breeds could be related to feeding and influence of the area localization. Because daily diet and

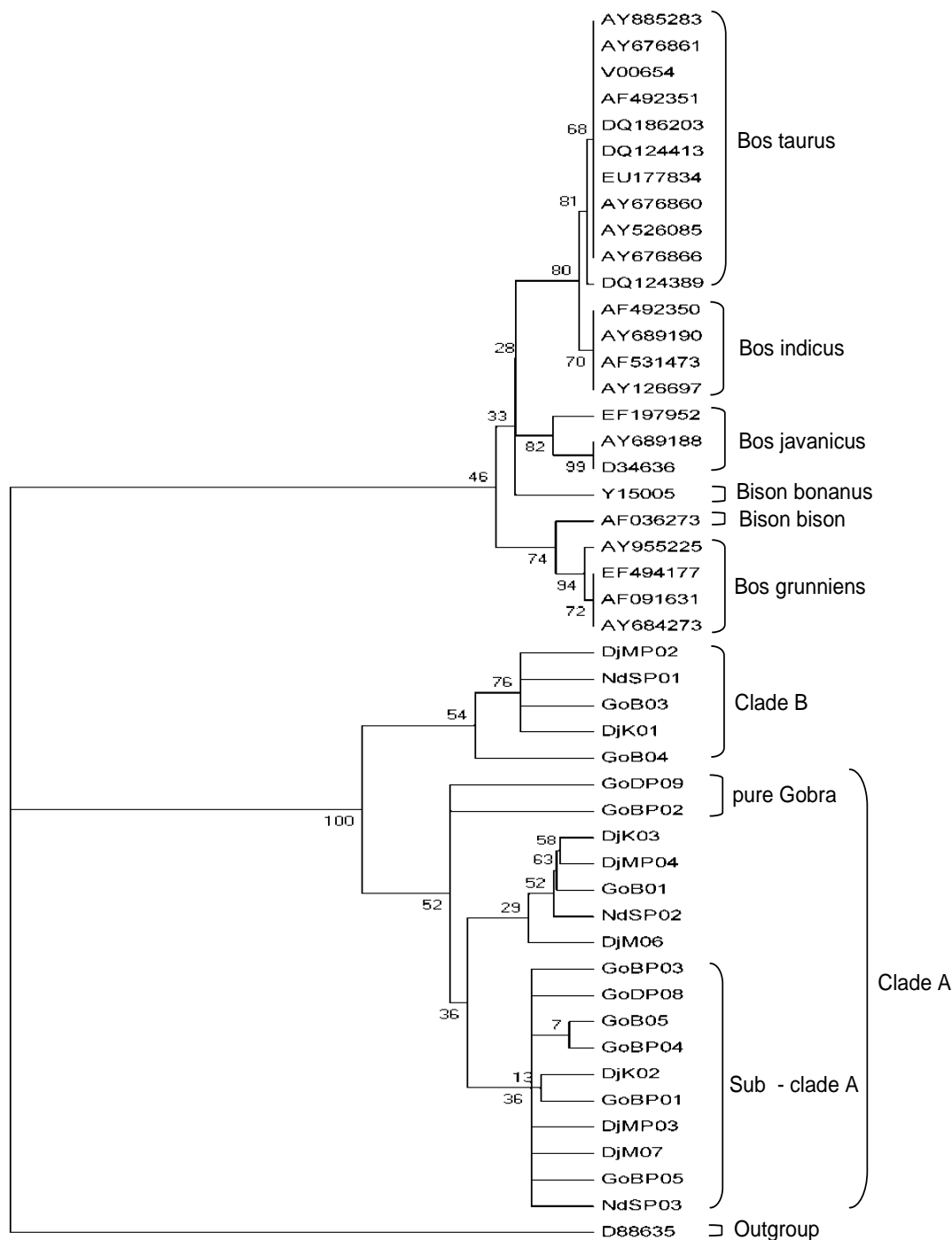


Figure 2. Phylogenetic relationships of Senegalese cattle breeds and six bovine species: neighbor-joining tree (tree consensus). The bootstrap values of the branches.

supplement products provided to each alleged purebred or crossbred may differ according to their production (milk, meat or labor). All the more we did not have information on the amount of daily feed; we supposed that these variations in sera protein concentration were due to feed ingested. Indeed the main factors of variation

of sera protein concentration were due to feed (Sawadogo, 1998). However the influence of the region also weighed because the nature of plant species that constitute the fodder may also vary according to geographical area. Thus the kind of plants determined the nutritional value of pasture (Friot and Calvet, 1973).

