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Trypanosomosis, genetic diversity and admixture in cattle breeds of Burkina Faso

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Table of contents

List of Tables

List of Figures

List of appendix

Dedication

To My Wife

Kouilga Bertille Marie Lydie Soudré / Léama

My Children

Koundja Flavie Juanita Soudré

Guilga Axelle Ophélia Gritschi Soudré

My Mother

Clémentine Soudré / Kaboré

My Brothers and Sisters

Jean L. Kalkoumdo, Dominique, Didier, Pélagie and Nicole

In Memory of

My Father, **Youssouf Emmanuel Soudré** *R.I.P.*

My Brother, **Roland Soudré** *R.I.P.*

Abbreviations and Acronyms

AAT:	African Animal Trypanosomosis
ALD:	Admixture Linkage Disequilibrium
Boku:	University of Natural Resources and Applied Sciences
BTAX:	*Bos taurus* chromosome X
CIRDES:	Research-Development International Centre for Husbandry in sub-Humid Area
cM:	centiMorgan
DDT:	Dichloro-Diphenyl-Trichloroethane
ECOWAS:	Economic Community of West African States
FAO:	Food and Agriculture Organization of United Nations
GDP:	Gross Domestic Product
HAT :	Human African Trypanosomosis
Hexp:	Expected heterozygosity
Hob:	Observed heterozygosity
HWE:	Hardy-Weinberg Equilibrium
ILCA:	International Livestock Centre for Africa
ILRAD:	International Laboratory for Research on Animal Diseases
ILRI:	International Livestock and Research Institute
INSD:	Institut National de la Statistique et de la Démographie
ISAG:	International Society for Animal Genetics
ISCTRC:	International Scientific Council for Trypanosomosis Research and Control
LD:	Linkage Disequilibrium
MCMC:	Markov Chain Monte Carlo
MECV:	Ministère de l'Environnement et du Cadre de Vie
MED:	Ministère de l'Economie et du Développement
MEE:	Ministère de l'Environnement et de l'Eau.
MNA:	Mean Number of Alleles
MNE:	Mean Number of Effective alleles
MRA:	Ministère des Ressources Animales

NCBI:	National Centre for Biotechnology Information
OEAD:	Austrian Agency for International Cooperation in Education and Research
PAAT:	Programme Against African Trypanosomosis
PATTEC:	Pan African Tsetse and Trypanosomosis Eradication Campaign
PCR:	Polymerase Chain Reaction
QTL:	Quantitative Trait Loci
RT:	Room Temperature
SAS:	Statistical Analysis System
SIT:	Sterile Insect Technique
SNP:	Single Nucleotide Polymorphism
TNA:	Total Number of Alleles
UNDP:	United Nations Development Programme
UNECA:	United Nations Economic Commission for Africa
UPGMA:	Unweighted Pair Group Method with Arithmetic mean
WAEMU:	West African Economic and Monetary Union

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Summary

Located in the Southern part of the Sahara, Burkina Faso is one of the tsetse challenged African countries. Therefore, trypanosomosis is an important disease affecting mostly animals. Despite several methods applied for many years, the control of this disease remained a big constraint to livestock productions. Among the indigenous cattle within the country, a taurine breed called Baoule which is located in the Southern part is known as a trypanotolerant breed. An introgression of zebuine cattle which has bigger body size but is susceptible to trypanosomosis in the tsetse challenged areas has been favored by droughts events, the shift of the Northern limit lines of tsetse flies and the development of cotton culture.

The objectives of this study were to assess the importance of trypanosomosis among diseases in cattle in Burkina Faso, mainly in tsetse challenged areas; to capture information how farmers apply methods to control the disease; to assess the structure, genetic diversity and level of admixture of cattle populations across the tsetse belt in Burkina Faso; to investigate the breed composition of crossbred animals across the genome versus that in trypanotolerance candidate gene regions. A survey has been carried out in 3 regions of Burkina Faso, one tsetse free region in the North and 2 tsetse challenged regions in the South-West and the West. Out of 29 villages chosen, 5 were situated in the tsetse free region. 134 Cattle breeders were interviewed individually with a questionnaire consisting of opened and closed questions. Apart from the survey 1045 blood samples have been taken from Zebu, Baoule and Baoule×Zebu populations for genotyping. 25 DNA microsatellite positions of 22 autosomes were applied on 450 animals from both sexes as well as 26 microsatellites on the X-chromosome of 300 males. The results indicate that among the 16 diseases mentioned by cattle breeders, trypanosomosis is the most important one in tsetse challenged areas. Overall, 54.55 % and 70.91 % respectively in the South-West and the West ranked trypanosomosis as the priority disease. Chemoprophylaxis/chemotherapy is widely used as a control method as well as insecticides to fight the flies. Isometamidium was used by 54.49 % of respondents versus 46.51 % for diminazene. The most decreased efficacy trypanocid to farmers' opinions was diminazene (85.55 %) compared with isometamidium (14.55 %). Pure Zebu cattle are much more susceptible to the disease than the taurine Baoule cattle or Baoule×Zebu. Zebu cattle are preferred by cattle breeders for their body size and draft power.

From the study of microsatellites on autosomes, the mean of observed alleles per locus was 12.44 ± 4.31 while the mean of expected alleles was 4.67 ± 1.48. The heterozygosity was ranged from 0.34 to 0.76 and 0.36 to 0.87 respectively for observed and expected heterozygosity across loci. The average heterozygosity across population was 0.73 ± 0.10. The mean estimates of *F*-Statistics were F_{IS} = 0.117 ± 0.019, F_{IT} = 0.158 ± 0.019 and F_{ST} = 0.047 ± 0.005. The proportions of membership showing the admixture level were ranged from 66 % - 98 % of individuals assigned to Zebu breed and 24 % - 85 % of individuals assigned to Baoule breed. The dendrogram showed the Baoule South-West clearly segregating from the other populations, Baoule×Zebu being intermediate genetic groups between Baoule South-West and Zebu North populations.

In the X-chromosomal microsatellite study, the average spacing between adjacent loci was 6.02 ± 6.23 cM and 232 alleles have been detected. The mean number of observed alleles per locus was 8.92 ± 3.59 vs. 4.46 ± 2.64 of effective alleles per locus and 0.69 ± 0.18 for the gene diversity. The average switching has been estimated at 0.1148 ± 0.076. That led to estimate the age of admixture at 69 ± 43 years from 2007 considering a generation length of 6 years in cattle.

The fairly moderate genetic differentiation among indigenous cattle populations in Burkina Faso across the loci makes it possible to genetically improve these breeds for production, conservation and diversity.

64 tryanotolerance candidate SNPs have been genotyped from which 9 blocks were constructed with 32 SNPs. The analysis of the blocks confirmed 5 blocks being potentially involved in the disease control, based on breed composition in these regions versus breed composition in the residual genome. This was confirmed by the deficiency of Zebu alleles in the crosses ranging from -0.15 ($p = 0.0238$) to -0.34 ($p<0.0001$). These blocks are located within QTL regions controlling the disease.

Crossing susceptible breeds with the trypanotolerant ones like the Baoule cattle can help to reduce trypanosomosis occurrence in cattle. This can then be used as part of an integrated control method. More research is needed on the best level of breed composition, involving on farm research and selection of a set of ancestry informative markers to cheaply identify the breed composition of individual animals.

Key words: Admixture, Baoule, Baoule×Zebu, Burkina Faso, breeder, SNP, trypanocid, trypanotolerance, trypanosomosis, Zebu.

Trypanosomose, genetische Diversität und Admixtur von Rinderrassen in Burkina Faso

Zusammenfassung

Im südlichen Teil der Sahara gelegen gehört Burkina Faso zum Verbreitungsgebiet der Tsetse-Fliege in Afrika. Die Trypanosomose, die von der Fliege übertragen wird und an der vor allem Tiere erkranken, stellt ein schwerwiegendes Problem dar. Obwohl bereits seit Jahren verschiedene Maßnahmen gesetzt werden, die Krankheit unter Kontrolle zu bringen, ist die Produktion landwirtschaftlicher Nutztiere großen Einschränkungen unterworfen. Unter den bodenständigen Rindern ist die „Baoule“ genannte taurine Rasse aus dem südlichen Teil des Landes als trypanotolerant bekannt. Die Einführung zebuiner Rinder, die von größerem Wuchs aber empfänglich für Trypanosomen sind, in Tsetse-Gebiete wurden durch Dürre, die Verschiebung der nördlichen Tsetse-Linie und die Entwicklung der Baumwollkulturen begünstigt.

Die Ziele dieser Arbeit waren, die Bedeutung der Trypanosomose als Krankheit beim Rind in Burkina Faso, besonders in Tsetse-Gebieten, einzuschätzen, Informationen über die Anwendung von Bekämpfungsmaßnahmen seitens der Landwirte zu sammeln, Struktur, genetische Diversität und Grad der Durchmischung von Rinderpopulationenen im Tsetse-Gürtel in Burkina Faso zu erfassen und anhand der Untersuchung von Markergenen die Rassenzusammensetzung von Kreuzungstieren im Gesamtgenom einerseits und in den Kandidatengenregionen für Trypanotoleranz andererseits zu vergleichen. In drei Gebieten von Burkina Faso, einer von Tsetse-Fliegen freien Region im Norden des Landes und zwei belasteten Regionen im Westen und Süd-Westen wurden Erhebungen durchgeführt. Von 29 ausgesuchten Dörfern waren fünf in der von Tsetse-Fliegen freien Region gelegen. 134 Rinderzüchter wurden anhand eines Fragebogens, der aus offenen und geschlossenen Fragen bestand, einzeln befragt. Neben der Erhebung wurden 1045 Blutproben von Zebu, Baoule und Baoule×Zebu Kreuzungstieren für die Genotypisierung entnommen. 25 DNA-Mikrosatelliten, die auf 22 verschiedenen Autosomen positioniert sind, wurden zur Untersuchung von 450 Tieren beiderlei Geschlechts eingesetzt, zusätzlich 26 X-chromosomale Mikrosatelliten bei 300 männlichen Tieren. Die Resultate lassen erkennen, dass von den 16 von den Rinderzüchtern benannten Krankheiten in Tsetse-Gebieten Trypanosomose die wichtigste ist, sie rangiert mit 54.55 % im Süd-Westen bzw. 70.91 % im Westen. Der Einsatz von Chemoprophylaxe und Chemotherapie zur Krankheitskontrolle ist weit verbreitet, ebenso wie der Einsatz von Insektiziden zur Fliegenbekämpfung. Von 54.49 % der Befragten wurde als Trypanozid Isometamidium eingesetzt, von 46.51 % Diminazen. Nach Einschätzung der Landwirte hatte Diminazen die weit geringere trypanozide Wirksamkeit (85.55

%) im Vergleich mit Isometamidium (14.55 %). Reine Zeburinder sind wesentlich empfänglicher für die Krankheit als die taurinen Baoule oder Baoule×Zebu Kreuzungstiere. Zebu werden von den Bauern wegen ihrer Großrahmigkeit und Zugkraft bevorzugt.

Die Untersuchung der autosomalen Mikrosatelliten ergab eine durchschnittliche Anzahl beobachteter Allele pro Locus von 12.44 ± 4.31, während der Durchschnitt der erwarteten Allele bei 4.67 ± 1.48 lag. Die beobachtete Heterozygotie über die Loci bewegte sich zwischen 0.34 to 0.76, die erwartete Heterozygotie zwischen 0.36 to 0.87. Die mittlere Heterozygotie über die Population war 0.73 ± 0.10. Die durchschnittlichen Schätzungen der *F*-Statistik waren F_{IS} = 0.117 ± 0.019, F_{IT} = 0.158 ± 0.019 und F_{ST} = 0.047 ± 0.005. Die proportionalen Anteile der Rassen, die das Ausmaß der Durchmischung zeigen, bewegten sich von 66 % - 98 % für Individuen die Zebu-Rassen zuzuordnen sind und 24 % - 85 % für Individuen die der Baoule-Rasse zuzuordnen sind. Das Dendrogramm zeigte die Baoule Süd-West deutlich segregierend von den anderen Populationen, die Baoule×Zebu Kreuzungstiere erscheinen als mittlere genetische Gruppe zwischen den Baoule Süd-West und Zebu Nord Populationen.

In der Studie der X-chromosomalen Mikrosatelliten wurde ein durchschnittlicher Abstand zwischen benachbarten loci von 6,02 ± 6.23 cM und 232 Allelen erhoben. Die Anzahl von beobachteten Allelen pro locus war 8.92 ± 3.59 gegenüber 4.46 ± 2.64 effectiver Allele pro locus und 0.69 ± 0.18 für Diversität der Gene. Das durchschnittliche switching wurde mit 0.1148 ± 0.076 geschätzt. Das daraus berechnete Alter der Admixture liegt unter der Annahme von einem Generationsintervall von 6 Jahren beim Rind bei 69 ± 43 Jahren im Jahr 2007. Die relative geringe genetische Differenzierung der unterschiedlichen lokalen Rinderpopulationen in Burkina Faso ermöglicht eine züchterische Bearbeitung zur Verbesserung der Produktion.

64 Trypanotoleranz-Kandidaten-SNPs wurden genotypisiert, 9 Blöcke mit 32 SNPs wurden daraus konstruiert. Bei der Analyse der Blöcke konnten, basierend auf den Rassenkompositionen in diesen Regionen verglichen mit den Rassenkompositionen im verbleibenden Genom fünf Blöcke als potentiell involviert in die Krankheitskontrolle abgesichert werden. Die Absicherung erfolgte auf Grund eines Defizites von Zebu-Allelen in den Kreuzungen im Bereich von -0.15 ($p = 0.0238$) bis -0.34 ($p<0.0001$). Diese SNP-Blöcke liegen innerhalb von krankheitsassoziierten QTL-Regionen.

Die Kreuzung empfänglicher mit trypanotoleranten Rassen wie dem Baoule-Rind kann dabei helfen, das Auftreten von Trypanosomosis beim Rind zu reduzieren und Teil eines integrativen Kontrollverfahrens sein. Weiterführende Forschung, auch Feldforschung, ist nötig, einerseits um die bestmögliche Komposition der Rassen zu finden, andererseits für die Auswahl eines Sets herkunfts-informativer Marker, mithilfe derer die Rassenkomposition einzelner Tiere kostengünstig festgestellt werden kann.

Introduction

Cattle are considered to have been domesticated 8000 to 9000 BP (MacHugh *et al.*, 1997; Troy *et al.*, 2001; Helmer *et al.*, 2005; Taberlet *et al.*, 2008) during the Neolithic phase from a wild ancestor now extinct *(Bos pimigenius primigenius)* in the Near East. *B. primigenius primigenius* was thought to be the ancestor of the humpless taurine *(B. Taurus)*, while *B. primigenius namadicus* the wild ancestor of *B. indicus* (the humped zebu) (Loftus *et al.* 1994a; Bradley *et al.* 1996; Bradley& Magee 2006) was supposed to originate from the Southern Asian subspecies of aurochs. Those arguments asserted two cattle domestication events in Asian continent. However, other authors hypothesized that *B. indicus* populations diverged from *B. taurus* cattle at a later date through breeding and selection (Epstein, 1971; Epstein and Mason, 1984; Payne, 1991; Loftus *et al.*, 1994b). Therefore they considered a unique cattle domestication event. Added to this controversial debate of considering 2 independent or 1 domestication event in the Asian continent Bradley *et al.* (1998), Troy *et al.* (2001), Hanotte *et al.* (2002) and Zeder *et al.* (2006) revealed the African origin of cattle. That makes the assessment of cattle origin much more complex and the relationships of the two types of cattle always contentious with various hypotheses having been proposed to account for the morphological and genetic differences observed between the two subspecies.

Beyond the controversy, it is agreed that domesticated cattle are a major component of pastoral economies throughout the world. Through milk, they provide the bulk of the animal protein consumed by many human societies, and contribute other important commodities including meat, hides, traction and dung. However many constraints to livestock do exist. One of the major constraints to livestock and mixed livestock farming in Africa is African Animal Trypanosomosis (AAT) according to many studies (Naessens *et al.* 2002; Waaij *et al.*, 2003; Courtin *et al.*, 2008). Indeed it affected approximately 11.5 million sq. km in West and Central Africa and about 38 % of African cattle are considered to be at risk of contracting the disease. Annual losses in meat production are estimated at US$ 5 billion (Agyemang, 2005).

In Burkina Faso, between 1989 and 1992 livestock losses ranged from 75 % to 85 % in individual herds (Kamuanga *et al.*, 1995). Nearly 6 million animals have been treated in 2005 (MRA, 2006) with most treatments in cattle, 97.95 % of the whole treatments. Among the animals, the Baoule like most taurine breeds in West Africa in general inherited resistance to

trypanosomosis (trypanotolerance) that allows them to inhabit areas infested with tsetse flies (*Glossina spp*.). By contrast, Zebu cattle possess no innate resistance to trypanosomosis and have only started to penetrate the tsetse infested regions with the assistance of veterinary prophylaxis and other control methods (Taylor, 1998; Steverding, 2008; Achukwi & Musongong, 2009). To our knowledge, there is no vaccine available up today.

These migrations of zebu cattle pose a serious threat to the genetic integrity of valuable trypanotolerant populations of taurine cattle in the southern areas of West and Central Africa (MachHugh *et al.*, 1997). In Burkina Faso, the migrations have been favored by many drought episodes that occurred (Paturel *et al.*, 1998) and the shift of the Northern limit lines of tsetse flies (Courtin *et al.* 2010).

A survey has been conducted in the North, the South-West and the West of Burkina Faso in order to assess the importance of trypanosomosis regarding other diseases that affect the herds, to state the different methods used among the existing methods to control trypanosomosis in the study area, and also to state the method used by farmers to handle resistance to trypanocidal drugs.

Apart from the survey a genotypic characterization has been carried out on the three regions. That characterization is an accepted method and being widely used for documenting domestic animals genetic resources. It has been as a method of describing and classifying livestock breeds using measures of genetic distances between populations (Nei *et al.*, 1978; Reynold *et al.*, 1983) based on molecular genetics tools. Among molecular techniques, microsatellites have been and still are favourite markers for population genetic studies due to their high number in the genome, extremely high degree of polymorphism. They are easy to automate with the possibility of multiplex application and easy to detect. They have been effectively exploited to understand bovine domestication and migration pattern (Bradley *et al.*, 1994; Loftus *et al.*, 1994; Edwards *et al.*, 2000) and to evaluate genetic diversity and relationships among cattle populations (MacHugh *et al.*, 1997; Canon *et al.*, 2001; Ibeagha-Awemu *et al.*, 2004; Zerabruk *et al.*, 2007; Dadi *et al.*, 2008; Kugonza *et al.*, 2010). In this study the markers have been used to not only study the diversity but also the level of admixture of the cattle and estimate the age of admixture of the crosses between the Baoule breed and the Zebu.

The thesis is presented in 5 main sections:

A background gives general information about Burkina Faso. This information includes a view on the location of the country, its climate, hydrography, vegetation that have a big influence on the main activities undertaken in the country. With this respect the agriculture in general of Burkina is described with a special focus on livestock. The background also comprises information about African Animal Trypanosomosis, the way it is transmitted as well as the existing control methods applied over the world and also in Burkina Faso.

The second section gives information about the material used for the study and the methods that have been used to collect the data from the field, in the laboratory, the softwares and the analyzing methods.

The achievements of the study have been presented in the 3rd section. Indeed, the survey results showed how severe is the trypanosomosis compared to other diseases, the mostly control methods used by interviewed farmers, the breed status regarding the disease and so on. In the same section the cattle populations' diversity has been described, the level of admixture and the age of admixture have been also assessed. Trypanotolerance candidate regions have been confirmed.

The results have been discussed in the last section. Comparisons of our results to previous studies in different countries with different breeds and management systems have been made. Interpretations based on personal knowledge and literatures have been given.

1 – Background

1.1 – Burkina Faso

Burkina Faso is a Sahelian country with an economy almost totally based on agriculture, livestock and forestry. Considering the annual average population growth (2.8%) reported by UNDP (2008), the population was estimated in 2009 at 15,224,780 inhabitants (INSD, 2010a). The population is relatively young, 47.98 % of the population was under 15 years in 2009. According to UNECA (2006) and FAOSTAT (2010), around 92 % of the population is involved in agricultural activities. The agriculture sector contribution to the Gross Domestic Product (GDP) was estimated at 32 % in 2008 by FAO (2010). The conditions in Burkina Faso, like in the other Sahelian countries, are not really favorable for agricultural production. Despite this situation, the farmers are trying to boost the economy as well as they can, using mostly rudimentary means.

Burkina Faso is one of the 8 member states of the West African Economic and Monetary Union (WAEMU) and the 15 member states of the Economic Community of West African States (ECOWAS) West African organizations with which it exchanges many products such as livestock.

1.1.1 – Geographical location and relief

Burkina Faso is situated in West Africa at 9°20'S to 15°N and 5°30'W to 2°30'E. It is land-locked without any sea border, the country is around 500 km far from the Atlantic Ocean. It is limited to the North and the West by Mali, to the East by Niger, to the South East by Benin and to the South by Togo, Ghana and Côte d'Ivoire. The area of Burkina Faso is 274,200 square kilometre (sq. km). It has been divided into 13 regions, 45 provinces, 335 districts and 8,500 villages.