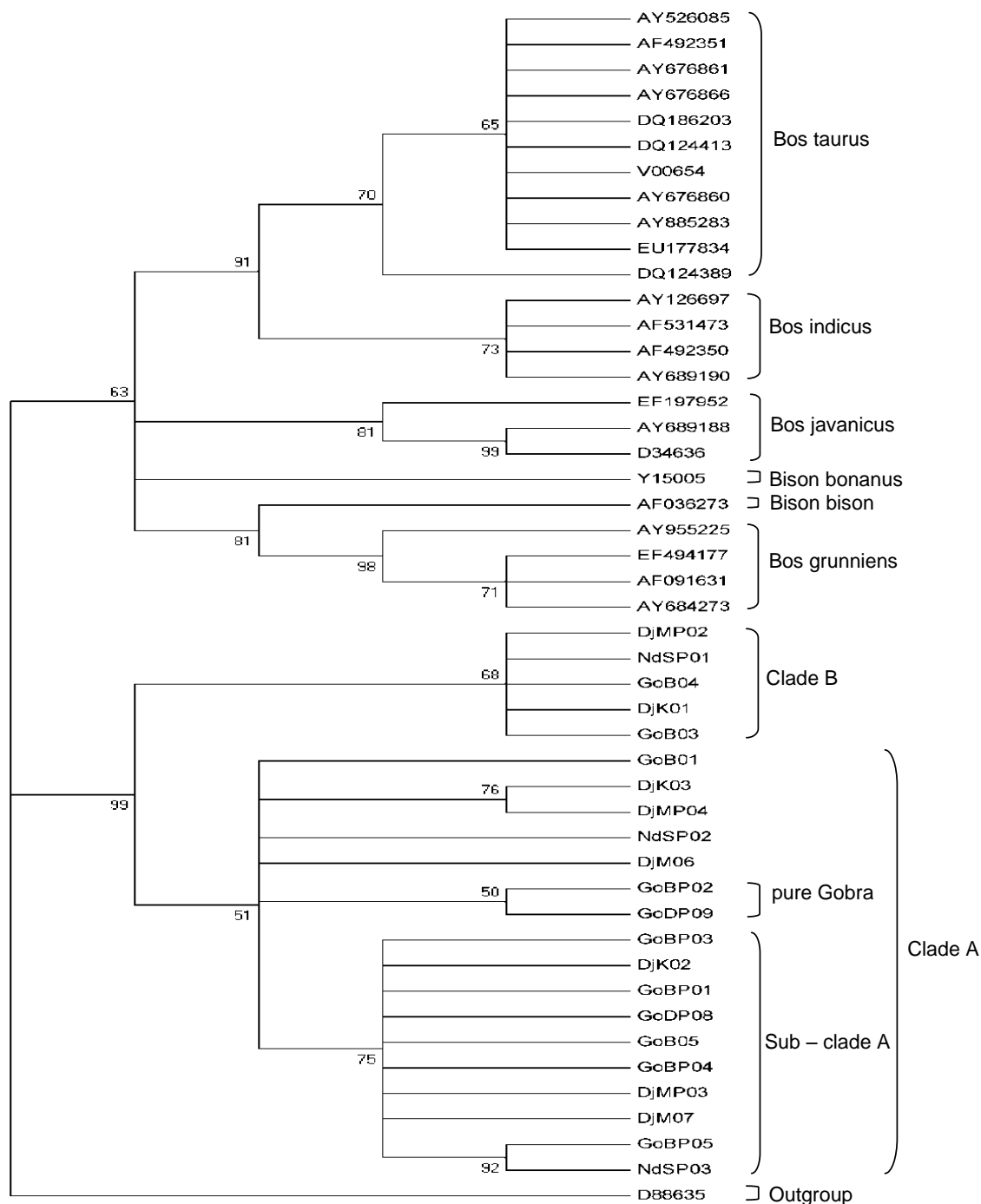


Figure 3. Phylogenetic relationships of Senegalese cattle breeds and six bovine species: parsimony tree sequences (condensed tree). The bootstrap values of the branches.

Experiments in cows in stalls, shown that albumin was sensitive to variations in the external temperature during the course of a day (Labouche, 1964). And variations in serum albumin observed only between supposed purebred Gobra of Birkelane and supposed purebred N'dama of Santamba would be due to the variation of

environmental temperature during the daytime. Because samples were collected in supposed purebred Gobra in the morning and the middle-morning in assumed purebred N'dama, before the cattle left to pasture. These same differences in serum albumin were mentioned by Condry and Carr (1961), in South Africa, between the

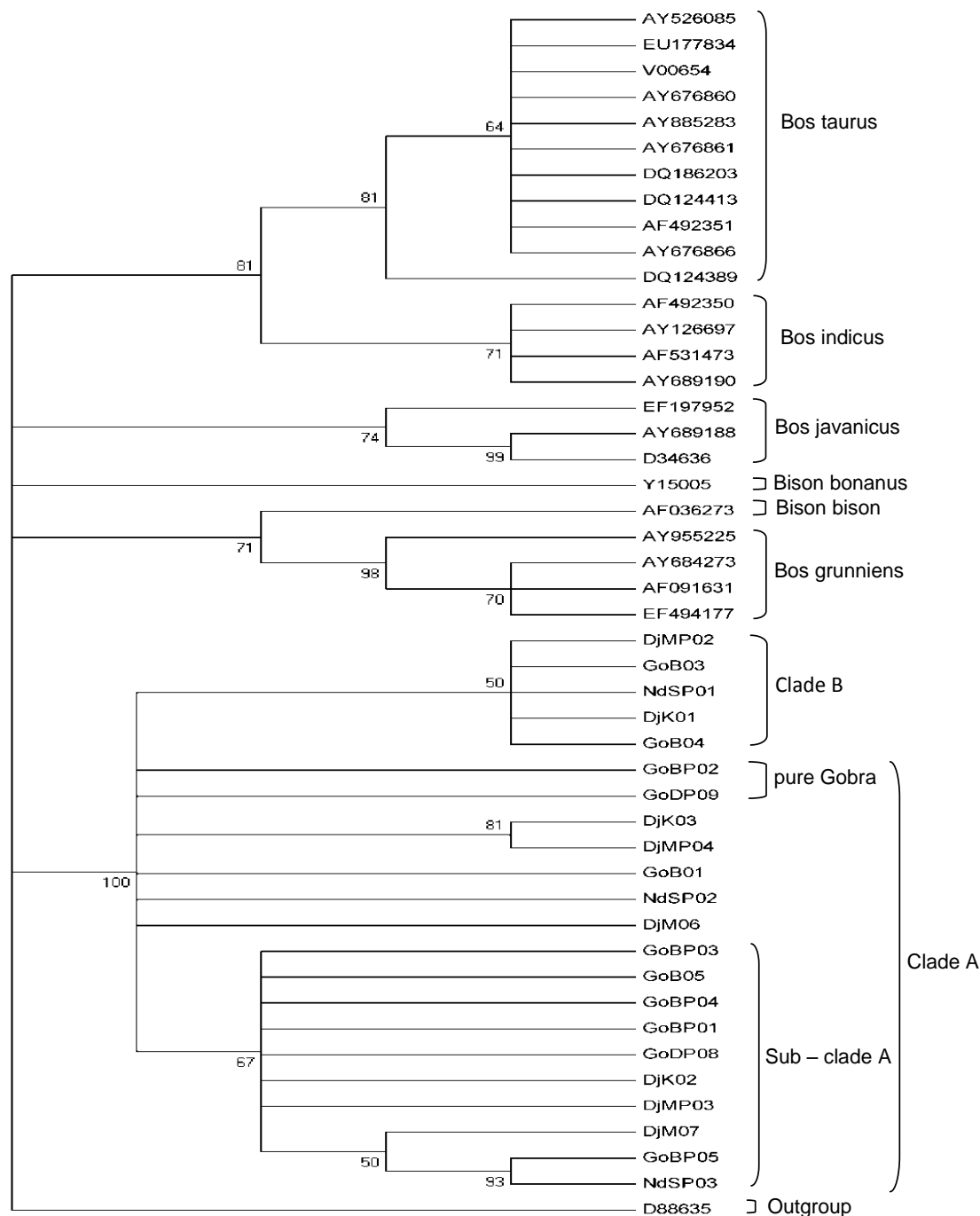


Figure 4. Phylogenetic relationships of Senegalese cattle breeds and six bovine species: maximum likelihood tree (tree condensed). The bootstrap values of the branches.

Ngami breed and those Africander and Mashona.

In dairy cows, the concentration of sera cholesterol is particularly high due to partially use of cholesterol in mammary glands for the synthesis of milk's fats. Unlike the triglycerides that are most used by the mammary gland (Moore and Christie, 1981 in Mazur and Rayssiguier, 1988). However, variations in cholesterol concentration

observed were not linked to diet, because ruminant feed didn't contain cholesterol. Owing the genetically potential variability of dairy related to breed, serum cholesterol concentration also fluctuated following each breed.

However, variations in magnesium concentration could be related to feeding. Because milk is poor in magnesium (Sawadogo, 1998), some cattle can received sometimes

mineral supplements for their need of production.

However, serum calcium did not vary between breeds as mentioned by Ghislain et al. (1985), fodder systems didn't influence serum concentrations of calcium in dairy cows. This maintenance of serum calcium in all breeds was due to mechanisms of endocrine regulation (Trumbleson et al., 1973).

Serum phosphate concentration did not also show variation and this may be due to dietary intake. In effect as the breeds had in common a natural diet and 87.03% of cattle were used for breeding and dairy production, so the natural fodder was sufficiently rich in this element to meet the needs of dairy cattle. However, this maintenance of serum phosphorus may also be involved in the physiological regulation, if the inputs did not cover requirements. And the exchange between the blood and the skeleton can regulate inputs and their use (Payne, 1983).