The majority (about 75%) of the Burkinabè territory lies on Precambrian crystalline platform turning the overall relief flat. The average altitude is 400 m above sea level (a.s.l.), while the highest altitude is Mount Ténakourou (749 m a.b.s.l) in the South-West.

Two main topographic domains cover the territory of the country: a large peneplain covering ¾ of the country and a sandy massif in the South. Besides the two domains, one can notice a few specific forms of relief such as dunes, chains of hills and/or small mountains, crusted tables and a few cliffs.

The Southern part is less arid than the North, wooded savannah, gradually drying out into sand and desert in the North. The Sahara desert is relentlessly moving south, however, stripping the savannah lands of trees slowly turning the thin layer of cultivable soil into sun blackened rock-hard *lakenite*.

1.1.2 – Climate and hydrography

In general, Burkina Faso is characterized by a tropical climate of the Sudanese and Sahelian type. The wetness is very changeable. From an average of 400 mm in the North (Sahelian climate) it can go up to 1,200 mm in the South-West (MECV, 2001). The rainy season varies from 3 months to 7 depending on the regions. The end of the rainy season occurs by the end of September in the North and by the end of October in the South. The South, the South-West and West regions are better provided with rainfall than the others.

The temperatures are always higher than 0°C, monthly average temperatures rarely go beyond 30°C.

The hydrographic network includes three big international basins (MECV, 2001) which are: Volta basin (178,000 sq. km), Comoé basin (1,700 sq. km) and Niger basin (79,000 sq. km). Those basins are fed by many rivers such as Mouhoun, Nakambé, Nazinon in the central, Comoé in the South-West, and Niger.

Many streams, lakes and hydraulic dams are also found. Most of them are seasonal flow. Only three streams, Mouhoun, Comoé and Pendjari and are perennial but not navigable all year.

The hydraulic dams constitute 80% of the stocking capacity of the country's surface water resources. That represents 4208.7 cubic meters of surface water resources.

The underground water is estimated at 113.5 billion cubic meter but only 9.5 billion cubic are workable.

1.1.3 – Vegetation

Two main domains characterize the vegetation in Burkina Faso:

1.1.3.1 – The Sahelian domain

It is a grassy, bushy, shrubby and thicket steppe usually quite sparse. Ligneous species come locally sometimes together to form more or less penetrable bushes.

North of 14°N is the North Sahelian sector characterized by a batch of Saharan and Sahelian species which are found very rarely in the areas further south. The wetness in the sector is less than 600 mm, the dry season varies from 8 to 9 months.

The South Sahelian sector lies in 13°-14°N. The wetness is between 600 and 750 mm with 4 to 5 months rainy season. The Saharan and Sahelian based flora species enriches itself with Sudanese components. The vegetation physiognomy is always steppe type, demarcating the extension limit of savannah north by the thirteenth parallel.

1.1.3.2 – The Sudanese domain

The Sudanese savannah gradually takes over the steppe formations. This sector is intensively cultivated. The herbaceous ground cover fills out higher the ligneous species. From North to South, decline herbaceous, shrubby, bushy and thicket tend eventually towards a clear forest in the extreme South-East.

The Sudanese-North situated in 12°-13°N is a very populated sector. The savannah shows a regular rustic landscape of park type, predominated by big trees belonging to protected agro-forestry species. In the grassy stratum, the share of perennial species intensifies. In the ligneous one can notice a more important shrubby stratum. There are dry pockets of dense forests constituting "sacred woods". Those woods represent vestiges of ancient forest climates saved from clearing because they were protected by customary practices. The wetness varies from 750 to 1,000 mm and 6 to 7 months of dry season. The gallery-forests are dominated by Sudanese species.

The Sudanese-South is characterised by less arid climates. It includes the densest forestry formations. The rainy season varies from 8 to 6 months with 1,000 to 1,400 mm precipitation. This sector is distinguishable from the wooded savannah, the gallery-forests. The Sudanese domain is generally associated with the tsetse fly spread in Burkina Faso.

1.1.4 – Role of Agriculture and Livestock sector in the economy

Agriculture is the basic sector of Africa's economy. It is the most important activity in Burkina Faso, the main part of rural population occupations. Consequently, much attention is dedicated to issues pertaining to sustain agricultural development.

Agriculture is made, as in many African countries, on small fields with trees and low density of seedbeds. The arable land was estimated in 2007 at 5,202,120 ha (FAO, 2010). Agricultural productions in Burkina Faso are very irregular and highly dependent on meteorological and phytosanitary conditions. They are dominated by food-producing, cereals production in 2009/2010 was 3,626,637 tonnes (INSD, 2010b). The main cash crop is cotton, cotton and cotton fabric exports were estimated at 28.51 % of all exports 2009 (INSD, 2010c).

Agriculture is traditional, manual using human energy. Mechanization is less developed; the majority of the farmers are still using rudimentary means such as hoe, digging sticks, machete. But more and more the traction animal plough is used. Traction animals are mostly camel and donkey in the Sahelian domain, and cattle in the Sudanese domain, particularly in the cotton zone. According to Hauchart (2006) 77.3% of farmers in cotton zone are using draft animals.

Thus, animal is integrated to agriculture. In this integration, livestock is used for cultivation and transport of various products and materials. The manure from the animals is used as fertilizer, while crop residues are preserved and stored to feed animals.

In Burkina Faso 30% of the population are livestock keepers and 92% of them are living in rural areas (MED and MRA, 2004). Burkina Faso is among the best breeding countries in West Africa. Livestock is characterized by its importance and diversity, but also by an extensive and dominant production system. That system is well adapted to the seasonal and intra annual variability of pastoral resources.

Livestock production contributes to nutritional and food security as well as to poverty alleviation in Burkina Faso. It is the fourth main source of income of the country (gold: 46.25%, cotton: 28.51%, sesame seed: 5.45% and livestock: 1.61% of total exports). Livestock contribution to foreign currency earnings is estimated at 15% of GDP. But this contribution according to Nianogo and Thomas (2004), is most of the time underestimated as many of livestock products (skin and hide for instance) are accounted for in other sectors and as several by-products (manure, draft power).

The main species reared in Burkina Faso in economic terms are ruminants (Table 1). Livestock also play important cultural function, particularly for important traditional (dowry) or religious events and ceremonies, and serves as a source of prestige for traditional chiefs and merchants.

Table 1: Livestock size and growth rate in Burkina Faso

	Cattle	Sheep	Goat	Donkey	Horse	Camel	Pig	Poultry	
								Hen	Guinea fowl
Size	8,072,420	7,770,083	11,633,992	1,009,615	37,810	16,331	2,083,127	28,267,052	7,092,122
Growth rate(%)	2	3	3	2	1	2	2	3	

Source: MRA, 2008

1.1.5 – Livestock/cattle production system in Burkina Faso, especially cattle

While livestock keeping is possible in the whole country, large part of the livestock is found in Sahelian domain (MED and MRA, 2004).

1.1.5.1 – Transhumant/Sedentary systems

In Burkina Faso, most of the herders are mixed crop-livestock farmers. Most of the animals found in the systems above are indigenous breeds. Through natural selection over the years, the breeds got adapted to harsh environmental conditions and management systems.
Two main systems coexist at most species level: the improved systems and the traditional systems.

1.1.5.2 – Improved or intensive production system

An intensive dairy production is growing in importance in Burkina Faso, mostly in peri-urban areas of some big cities such are Ouagadougou, Bobo – Dioulasso and Dori. Those small livestock enterprises have invested imported dairy genes (pure or cross-breds imported from others countries of Africa, Europe or America). That is to meet the high demand for milk products. In 2007, 393 tonnes milk whole and fresh from cow and 2919 tonnes of milk whole dried have been imported to Burkina Faso (FAOSTAT, 2010).
Fattening cattle for sale are kept in rural areas as well as in peri-urban and urban areas. Several thousand bulls are kept in a stable to receive concentrate feeds indicated above and variable proportions of forage requirement in a feed bin. Sometimes the animals are grazed during part of

the day in order to minimize feed costs. The animals are mostly sold in the neighbouring countries. According to MRA (2008), 409,332 cattle have been exported in 2006.

These intensive meat and dairy productions can be very productive and generate a much needed income for farmers and national economy. At the same time these productions are reducing the need for meat and milk from the sub-region.

In the improved system, the pregnant, lactating and fattening animals receive natural or cultivated forage and variable amounts of concentrate feeds (cottonseed, cottonseed meal or cake, groundnut meal, cereal bran, etc.).

1.1.5.3 – Traditional or extensive systems

In general the traditional systems are extensive that use less materials inputs. They are mostly based on natural resources. The pasture was estimated in 2008, (FAOSTAT, 2010) at around 22 % (6,000,000 Ha permanent meadows and pastures) of Burkina Faso area. The animals in these systems essentially depend on pasture for their survival and production. They receive less or no concentrate feeds compared to those in intensive production. One can distinguish two types or sub-systems that are sedentary and transhumance. In the sedentary sub-system, livestock owners are mostly crop producers. They live essentially on their crops. Those owners have two up to ten animals. Some of them use cattle for cultivation and transport. The animals in such systems are generally reared in the villages and sometimes move not far away. Also they give value to areas that are unsuited for crop production (too poor land, too rocky or too sloping). However, when the herd size is larger a part of the animals are committed to someone (most of the time a Fulani) who may move for another villages far away. Indeed when the number of animals exceeds the carrying capacity of the land, the herders periodically leave their villages. Herders then move generally part or the herd to cultivated areas after grain harvest in order to benefit from standing crop residues. Eventually the animals are moved to remote but more humid regions where both feed and water are assured. Such favourable areas are found in sub humid and humid zones, but also in the floodplains. This sub-system is mostly the lifestyle of Fulani herders. Those transhumant people live on milk from cattle and money made from the sale of dairy products and animals.

Following the great droughts of 1973-1974 and 1984, a large number of Fulani sold part of their cattle to sedentary farmers while others migrated from Sahelian to Sudanese areas where they

have settled for good. These "new" sedentary livestock owners have developed a system where they cohabite with crop production. Livestock moves into marginal or fallow lands during the rainy season in order to avoid conflicts with crop producers; then comes back after harvest, to benefit from crop residues left standing in cultivated fields, and may migrate to more remote areas in search for water and better quality folder. However, the mobility is generally of smaller amplitude and of shorter duration than those who come from North and Sahelian regions.

Given that the traditional systems depend above all on natural resources they are threatened by the space restriction. Therefore, the risks of conflicts with crop farmers and other natural resources users are higher. That is also a reason why some livestock owners are settled in sub humid and humid zones where there is more space however they go through diseases, one of the main constraints of livestock production. Indeed in those zones Zebu cattle (the main breed of the sahelian regions) are susceptible to trypanosomosis which depreciates them much.

1.2 – Trypanosomosis

Trypanosomosis is a parasitic disease. It is caused by infection with trypanosomes, blood-borne, flagellate protozoan parasites (Picture (Pict.) A1 in Appendix) transmitted by tsetse flies (*Glossina spp*.). The trypanosomes replicate in the tsetse fly (Pict. A2 in Appendix) and are transmitted through saliva when the fly feeds on animal. Trypanosomosis is also mechanically transmitted by tsetse flies and other biting flies through the transfer of blood from one animal to another.

There are thirty one species or subspecies of tsetse flies in Africa classified in three groups, Moristans group living in wooded savannah; Palpalis group living in forest galleries or in marginal areas of forests; and Fusca group living generally in equatorial forests. Tsetse flies of the two first groups are met in Burkina Faso in the Sudanese domain where the density of the vegetation is higher and many rivers are encountered. The climate or microclimate in those areas is favourable to tsetse flies life. The main species of the flies met in Burkina Faso are *Glossina palpalis gambiensis*, *G. tachinoides*, *G. morsitans submorsitans*. They have been reared in 1980's for the Sterile Insect Technique (SIT) (Bauer *et al.*, 1984; Bouchon and Cognet, 1984). But according to McDermott *et al.*, (2003), *G. morsitans submorsitans* is less and less encountered in field.

Tsetse flies are not exclusively vectors of trypanosomosis. Other biting flies implicated in trypanosomosis are Haematopota, Liprosia, Stomoxys and Chrysops (Roder *et al.*, 1984).

Trypanosomosis in human being is sleeping sickness also known as Human African Trypanosomosis (HAT). The incidence of the disease has been on the rise with approximately 50,000 deaths in 1998 and 55 million people are considered to be at risk (Agyemag, 2005) HAT is caused by *Trypanosoma brucei rhodensiense* or *T. b. gambiense* infections (Kemp and Teale, 1998). In America human trypanosomosis is called chagas.

In African animals the disease is called African Animal trypanosomosis (AAT) or *Nagana* (a Zulu word meaning "to be depressed"). The disease in cattle is very often caused by three trypanosome species (O´Gorman *et al.*, 2006): *T. congolense, T. vivax and T. b. brucei*.

Zebu (humped) cattle are extremely susceptible while the taurine (humpless) cattle of Africa show remarkable tolerance (Taylor *et al.*, 1996; O´Gorman *et al.*, 2006). This tolerance enables the animals to live and be productive in tsetse challenged areas where other breeds can only survive with drugs and care.

1.2.1 – Description of the disease

Trypanosomosis is generally a chronic evolving disease which is usually fatal if appropriate treatment is not established. The incubation period varies from 4 to 40 days, depending of the involved trypanosome.

1.2.1.1 – AAT clinical signs and symptoms.

There are many hemoparasites (*Babesia spp., Theileria spp., Anaplasma spp., Ehrlichia spp*.) and also cases of simultaneous infection with more than one trypanosome species that it is sometimes difficult to conclude which clinical signs are attribute to a given parasite. Thus, different signs are observed in trypanosomosis. Also, depending of the trypanosome, the signs are different. But the cardinal clinical sign observed is anaemia that is characterised by decrease in packed cell volume (PCV), haemoglobin, red blood cell, and white cell levels. Parasitemia can also be observed at clinical level. The most common symptoms are: presence of intermittent fever, oedema and loss of condition; depression, lethargy, weakness, anaemia, salivation, lacrimation, nasal discharge, change of hair colour (black to metallic brown), accelerated pulse and jugular pulsation, hard breathing. The back of a suffering animal is often arched and the

abdomen “tucked up”. As said above the symptoms are variable according to the trypanosome that infected the animal, but most of those symptoms are observed with *T. congolense*. Abortion may be seen, and infertility of males and females is a common sequel. The disease can evolve from peracute, acute to chronic disease in cattle and the other susceptible animals (sheep, goats, horses, camels, pigs, dogs) depending again on the trypanosome.

1.2.1.2 – Diagnosis

The diagnosis of AAT can be conducted in field or at laboratory level. But the disease cannot be diagnosed with exact certainty through detection of parasites by microscopic examination of blood or by various serological reactions.

The evolution of the disease varies widely according to the trypanosome involved and the animal species or breed affected. In cattle the trypanosome usually causes asymptomatic infection. It's one of the reasons of not exact diagnostic and also because the symptoms are common with other diseases.

The conventional techniques of microscopic examination for the presence of trypanosomes are widely used. Microscopic examination consists of observing the presence of the parasites on a wet-mount of blood on slides, thick blood films stained with Giemsa or smears of buff coat (obtained by microhematocrit centrifugation). Lymph node smears are also used for examination. The parasites may be hard to demonstrate especially when parasitemia is low. In laboratory, different examinations are performed with serum, blood with anticoagulant EDTA, dried thin and thick blood smears, and smear of needle lymph node biopsies.

1.2.2 – Economic importance of AAT

People raise livestock for, largely, economic purposes. Given that trypanosomosis affects the health and productivity of livestock, therefore trypanosomosis has economic impact on farmers. That also affects the economy of the different countries where trypanosomosis is rife. The total land surface for the countries in West and Central Africa affected is approximately 11.5 million km^2 and represents about 37% of Africa as a whole involving 37 countries (Agyemang, 2005). To this purpose Kristjanson *et al.* (1999) assert that trypanosomosis is the most important constraint to livestock and mixed crop-livestock farming in tropical Africa. It is estimated that some 46 million of cattle are at risk. Annual losses in meat production are estimated at US$ 5

billion. If losses in milk, manure production and traction could be prevented, the benefit from livestock and mixed agricultural developments in tsetse infected Africa could amount to US$ 50 billion annually (Agyemang, 2005). It costs at least US$ 35 million in treatment annually, 17.5 and 38% of African cattle at risk million treated per year (Kristjanson *et al.*, 1999).

In Burkina Faso, most of the animals at risk are located in the sub-humid zones that represent 32% of the total land area (Ouedraogo-Kone, 2008). In contrast, risk of trypanosomosis is lower in the Sahelian domain (Sow *et al.*, 2008) where it even doesn't exist in some areas.

In the country, nearly six million animals have been treated in 2005 (Table 2) with most treatments in cattle. According to owners´ estimates in Southern Burkina Faso, the livestock losses ranged from 75% to 85% in individual herds between 1989 and 1992 (Kamuanga *et al.*, 1995).

In Burkina Faso, many efforts and resources have been invested in the control of trypanosomosis and the country benefit from the presence of the Centre International de Recherche-Développement sur l´Elevage en zone Subhumide (CIRDES) to apply many techniques developed in the centre.

Table 2: Trypanosomosis treatments in 2005 in Burkina Faso

	Cattle	**Sheep**	**Goat**	**Donkey**	**Horse**	***Total***
Curative treatments	3,436,600	57,690	4,956	15,521	334	***3,515,101***
Preventive treatments	2,411,932	12,337	1,254	29,830	502	***2,455,855***
Total	***5,848,532***	***70027***	***6210***	***45351***	***836***	***5,970,956***

Source: MRA, 2005

1.2.3 – Different approaches to control the disease

Many organisations have been involved in tsetse and trypanosomosis controls such as Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) launched in 2001 (Schofield and Kabayo, 2008), International Livestock Research Institute (ILRI), International Livestock Centre for Africa (ILCA), International Laboratory for Research on Animal Diseases (ILRAD), International Scientific Council for Trypanosomosis Research and Control (ISCTRC), Programme Against African Trypanosomosis (PAAT), CIRDES. So many organisations involved give evidence that trypanosomosis is not easy to fight. But also as Agyemang (2005)

argued, tsetse-trypanosomosis problem should be seen in the context of rural poverty and agricultural development because it has direct severe implications on current and future land use and related development.

Thus, several methods have been used to control tsetse flies and trypanosomosis in Africa. The first control of tsetse flies has taken place in 1900's according to Challier (1984). Methods used in Burkina Faso (like trypanocidal drugs, insecticides, bush clearing, traps and targets, SIT, trypanotolerant animals, etc.) are part of the former and currently used methods. Unlike other insects, for tsetse flies there are no seasonal interruptions in their life that make the control much more difficult. Indeed, many methods have been used or still in use to control trypanosomosis through the vectors or the parasites themselves. Some of them are usable at farmers' level but others need involvement of governments and partners as well.

1.2.3.1 – Vector control

1.2.3.1.1 – Indirect tsetse control methods

1.2.3.1.1.1 – Bush clearing

The microclimate established by plant cover provides a suitable combination of temperature and humidity for the tsetse flies that are sensitive to the variation of those factors. Since people have known that tsetse flies are concentrated in certain areas. That leads to numerous bush-clearing all over West and East Africa.

Destruction of the vegetation can be done manually, by felling the trees or by mechanical means. Thus when the vegetation is cleared, changes occur in the microclimate that drastically alter and maintain the area unsuitable for tsetse fly habitation. Despite the apparent success of this method, it is accepted that bush-clearing is unsuitable as a long term control measure. Regardless of the technique used, the destruction of vegetation presents drawbacks such as the increase of soil erosion, which is liable to cause sterility in the cleared land. There is also the fact the cleared lands can be quickly reinvaded.

This method is now rarely employed in large scale but still around the enclosures and the houses.

1.2.3.1.1.2 – Eliminating wild animals

More than thirty species of wild animals can be infected with pathogenic trypanosomes. Many of those wild animals remain carriers. Ruminants are widely known to be active reservoirs but also wild Equidae, lions, leopard, wild pigs are susceptible and can also serve as carriers. Studies of those hosts have shown that tsetse fly obtain much its nourishment from small number of wild animal species, which differ for each fly species. A control method has therefore been developed with the aim of eliminating the preferred hosts. The method has been employed to a considerable extent in some countries of East and Southern Africa at the cost of massive destruction of big game.

However, the elimination of wild animals, even if restricted only to host species, is not easy to accomplish. Especially when it involves destroying small animals like warthogs, bush pigs and small antelopes. Which are the favourite hosts of many tsetse species. It has also been observed that tsetse is not solely dependent on specific animal species. When the preferred hosts disappear it can feed on other species.

Those difficulties and the increasing concern for wildlife protection made that the control of tsetse fly by the selective elimination of wild animals is not recommended nowadays but also the wild animals are somehow rare.