Because only 4 biochemical parameters were significantly varied between breeds, this indicated that there was not a biochemical profile which is specific to a supposed purebred or crossbred. All the more these variations were in major part due to environmental factors (feeding, temperature). Although serum biochemical parameters of cattle breed variation was not enough to characterize population. However the concept of purity of local breed and the relationships can be demonstrated by the analysis of partial Cyt B sequences.

Molecular analysis of Cyt B sequences highlighted the evolutionary relationships between breeds and the purity of the local breed. The variability of mitochondrial DNA sequences provided favorable information for the origin of the species of livestock.

The high haplotype diversity observed in all breeds populations (Table 4), showed the existence of different haplotypes individual of supposed purebreds and crossbreds. Similar studies conducted in cattle (Bradley et al., 1996), in sheep (Hiendleder et al., 2002; Meadows et al., 2005; Karpinski et al., 2006), among goats (Luikart et al., 2001) and pigs (Giuffra et al., 2000) showed that the presence of different haplotypes in domesticated breeds was related to the multiple maternal origins. This joined the hypothesis in phylogenetic analyzes and according to which the supposed pure N'dama, Gobra and Djakoré had distinct maternal origins. This finding is in accordance as well as with inter-breeds genetic distances (Table 5) and showed that there was considerable genetic diversity among our supposed purebreds. In addition the fact that they had no direct affinity with European or Asian or American *Bos taurus*, Asian *Bos indicus*, *Bos javanicus* and *Bos grunniens*, showed that the Senegalese cattle breeds and these species have been domesticated separately in different homes of domestication. In short, the divergence between livestock reflected that the domestication had separated most maternal lineages and favored introgression between livestock populations

(MacHugh and Bradley, 2001).