1.2.3.1.2 – Direct tsetse control methods

1.2.3.1.2.1 – Insecticides

The treatment of tsetse infested zones with insecticides has been one the most common methods of eradication.

Insecticides may be applied from the ground or from the air.

The method consists in applying a persistent insecticide where it has the most chance of coming into contact with tsetse flies on their most frequent resting places. The preferred resting places depend on the tsetse species, the season and local ecological conditions. It is therefore essential to have knowledge of the biology of the species in the region to determine the types of vegetation to be treated.

The insecticides are applied in the dry season. That is to prevent it from being washed off by rain and also because the severe conditions prevailing in the dry season force the flies to concentrate on certain types of plant which provide a favourable microclimate as said before.

Whether aerial or from the ground residual insecticides such as organochlorines (Dichloro-Diphenyl-Trichloroethane (DDT), Diedrin, Endosulfan), pyrethroids (deltamethrin, permathrin and alphamethrin), and avermectins (ivermectin) are used to target areas where human and animals - to- fly contact are likely. Usual insecticides like DDT and Dieldrin both persist for several months on vegetation. Also some insecticides such as organochlorines bioaccumulate in the food chain and are highly toxic to mammals and other vertebrates. Despite being effective, the use of organochlorines and organophosphate are now banned for widespread outdoor spraying (Leak, 1999). Insecticides can be applied with pressurized sprayers, or motorized or high capacity vehicle-mounted sprayers, or aircraft.

Animals are also treated with insecticides in some areas like in Burkina Faso where flumethrin have been used pour-on for cattle (Bauer *et al.*, 1992). Insecticides can be also put in a solution in deep tank or a foot bath (Stachurski *et al.*, 2005) to fight against flies and other ectoparasites.

Depending on the means used, this technique has shown satisfactory results but tsetse flies reappeared in some treated zones. So, it is difficult to indefinitely isolate the treated area. The drawback of that technique is not only the high cost (when using a vehicle or an aircraft or helicopter, well-equipped and well-trained spraying team). But also environmental pollution, intoxication of animals (wild and domestic) fish, insects, etc. and the team members when they are not well-equipped and also third parties mostly when using aerial spraying. There also concerns about non-tsetse targets (ticks for example) becoming resistant to some insecticides, such as pyrethroids, used on livestock (Agyemang, 2005).

1.2.3.1.2.2 – Targets and traps

Traps and targets are mechanical devices used to kill or weaken tsetse flies through insecticides or various trapping methods. The taps and targets attract tsetse flies by taking advantage of their host-seeking behaviours, visual and olfactory stimulation. Those devices are usually deployed in and around areas where human-fly or animal-fly contacts are greatest, such as streams frequented by villagers or animals, or fringes of cultivated fields. The developments of potent attractants as well as the production synthetic pyrethroid insecticides are making this form of control technique

highly successful (Wall and Langley, 1991) because tsetse flies have a low rate of reproduction and require a little sustained mortality pressure to bring about a reduction in population from an area. But given the migration of the tsetse flies, an area where the population had been reduced or even eradicated can be reinvaded by tsetse flies. Added to that, targets and traps have a limited land covered.

1.2.3.1.2.3 – Sterile Insect Technique

One of the modern methods for controlling tsetse flies by non-insecticidal means is SIT (Kamuanga *et al.*, 2001; Schofield and Kabayo, 2008). It relies on the mating of wild females with sterile male flies (male sterilized by irradiation or chemistry or physiologically). Physiologically, female tsetse flies are only required to mate once their lifetime (female lives longer than male) and store sperm in its spermathecae in sufficient quantity. Thus, such quantity can fertilize over its entire reproductive life. Then mating with a sterile male would result in no offspring. However, SIT was considered to be impractical for control of high-density tsetse population above 1000 males per square mile due to the large number of sterilized males that would be required. For SIT to be effective, it has been estimated that 10% of the females in the population need to be inseminated, and in order to achieve that, the number of sterile males released must constitute 80% of the male population (Rogers and Randolph, 1985).
So, SIT has limitations like effectiveness for continent wide, the high cost and time associated with mass rearing the large numbers, and the potential for re-invasion of areas cleared of tsetse (Molyneux, 2001).

1.2.3.1.2.4 – Application of transgenics

Tsetse flies harbor three bacteria that are *Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia*. The transgenic technique uses the third one that harbored in certain tsetse species (Askoy *et al.*, 2002). It consists in injecting transformed symbiont (*Wolbachia* in this case) into tsetse flies. As it is known a common reproductive abnormality *Wolbachia* induce, termed cytoplasmic incompatibility (CI) which when expressed results in embryonic death due to disruptions in early fertilization events (Bandi *et al.*, 1999). In an incompatible cross, the sperm enters the egg but does not successfully contribute its genetic material to the potential zygote. It results then, in most species, in a low number of viable eggs.

The goal is to replace the natural susceptible population with their engineered refractory counterparts. Genetically engineered females have a reproductive advantage, over their uninfected counterparts, because they produce progeny after mating with both *Wolbachia*-infected and uninfected males.

This approach is limited by the fact that the antibiotic treatment results in the clearing of all bacterial symbionts. The clearing including the obligate symbiont *Wigglesworthia*, in the absence of which, the flies become sterile (Aksoy *et al.*, 2002).

1.2.3.2 – Parasite control

1.2.3.2.1 – Chemotherapy and chemoprohylaxis in cattle

The use of drugs for the prevention and treatment of trypanosomosis has been important for many decades. In West Africa, those drugs protect 17 million head of cattle from trypanosomosis (Kristjanson *et al.*, 1999).

The often and widely used chemotherapeutic drugs are diminazene aceturate (Berenil), isometamidium chloride (Samorin, Trypamidium) which are effective against AAT. There is also humidium salts (Ethidium, Novidium).

Isometmidium chloride is also a chemoprohylactic drug that has been in use for over than twenty years. It gives protection for 3 - 6 months. Added to this, older drugs such as the quinapyramine derivates Antrycide and Antrycide Prosalt are still in use and give effective protection up to 3 months and also purithidium bromide for a protection up to 6 months.

The action of the different trypanocides varies according to the animal species infected and the trypanosomes involved. Therefore using a trypanocide without knowing the trypanosome involved (which is not possible in some areas) leads many times to no or less effectiveness.

Also, the trypanosomes have developed resistance to each drug introduced that has tremendously complicated this approach to controlling the disease. This is a particular concern for smallholder crop. Livestock farmers in the cotton-zone of West Africa studies report increasing resistance to trypanocide drugs in many countries (Affognon *et al.*, 2006; Grace, 2006; McDermott *et al.*, 2003). For Diall *et al.* (2005), trypanocide resistance has become a major handicap to the control of trypanosomosis in West Africa.

Drugs do provide protection, in some cases it may last up to six months, but all of them frequently give rise to drug resistant against trypanosome strains. The drug resistance occurs when the trypanosomes are in contact with a trypanocide administered in a subcurative dose not enough to ensure the destruction of the parasites.

Although extensively used in trypanosomosis control, chemoprophylaxis and chemopherapy are expensive (Kristjanson *et al.*, 1999), time-consuming, unsatisfactory long-term solution. Additionally, the parasite is able to switch the variant surface glycoprotein (VSG) coat that it is covered with from one to another resulting in waves of parasitemia so that it can't totally be eliminated by antibodies (Taylor *et al.*, 1996; O'Garman *et al.*, 2006). That is one reason of not finding an effective vaccine against *trypanosoma spp*..

1.2.3.2.2 – Breeding trypanotolerant animals

Some African cattle breeds are recognized to be more resistant to African trypanosomosis that others. This *Bos taurus* has been domesticated in West Africa many century ago (Hanotte *et al.*, 2002). Among the breeds, there are N´Dama and West African humpless short-horned cattle (Muturu, Baoule, Laguna, Samba, and Dahomey depending on the region). They have existed in the tsetse challenged zones for long and therefore acquired an immunology phenomenon (trypanotolerance) that has a genetic basis (Naessens *et al.*, 2002; Agyemang, 2005). These animals have a capacity to rid themselves of trypanosome parasites and maintain low parasitemia.

Thus, trypanotolerant animals have been introduced in other tsetse affected countries of Africa in order to exploit their genetic advantage. They have been reared in pure system or crossed to other breeds. Trypanotolerant breeds represent a small proportion (6%) of the cattle population of Africa and 17% of the cattle population in the tsetse challenged areas (Agyemang, 2005).

Compared to others, the option of using those breeds in breeding systems, thus reduces or eliminates the use of chemicals to control trypanosomosis vector and other parasites, contributes positively to a balanced ecosystem health. Unfortunately most of these taurine cattle types are very small in body size, with height at withers of less than 100 cm. This makes them much less useful for ploughing purposes than the susceptible Zebu populations found nearby.

2 – Materials and Methods

2.1 – Study areas

The study was conducted in three geographically different regions but four regions accordingly with Burkina Faso official cut-out. The West in this study comprised Hauts Bassins region (Houet and Kénédougou provinces) and Cascades region (Léraba province). The Léraba province has been added to the previous list because one year before the survey there has been an important epidemic event of trypanosomosis in there (Dr. Dominique Ilboudo, personal communication). This event had not been well documented but preliminary results of laboratory investigations confirmed it.

Average annual rainfalls are between 575 mm in the North and more than 1200 mm in the West (Figure 1). The vegetation belongs to the South-Sahelian and the South-Sudanese sectors as described above. The South-Sahelian sector is situated nearby the tsetse belt area and the South-Sudanese sector in tsetse challenged area. The trypanosomosis prevalence ranges from 6 to 26.7 % (McDermott, 2003).

The livestock production system is widely traditional and mixed with crop production. The main breed in the challenged area was taurine cattle (trypanotolerant) but since the first great droughts, some farmers settled with Zebu cattle (trypanosusceptible) despite the different drawbacks of trypanosomosis and other diseases encountered. The proportions of cattle stocks in the study sites varied from 3.77% of the whole Burkina Faso cattle stock in the South-West to 14.195% in the West (cattle stock of Hauts Bassins in the West is the second biggest stock after Sahel region) (Figure 2).

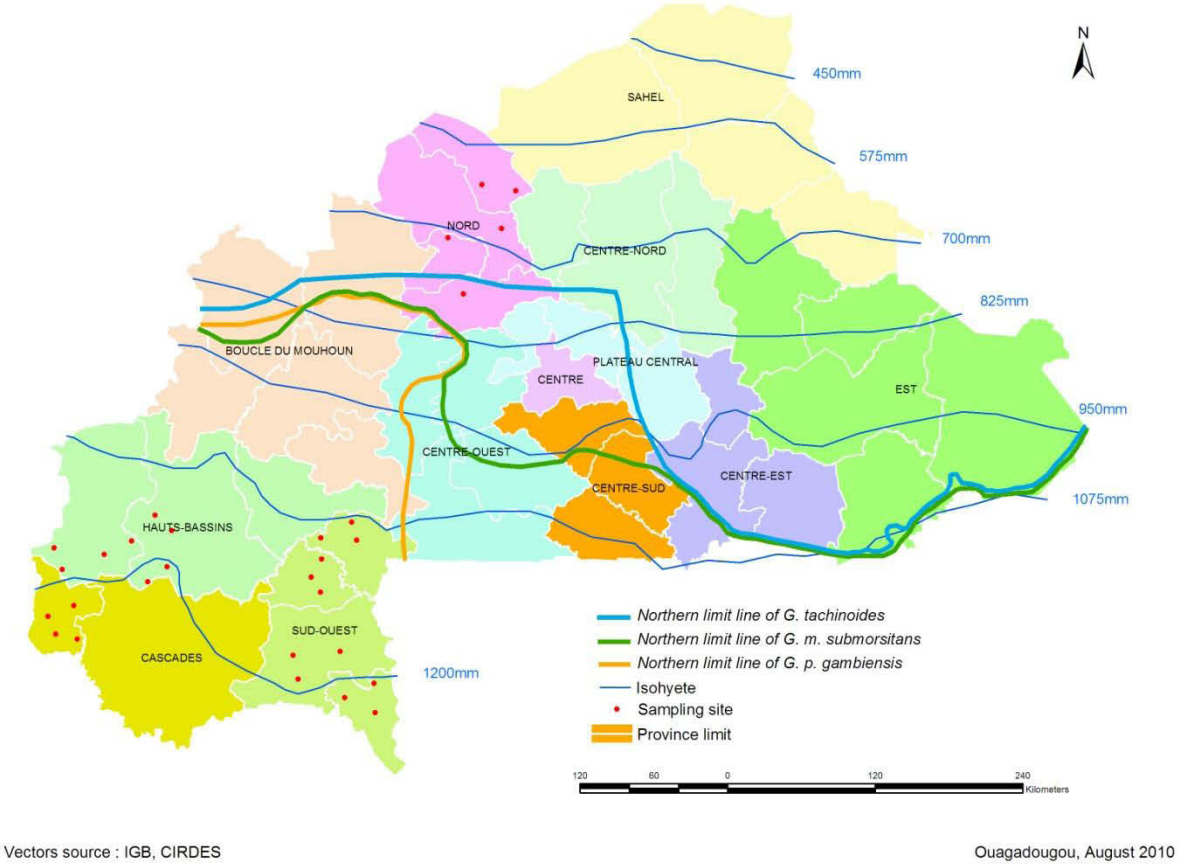

Figure 1: Sampling locations and tsetse limit lines

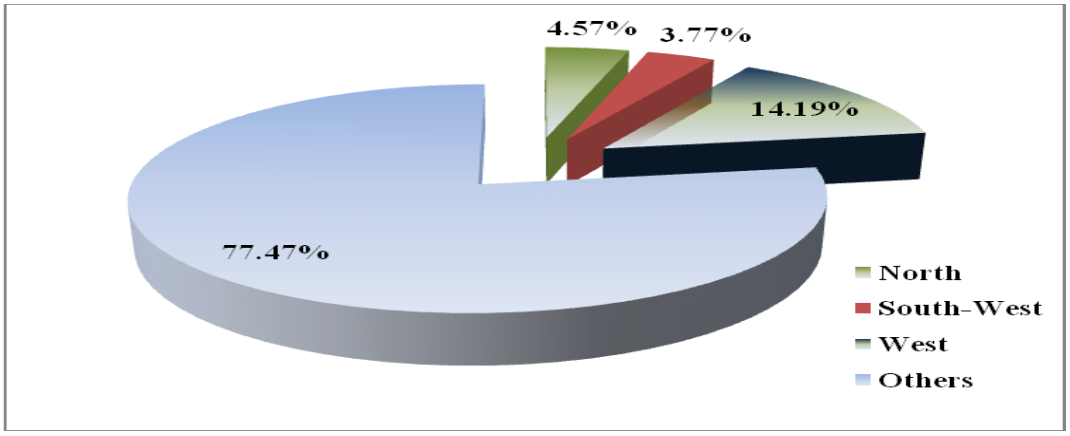

Figure 2: Cattle stocks proportion of the studied regions

2.2 – Data collection

2.2.1 – Sites design and farmers selection

Villages in each region were selected in close collaboration with the regional animal breeding staff. It was based on the availability of cattle but also the availability of the farmers to allow blood sampling from their animals. Among the 3 regions chosen for this study, the North was considered as a negative control regarding trypanosomosis. Only 5 villages were selected in the North. In the tsetse infested areas, 12 villages were selected in the South-West and 12 villages in the West as well.

An Etrex H Garmin global position (GPS) device employing a satellite navigation system was used for definition of the particular geographic location of the different villages that were sampled. Data for 29 geographic positions determined for each village includes altitudes, latitudes and longitudes of the locations.

The sites were situated between 12° and 14°N and 01° and 03°W in the Northern, between 09° and 12°N and 02°and 04°W in the South-western, and between 10° and 12°N and 04° and 06°W in the Western Burkina Faso (Table 3). The altitude varied from 277 m in Orkounou (South West) to 522 m in Koloko (West).

2.2.2 – Survey

A questionnaire consisted of 28 open ended and closed questions has been used to interview owners of the animals or herdsmen. They were asked individually about the composition of the herd, the different diseases affecting the herd, trypanocidal drugs in use, experiences on resistance with trypanocidal drugs and how resistance is handled, background (ancestry) of the sampled, etc. A total of 134 persons were interviewed, 24 in the North, 55 in the South-West and 55 in the West (Table 3). Farmers were identified regarding sedentary lifestyle. Thus, in addition to autochthonous in the 2 regions, sedentary migrants were interviewed as well as in the North. Autochthonous accounted for 55.22 % of interviewees. 41.67 % of interviewed migrants were settled from 11 - 20 years before the interview, the others settled from 1 - 10, 21 - 30 and more than 30 years.

The interviews were made in presence of an official local breeding technician in each village. A translator was needed when the researcher did not understand the local language. The survey was conducted from July 2007 to November 2007.

Table 3: Regions selected for the study, geographic coordinates of villages and number of interviewees

Region	Province	Village	Altitude (ft)	Latitude	Longitude	Interviewees
North	Yatenga	Nommon	981	13°49.557'	002°42.594'	4
		Séguénéga	1102	13°23.320'	001°59.062'	5
	Lorum	Sala	1137	13°47.959'	002°06.911'	4
	Zondoma	Marmisga	1113	13°22.482'	002°22.319'	6
	Passoré	Yako	1115	12°56.738'	002°17.415'	5
South-West	Poni	Gaoua	1091	10°18.206'	003°10.319'	3
		Takouloula	924	10°06.911'	003°29.669'	4
		Loropéni	1120	10°15.230'	003°30.834'	4
	Ioba	Dano	929	11°09.328'	003°03.791'	5
		Oronkua	1006	11°14.681'	003°05.768'	5
		Bouni	895	11°07.960'	003°21.108'	5
	Bougouriba	Orkounou	910	10°45.626'	003°19.688'	5
		Dollo	853	10°52.914'	003°24.009'	4
		Djonkargo	1006	11°00.244'	003°19.368'	5
	Noumbiel	Midebdo	1057	09°58.595'	003°12.065'	4
		Batié	1043	09°52.711'	002°54.708'	4
		Kour	962	10°08.639'	002°54.634'	6
West	Kénédougou	Koloko	1877	11°08.746'	005°17.550'	5
		Kagnabougou	1700	10°55.040'	005°14.779'	5
		Lidara	1816	11°01.965'	004°56.422'	4
		Banflaguè	1409	11°09.143'	004°44.963'	4
	Houet	Nasso	1183	11°15.156'	004°24.873'	4
		Toussiana	1582	10°50.579'	004°36.361	4
		Péni	1696	10°58.126'	004°29.461'	5
		Soungalodaga	1078	11°22.502'	004°33.241'	5
	Léraba	Sindou	1266	10°40.061'	005°10.537'	4
		Loumana	1170	10°33.962'	005°21.289'	5
		Sobara	1113	10°26.926'	005°17.874'	5
		Kasséguera	1031	10°24.013'	005°07.399'	5

2.2.3 – Blood sampling

Blood samples were taken from 1045 animals that belonged to 3 assumed breeds. Out the total 471 were males versus 574 females. An average of 8 animals was sampled within each herd in each village. Blood was collected with needle from jugular vein (Pict. A3 in Appendix) in EDTA tubes. The whole blood of each individual was dropped onto a Whatman FTA card according to

Whatman protocol BD01 (www.whatman.com) and dried. FTA cards are impregnated with a patented chemical formula that lyses cell membranes and denatures proteins on contact. Nucleic acids are physically entrapped, immobilised and stabilised for storage at room temperature. FTA cards protect nucleic acids from nucleases, oxidation, UV damage and microbial and fungal attack. Infections pathogens in samples applied to FTA cards are rendered inactive on contact. The cards were labelled with sample identification (province, village, farmer number, animal number) enclosed in pouches and stored till punching day.

The distribution of those breed Baoule (B), Zebu (Z) and of Baoule×Zebu (BZ, crossbreed) and herds are presented in Table 4 while the pictures of representative of each breed are presented in Appendix (Pict. A4; A5 and A6).

Table 4: Frequencies of herds' types (breeds in) of the interviewees in the different regions and provinces

		Types of herds (n)							
Regions	Provinces	B	BZ	BZ+Z	B+BZ	B+BZ+Z	B+Z	Z	Total
North	Lorum	0	0	0	0	0	0	4	4
	Passoré	0	0	0	0	0	0	5	5
	Yatenga	0	0	0	0	0	0	9	9
	Zondoma	0	0	0	0	0	0	6	6
South-West	Bougouriba	6	1	1	2	4	0	0	14
	Ioba	1	0	1	5	8	0	0	15
	Noumbiel	5	0	4	0	1	2	3	15
	Poni	3	0	1	1	5	1	0	11
West	Houet	0	8	7	1	2	0	0	18
	Kénédougou	0	1	3	5	9	0	0	18
	Léraba	0	6	0	7	6	0	0	19
Total		15	16	17	21	35	3	27	134

B = Baoule; BZ = Baoule×Zebu; Z = Zebu

2.2.3 – DNA isolation

Harris punch of diameter 3 mm has been used to remove sample discs from the spotted cards (**Whatman FTA Protocol BD09**) (Pict. A7 and A8 in Appendix).