Indeed, the links between individuals of clade A assumed the fact that these would share identical molecular states but having undergone different evolutionary phases, different kind of mutations.

However, the links shared by the alleged purebred Gobra, GoBP02 (Birkelane) and GoDP09 (Dahra), showed that these derived from the same maternal sub-lineage that was pure Gobra and therefore they were pure. These individuals despite accounting from different geographical origins were joined together in phylogenetic tree (figure 3). The low evolutionary distance which characterizes them (Table 5), was the evidence that their characters would share ancestral homologous. It takes in account that Gobra of Birkelane (GoBP02) could be originated from the north and its presence in this locality was due to commercial transactions of cattle in the livestock weekly markets or the migration of livestock keepers.

Furthermore, the distribution in the trees (Figures 2, 3 and 4), showed that the individuals of supposed pure N'dama of Santamba belonged to different maternal origins. Probably, the supposed pure N'dama had a result of interbreeding frontier. As this locality is close to the frontier of Senegal and Gambia, so we assumed that former migrations of local populations might have favored a crossbreeding between these and other purebreds or crossbreds from the Gambia. This can generate individuals morphologically similar to pure N'dama. This fact corroborated with intra-breed genetic distances observed in the supposed pure N'dama (Table 4). The divergence related to the variability of shared characters could be explained by an observed variation in the color of their coat which differs from one individual to another of the hereditary probably different. The gene which encoded the coat color was a gene with visible effects, therefore constrained by environmental factors, resulting in several forms of expression: seat of the intra-breed variability.

However, the dispersal of individuals of supposed purebred Djakoré from Mbadakhoun showed that they were from several maternal sub-lineages. So we assumed that they were from different crossbreeding because this locality was in the center of "Bassin arachidier", region where encountered several breeds cattle from different geographical areas of Senegal.

The distribution of bovine haplotypes in different phylogenetic trees has given a monophyletic group (clade B). The reason we can deliver from this group is the fact that these haplotypes were from a single maternal lineage, which was not a purebred seen to the diversity of descendants. In addition, they weave relations reflect the fact that they shared synapomorphies (characters been inherited from a common ancestor but has undergone innovations in the mechanism of evolution), that they have acquired during the evolution through domestication or crossbreeding.

Finally, all these observations lead to a conclusion that among the Senegalese local cattle breeds, only the breed Gobra retained its state of purity and the other breeds N'dama and Djakoré had secondary lost their purebred status. This indicated that the strength of gene introgression between purebreds and crossbreds in frontier localities and those centers of "Bassin arachidier" is very important. Thus, the Cyt B gene is a very favoring of introgression between breed populations and this by its high mutation rate and its exclusively maternal heredity, allowing it to assess the phylogenetic relationships between subspecies or sub-populations of livestock. These introgressions indicated more that the mechanisms of isolation pre or post-copulatory didn't affect these breeds.

ACKNOWLEDGEMENTS

We are very grateful to BIOPASS laboratory of IRD of Dakar for providing assistance for this study. We appreciated the good collaboration of our colleagues of the Biochemistry laboratory of the Ecole Inter-Etats des Sciences et Médecine Vétérinaires de Dakar. We thank especially Dr MIGUIRI for helping us for the biochemical analysis of samples.