From the total of 1045 animals, 300 males and 150 females have been selected for genotyping. All 450 animals were surveyed with the autosomal markers while only males were used with sex-chromosome markers.

Genomic DNA was isolated from blood white cells according to a modified protocol of Whatman (**Whatman FTA Protocol BD08**) as follow:

3 discs of 3 mm each taken from spotted blood on FTA card were put in 1.5 ml standard tube. In the tube, 360 µl of Whatman FTA Purification Reagent containing 60µg/ml Proteinase K were added. Incubation has been made at room temperature (RT) overnight.

The day after a pipette was used to mix the contents up and down without vortexing. The used reagent was removed and discarded. After that, 360µl of Whatman FTA Purification Reagent without Proteinase K was put in the tube and incubated at RT for 30 min. During the incubation the content was mixed after 10 and 20 min. After 30 min the supernatant was removed. The wash without Proteinase K was repeated two more times.

The discs were again incubated 4 more times:

- First with 480 µl of 0.3M Sodium Carbonate for 5 min at RT,
- Second with 1200 µl of 0.5% SDS also for 5 min at RT,
- Third and fourth with 430 µl TE^{-1} buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) at RT for 15 min. During this incubation, the buffer was mixed after 5 and 10 min. Then the tube was briefly centrifuged to collect remaining buffer in one place and the last drops removed.

After a total of 8 washes the discs were left in the tube and dried at 65 °C for an hour.

The dried discs were then put in a new tube where 120 µl of 1% PCR buffer were added and incubated at 95°C for 15 min. The discs were squeezed and removed; 120 µl of double distilled water were added to the eluate which has been then used for PCR.

2.2.4 – DNA amplification

For the autosomal chromosomes, a total of 31 microsatellites primers have been chosen for the amplification of the genomic DNA. 15 of them were donated by the International Livestock Research Institute, Nairobi, Kenya. PCR conditions were optimized and all the 31 microsatellites tested for polymorphism. A final panel of 25 microsatellites was selected for genotyping of the cattle populations. 22 microsatellites out of them (BM1818, BM1824, BM2113, CSSM066, ETH3, ETH10, ETH185, ETH225, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL13, ILSTSS005, ILSTS006, INRA023, INRA032, TGLA53, TGLA122, TGLA126, TGLA227) were from a list recommended by the Food and Agriculture Organization (FAO) and the International Society for Animal Genetics (ISAG, http://ww.projects.rosalin.ac.uk) for use in cattle diversity studies. The others, namely AGLA293, ILSST033 and MGTG4B, were out of both the FAO and the ISAG list. The microsatellites were selected combining information from both the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) database and BOVMAP (http://locus.jouy.inra.fr/cgi-bin/bovmap/intro2.pl) as well. The selected microsatellite primers covered 22 autosomal chromosomes regions.

For the sex-chromosome, from an initial set of 30 microsatellite markers chosen for genotyping on the bovine chromosome X (BTAX), a final panel of 26 markers was kept for the genotyping process (Table 13). Indeed 26 primers were polymorphic out of the initial number and therefore held for the study. The loci physical positions were drawn using information available on the NCBI database.

64 single nucleotide polymorphism (SNP) markers were selected from Illumina bovine database. The information on best guesses of resistance candidate regions has been kindly provided by Dr. Miika Tapio (Biotechnology and Food Research of Finland) and Dr. Paul Boettcher (Animal Production and Health Division of FAO).

A PCR reaction mixture with the final volume of 22 µl included 10 ng template genomic DNA was used in both autosomes and sex-chromosome amplification. 8.05 µl of double distilled water, 3.20 µl of 10 x Buffer B (Mg^{2+} free containing 0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20), 2 µl of 2 mM dNTP-Mix, 1.60 µl of 25 mM $MgCl_2$, 0.5 µl of each forward and

reverse primers and 0.15 µl of 5 U/ µl FIREPoL® DNA polymerase. One primer in each pair was labelled FAM or TET.

In case of SNPs, PCRs were different. Indeed, the first PCR which was a normal has been run. The mastermix comprised 0.50 µl of 10 x Buffer, 0.1 µl of 25 mM dNTP-Mix, 0.40 µl of 25 mM $MgCl_2$, 0.5 µl of each forward and reverse primers and 0.20 µl of 5 U/ µl HotStar Taq DNA polymerase plus 3 µl (3 µg) of Salmon sperm to the all mastermix. The DNA amount added to the mastermix remained the same as in previous PCRs. The total volume was then 4 µl. A digestion was made after the PCR with shrimp alkaline phosphate (SAP) The SAP cleaves a phosphate from the unincorporated dNTPs, converting them to dNDPs and rendering them unavailable to future reaction. The SAP mix has been made from 1.5 µl of water (HPLC grade), 0.17 µl of 10 x SAP buffer, 0.30 µl of 1.7 U/µl SAP enzyme. 2 µl of the SAP mix was added to the normal PCR product for digestion. The digestion was followed by an iPLEX PCR. The iPLEX mix was made of 0.619 µl of water, 0.20 µl of 10 x iPLEX buffer, 0.2 µl of iPLEX-Termination mix, 0.041 µl of iPLEX-Enzyme and 0.940 µl of the extent primer. 2 µl of the iPLEX mix was added to the digested product.

The following cycling program was run for amplification: 5 min initial denaturation at 95°C followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1:30 min, extension at 65°C for 3 min and final extension step of 65°C for 5 min using Applied Biosystems 96-Well GeneAmp® PCR System 9700 thermal cycler.

The normal PCR of SNP study was run according the following protocol: 2 min initial denaturation at 95°C followed by 45 cycles of denaturation at 95°C for 0:30 min, annealing at 56°C for 0:30 min, extension at 72°C for 1 min and a final extension step of 72°C for 5 min and 4°C for 5 min.

The digestion was run on 45 min: 40 min at 37°C and 5 min at 85°C. While the iPLEX PCR run as followed: 030 min of initial denaturation at 94°C, denaturation again at 94°C for 0:05 min, annealing at 52°C for 0:05 min, extension at 80°C for 0:005 min. Annealing up to extension (80°C) was repeated 5 times, from the second denaturation to the extension 40 repeats as well. A final extension step of 72°C was run for 3 min ended by 4°C for ever. The normal PCR, the SAP digestion and the iPLEX PCR were performed on using Applied Biosystems 384-Well GeneAmp® PCR System 9700 thermal cycler.

2.2.5 – Genotyping process

The PCR product was then diluted 1/10 in distilled water. From the eluate 2 µl were mixed with 3 µl diluted ET Rox 400 MegaBACE (Pict. A9 in Appendix) size standard (0.25 µl of ET Rox 400 in 2.75 µl). With a total volume of 5 µl per sample, genotyping was performed on MegaBACE^{TM} 500, fluorescence – based DNA system utilizing capillary electrophoresis.
The alleles were called and scored under MegaBACE^{TM} Genetic Profiler Software Suite v2.2 system (Pict. A10 in Appendix).
The PCR product of SNP markers was diluted in 16 µl distilled water. A resin was added to the samples to desalt using a dimplate plate. The reaction product was transferred onto a Sequenom's SpectroCHIP® by the *MassARRAY* nanodispenser (*Samsumg*). The SpectroCHIP® was then analyzed by the Sequenom's MassARRAY system. Analysis of the spectra and others were performed using Sequenom's Typer 4.0 software.
The Sequenom's patented DNA analysis guide is available on
http://jmgroup.pl/kawaska/download/iPLEX%20Gold%20Application%20Guide.pdf

2.3 – Data analysis

2.3.1 – Survey data

Raw data were collected from questionnaires and stored in excel files. Analysis was done with SAS software (SAS, 2002). Frequency counts and means were calculated. Differences between regions were assessed statistically, using an overall chi-square ($\chi 2$) test at ($p < 0.05$). Then, if significant, individual pairwise comparisons were made between regions. The test of significance was performed only in regions when the number of answers to a given question was reasonable ($n \geq 5$). The prioritization of diseases was expressed as a weighted rank.

2.3.2 – Autosomal data

Estimates of total number of alleles, mean number of alleles, effective number of alleles, observed heterozygosity (*Hob*), unbiased gene diversity (expected unbiased heterozygosity, *Hexp*), and average heterozygosity (Ave. Het.) for each breed were obtained with POPGENE program version 1.31 (Yeh *et al.*, 1999). *Hexp* the most common measure of variability (Petit *et al.*, 1998; Caballero and Toro, 2002) was estimated using the algorithm of Levene (1949), which

is the same as Nei's (1987) unbiased heterozygosity. Ave. Het. was computed as a mean value of *Hob* and *Hexp* (using Levene (1949)) plus Nei's (1973) expected heterozygosity. Convert package version 1.31 (Glaubitz, 2004) has been used to determine the allele frequencies and detect breed-specific alleles.

Deviations from Hardy-Weinberg Equilibrium (HWE) probability exact test (with unbiased exact *P*-value of Guo & Thompson (1992)) was performed using GENEPOP package version 4.0.10 of Rousset (2008) according to the Markov Chain parameters, dememorization (1000), batches (100), iteration per batch (1000).

Using the variance – based of Weir & Cockerhan (1984), *F*-statistics (F_{IS}, F_{IT}, F_{ST}) for each locus and overall values were calculated using FSTAT version 2.9.3.2 (Goudet, 2002). Significance tests on the estimates *F*-statistics for each microsatellite locus were obtained by constructing 95% and 99% confidence intervals based on the standard deviations estimated by jackknifing across populations using FSTAT.

A dendogram was drawn based on Nei's (1978) genetic distance with the Unweighted Pair Group Method with Arithmetic mean (UPGMA) modified from NEIGHBOR procedure of PHYLIP program package version 3.5 and executed for multiple populations as implemented in POPGENE 1.31 (Yeh *et al.*, 1999). POPULATION 1.2.30 software (Langella, 1999) was used to construct a phylogenetic tree of populations with bootstrap on locus using Reynolds *et al.* (1983) least squares. That was run using UPGMA and 1000 trials. The tree was visualized with TreeView 1.6.6 software (Page, 1996).

The Bayesian clustering method, as implemented by the STRUCTURE 2.3.3 program released in year 2010 was used to assess the level of population admixture. The basic algorithm of the program was described by Pritchard *et al.* (2000), the extensions to the method were made by Falush *et al.* (2003 and 2007) and Hubisz *et al.* (2009). Breed information was available for the putative parental population then k = 2 parental populations were assumed. The program was run five times with burnin period of 5.10^4 iterations followed by 10^5 number of MCMC repeats after burnin. The admixture model was used with the sampling locations as a prior. Correlated allele frequencies model was used as well. The clustering has also been performed with Bayesian Analysis of Population Structure (BAPS) package version 5.3 (Corander *et al.*, 2008).