REFERENCES

- Bradley DG (1992). Genetic characterisation of cattle breeds in West and Central Africa. *Trypanotolerant Livestock Newsletter*, 5: 3-8.
- Bradley DG, MacHugh DE, Cunningham P, Loftus RT (1996). Mitochondrial DNA diversity and the origins of African and European cattle. *Proc. Natl. Acad. Sci.* 93(10): 5131-5135.
- Bradley DG, Magee D (2006). Genetics and the origins of domestic cattle. Dans M.A. Zeder, E. Emshwiller, B.D. Smith et D.G. Bradley, eds. *Documenting domestication: new genetics and archaeological paradigm*, pp. 317-328. Californie, Etats-Unis d'Amérique. University of California Press.
- Browers N, Stauffer JR, Kocher TD (1994). Intra and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling Cichlids (Teleostei: Cichlidae) from Lake Malawi, Africa. *Mol. Phylogenet. Evol.* 3: 75-82.
- Birungi J, Arctander P (2001). Molecular systematics and phylogeny of the Reduncini (Artiodactyla: Bovidae) Inferred from the analysis of mitochondrial cytochrome b gene sequences. *J. Mamm. Evol.* 8: 125-147.
- Condy JB, Carr WR (1961). Observations on the serum proteins of Africander, Mashona, and Ngami cattle. *Vet. Rec.* 73: 91-93.
- Doutressoule G (1947). *L'Elevage en Afrique occidentale française*. Paris: Larose, 298p.
- Farris JS (1970). Methods for computing Wagner trees. *Syst. Zool.* 34: 21-34.
- Felsenstein J (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368-376.
- Felsenstein J (1985). Confidence Limits on phylogenies: An approach using the bootstrap. *Evolution* 39(4): 368-376.
- Friot D, Calvet H (1973). Biochimie et élevage au Sénégal. *Rev. Elev. Vet. Pays Trop.* 26(4): 75-95.
- Ghislain P, Trembley AV, Pierre H (1985). Facteurs influençant le profil métabolique des vaches laitières. *Can. Vet. J.* 26: 306-311.
- Giuffra E, Kijas JM, Amarger V, Carlborg O, Jeon JT, Andersson L (2000). The origin of domestic pig: independent domestication and sub-sequent introgression. *Genetics* 154: 1785-1791.
- Gueye E, Nicolas A, Touré SM (1981). Couleur de la robe chez les N'Dama de haute Casamance, Sénégal. *Rev. Elev. Med. Vet. Pays Trop.*, 34(3): 275 - 279.
- Gueye M (2011). L'avenir de l'élevage africain : Le salut par la chaîne de valeur. *SOS Faim, défis sud*, 98: 26-28.
- Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, 41: 95-98.
- Hanotte O, Bradley DG, Ochieng J, Verjee Y, Hill EW, Rege JEO (2002). African pastoralism: genetic imprints of origins and migrations. *Science* 296(5566): 336-339.
- Hanotte O, Toll J, Iniguez L, Rege JEO (2006). Farm animal genetic resources: Why and what do we need to conserve. *Proceeding of the IPGRI-ILRI-FAO-CIRAD workshop: Option for in situ and ex situ conservation of AnGR*, 8-11 in press novembre 2005, Montpellier, France.
- Hassanin A, Ropiquet A (2004). Molecular phylogeny of the tribe Bovini (Bovidae, Bovinae) and the taxonomic status of the Kouprey, *Bos sauveli* Urbain 1937. *Mol. Phylogenet. Evol.*, 33: 896-907.
- Hiendleder S, Kaup B, Wassmuth R, Janke A (2002). Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. *Proc. R. Soc. Lond. Biol. Sci.* 269: 893-904.
- Karpinski M, Junkuszew A, Drozd A, Gruszecki TM (2006). A phylogenetic comparison of wild sheep (*Ovis musimon*) and domestic sheep (*Ovis aries*) represented by BCP synthetic line using mitochondrial cytochrome b gene sequence analysis. *Arch. Tierz. Dummerstorf.* 49: 310-316.
- Kikkawa Y, Yonekawa H, Suzuki H (1997). Analysis of genetic diversity of domestic water buffaloes and anoa based on variations in the mitochondrial gene for cytochrome b. *Anim. Genet.*, 28: 195-201.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989). Dynamics of Mitochondrial DNA Evolution in Animals: Amplification and Sequencing with Conserved Primers. *Proc. Natl. Acad. Sci.*, 86: 6191-6200.
- Kouamo J, Sow A, Leye A, Sawadogo GJ, Ouedrago GA (2009). Amélioration des performances de production et de reproduction des bovins par l'utilisation de l'insémination artificielle en Afrique Sub-saharienne et au Sénégal en particulier. *RASPA*. 7(3-4): 139-148.
- Labouche CL (1964). La protéinémie chez la vache. *Rev. Elev. Med. Vet. Pays Trop.* 17(4): 721-745.
- Lecompte E, Gannjoy L, Kerbis Peterhans J, Denys C (2002). Cytochrome-b- based phylogeny of the *Praomys* group (Rodentia, Murinae): a new African radiation? *C.R. Biologies* 325: 827-840.
- Librado P, Rozas J (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452.
- Loftus RT, MacHugh DE, Bradley DG, Sharp PM, Cunningham P (1994). Evidence for two independent domestications of cattle. *Proc. Natl. Acad. Sci.*, 91(7): 2757-2761.

- Luikart G, Gielly L, Excoffier L, Vigne JD, Bouvet J, Taberlet P (2001). Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proc. Natl. Acad. Sci.*, 98: 5927-5932.
- MacHugh DE, Bradley DG (2001). Livestock genetic origins: goats buck the trend. *Proc. Natl. Acad. Sci.* 98: 5382-5384.
- Mazur A, Rayssiguier Y (1988). Profil lipoprotéique de la vache laitière. *Ann. Rech. Vet.* 19: 53-58.
- Meadows JRS, Li K, Kantanen J, Tapio M, Sipos W, Pardeshi V, Gupta V, Calvo JH, Whan V, Norris B, Kijas JW (2005). Mitochondrial Sequence Reveals High Levels of Gene Flow Between Breeds of Domestic Sheep from Asia and Europe. *Journal of Heredity* 96(5): 494-501.
- Ndiaye AL, Balaam F (1977). Zébu du Sénégal. *Bulletin AASNS* Octobre 1977, 59: 15-19.
- Nei M (1972). Genetic distance between populations. *The American Naturalist*, 106: 283-292.
- Payne JM (1983). *Les maladies métaboliques des ruminants domestiques*. Paris: Ed. du point Vét. 190p.
- Payne WJA, Hodges J (1997). *Tropical cattle, origin, breeds and breeding policies*. Blackwell Science, Oxford, UK. p. 318.
- Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425.
- Sawadogo G (1998). Contribution à l'étude des Conséquences nutritionnelles sub-sahariennes sur la biologie du zébu Gobra au Sénégal. Thèse de Sciences, Productions Animales. Toulouse : Ecole Nationale Vétérinaire Toulouse, 202p.
- Shaw APM, Hoste CH (1991). Les échanges internationaux de bovins trypanotolérants. I. Historique et synthèse. *Rev. Elev. Med. Vet. Pays Trop.*, 221-228.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA 5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* 28: 2731-2739.
- Touré SM (1977). La Trypanotolérance, *Revue de connaissances. Rev. Elev. Med. Vet. Pays Trop.*, 30(2): 157-174.
- Trumbleson ME, Wingfield WE, Johnson HD, Campbell JP, Middleton CC (1973). Serum electrolytes concentrations, as a function of age, in female dairy cattle. *Cornell. Vet.*, 63: 58-64.
- Wendorf F, Schild R (1994). Are the early Holocene cattle in the Eastern Sahara domestic or wild? *Evolutionary Anthropology* 3: 118-128.
- Xuan TP, Georgescu SE, Manea MA, Hermenian AO, Costache M (2010). Genetic diversity and phylogenetic relationships of Romanian cattle breeds inferred from cytochrome b gene partial sequences. *Romanian Biotechnological Letters*, 15(2): 5154-5158.
- Zardoya R, Meyer A (1996). Phylogenetic performance mitochondrial protein coding genes in resolving relationship among vertebrate. *Mol. Biol. Evol.*, 13: 933-942.