2.3.3 – Sex - chromosome data

Estimates of total number of alleles, mean number of alleles, effective number of alleles, gene diversity for each locus, population and breed were obtained with POPGENE program version 1.31 (Yeh *et al.*, 1999). The gene diversity (H) was estimated according to Nei (1973).

The Bayesian clustering method, as implemented by the STRUCTURE 2.3.3 was used in the same way as for the autosomal data. program released in year 2010 was used to assess the level of population admixture. Breed information was available for the putative parental population, then k = 2 parental populations were assumed. Furthermore we assumed the scenario of Falush *et al.* (2003), stated that the admixture created an additional population that resulted in the cross-bred population.

To estimate the age of admixture in our populations we used the linkage model of Falush *et al.* (2003) as implemented in STRUCTURE. The model assumes admixture linkage disequilibrium (ALD). That ALD (the correlations that arise between linked markers) is occurring because each chromosome is composed by a set of "chunks" that are derived as unbroken units from one or another of the ancestral populations and all allele copies on the same chunk derive from the same population. The breakpoints between successive chunks are assumed to occur at random at a rate *r* (recombination rate) per unit of genetic distance and that the population of origin of each chunk in each individual is independently drawn. The linkage model takes also into account the correlations between markers that occur due to admixture (mixture LD) but ignore background LD. The background LD was also ignored by Freeman *et al.* (2006) when using ADMIXMAP to assess age of admixture of cattle breeds of Africa and the Near East.

The program was run five times with burnin period of 5.10^4 iterations followed by 10^5 number of MCMC repeats after burnin. The admixture model was used with the sampling locations as a prior information to determine the breeds admixtures proportions. Correlated allele frequencies model was used as well. To estimate the value of *r,* the rate of switching from one chunk to the next and the rate of drift away from ancestral population (Fst), the program was run five times as well with the same values of burnin period and number of repeats as in the admixture model. A probability of recombination rate of 0.01 per centiMorgan (cM) was assumed.

For *r,* it was considered *r* is equal to the probability of a recombination per genetic distance times the number of generations since the admixture took place (Falush *et al.* 2003).

2.3.4 – SNP data

From the 64 trypanosomosis resistance candidate SNPs, 32 SNPs were selected and put into 9 blocks (Table 5). The blocks have been constructed based on the distance between adjacent SNPs (0 to 2 cM spacing distance) on chromosomes. The other 32 SNPs have been skipped because either they were not lying in a block or had more than 20% missing alleles. Also, animals with more than 10% missing alleles were excluded from the final data set. From the blocks, breeds allelic proportions have been estimated using STRUCTURE 2.3.3 program. The run parameters were the same as in the autosomal data analysis. The relative excess or decrease in admixture contribution within the selected blocks by the zebu breed was computed using the approach of Tang *et al.* (2007). Differences between blocks Zebu allelic means land autosomal data were assessed statistically, using the non-parametric test of Wilcoxon signed-rank test on SAS (2002).

Table 5: SNPs markers and blocks design

Chr. Id.	SNP Id.	Map info	Distance (cM)	Block
7	ARS-BFGL-NGS-10447	5817122		1
7	ARS-BFGL-NGS-39011	5886680	0.07	1
7	ARS-BFGL-NGS-32423	5977524	0.09	1
7	BTB-00737899	59389495		2
7	BTB-00737840	59425067	0.04	2
7	BTB-01700292	59453636	0.03	2
8	Hapmap60967-rs29016114	55229628		3
8	ARS-BFGL-NGS-17993	55273003	0.04	3
8	UA-IFASA-7888	55556937	0.28	3
17	ARS-BFGL-NGS-10560	10587595		4
17	Hapmap6261-BTA-42054	10643642	0.06	4
17	ARS-BFGL-BAC-34362	10728397	0.08	4
20	BFGL-NGS-110436	20005762		5
20	BFGL-NGS-110667	21934672	1.93	5
20	BFGL-NGS-118577	21993303	0.06	5
20	Hapmap34321-BES11_Contig492_674	22068656	0.08	5
20	BTA-106532-N0-RS	22089392	0.02	5
21	Hapmap50722-BTA-52191	38052186		6
21	ARS-BFGL-NGS-100101	38116145	0.06	6
21	BTB-00817521	38142840	0.03	6
21	ARS-BFGL-NGS-92303	56530968		7
21	BTA-52592-no-rs	57573488	1.04	7
21	Hapmap45290-BTA-52591	57717565	0.14	7
21	ARS-BFGL-NGS-104549	57738923	0.02	7
23	UA-IFASA-9236	28828816		8
23	ARS-BFGL-NGS-31638	28890490	0.06	8
23	BTA-56168-no-rs	28915218	0.02	8
23	ARS-BFGL-NGS-80691	28943537	0.03	8
26	ARS-BFGL-NGS-43159	23170881		9
26	BFGL-NGS-110383	23191018	0.02	9
26	BTA-28185-no-rs	23362211	0.17	9
26	BTB-00932483	23406739	0.04	9

Chr.: chromosome; Id.: identity

3 – Results

3.1 – Survey results

3.1.1 – Most important diseases in the study areas

Table 6 provides a list of frequencies of each type of disease within regions. The diseases listed were those invoked by the interviewees. Hemorrhagic Septicaemia was present in the all regions but was mostly cited in the North. The proportions ranged from 18.18 % - 91.67 % respectively in the South-West and the North. Overall, 5 diseases out of 16 were mentioned in the North. This indicates that animals in the semi-arid area are less prone to disease development and spread than in the humid zones. Cowdriosis was the least appealed disease in the North. It has not been mentioned anywhere else. Trypanosomosis was almost absent in the North compared to the other regions (98.18 % in the West and 87.27 % in the South-West) with a significant difference ($P<0.05$) between the 2 regions. Some interviewees (especially transhumant breeders) knew the disease but it was not occurring in the North. Trypanosomosis is the most cited disease in the tsetse challenged regions of the South-West and the West compared to other diseases. The parasitic diseases (trypanosomosis and diseases caused by internal and external parasites) in general were very much present in the South-West and West due to the favourable conditions. Least invoked diseases were scab and brucellosis (01.82 %) in the South-West and ear infections (01.82 %) in the West. Anthrax, scab, tuberculosis and brucellosis were seen to be important in the South-West, while in the West they have not been mentioned. The same is true with ear infections in the West. Anthrax was probably mentioned because a year before the survey many persons in the South-West died from it. The pair wise comparison revealed that the differences between regions in foot and mouth disease (FMD) were not statistically significant.

Table 6: Most important diseases encountered in the study areas as stated farmers (%)

	Percent (%)			
Disease	**North**	**South-west**	**West**	**Overall**
Hemorrhagic Septicaemia	91.67a*	18.18b*	41.82c*	41.04
Gas Gangrene Infections	66.67a*	36.36b*	18.18c*	34.33
Contagious Bovine Pneumonia	70.83a***	09.04b***	07.27	19.40
Foot and Mouth Disease	37.50a	27.27a	43.64a	35.82
Cowdriosis	04.17	00.00	00.00	00.75
Trypanosomosis	00.00	87.27a*	98.18b*	76.12
Internal Parasites	00.00	16.36a	12.73a	11.94
Lumpy Coat Disease	00.00	09.09a***	41.82b***	20.90
Mastitis	00.00	03.64	03.64	02.99
External Parasites	00.00	16.36a	10.91a	11.19
Lumpy Skin Disease	00.00	05.45	20.00	10.45
Ear Infections	00.00	00.00	01.82	00.75
Anthrax	00.00	29.09	00.00	11.94
Scab	00.00	01.82	00.00	00.75
Tuberculosis	00.00	05.45	00.00	02.24
Brucellosis	00.00	01.82	00.00	00.75

Percents with alphabetic letters showed ≥ 5 observations and hence compared; Percents in the same row showing different values estimated within the disease are different if the letters are different. *: significant at P<0.05, ***: P<0.0001

33.1.2 – Prioritization of diseases

Farmers were asked to rank the worst diseases according to what was happening in their herd. For this exercise, 1, 4 and 1 interviewees respectively in the North, the South-West and the West did not reply. These farmers might have less contact with the cattle and very little knowledge about cattle diseases (owner or household head but not herdsman) or started to keep cattle a few months before the survey compared to those who responded.

The results of the exercise are shown in Table 7. The worst disease in the North was hemorrhagic septicaemia (79.17 % of respondents) ranked 2.5 while the worst in tsetse challenged area was trypanosomosis, 54.55 % and 70.91 % respectively in the South-West and the West. The difference between the South-West and the West was not statistically significant. In overall ranking, trypanosomosis came on the top for the majority of respondents (51.49 %). Anthrax was mentioned in South-West because months before the survey people died in the region because of the disease. That made this disease very particular this year but still less worse than trypanosomosis according to the proportion. The least frequently mentioned diseases according to the list of first ranks were lumpy skin disease and lumpy coat disease in overall study and the West as well. In the North and the South-West such rarely mentioned diseases were gas gangrene infections and internal parasites diseases respectively. Most of the diseases which gained higher percentages in Table 6 were reported in the list of first ranked disease in Table 7.

Table 7: Diseases ranked as worst diseases in the study area, frequencies (n), percent (Pct) and weighed ranks (R, lower numbers indicate higher priority)

	North			South-west			West			Overall		
Disease	**n**	**Pct**	**R**	**n**	**Pct**	**R**	**n**	**Pct**	**R**	**n**	**Pct**	**R**
Hemorrhagic Sept.	19	79.17[a]	2.5	03	05.45	15.5	08	14.55[b]	6.5	30	22.39	6.5
Gas Gangrene Infect.	01	04.17	11.5	05	09.09	11.5	02	03.64	11.5	08	05.97	11.5
Contagious Bov. Pn.	03	12.50	6.5				01	01.82	18.5	04	02.99	21.5
Foot and Mouth Dis.				03	05.45	15.5	02	03.64	11.5	05	03.73	18.5
Trypanosomosis				30	54.55[a]	2.5	39	70.91[a]	2.5	69	51.49	2.5
Internal Parasites				02	03.64	21.5				02	01.49	23
Lumpy Coat Disease							01	01.82	18.5	01	00.75	24.5
Lumpy Skin Disease							01	01.82	18.5	01	00.75	24.5
Anthrax				08	14.55	6.5				08	05.97	11.5

Percents with superscript letters showed ≥ 5 observations and hence compared; Percents in the same row showing different values estimated within the disease are different if the letters are different ($P<0.05$).

3.1.3 – Trypanosomosis control methods

Various trypanosomosis control methods were reported by farmers. Chemoprophylaxis/chemotherapy was used by 74.63 % of farmers, insecticides by 9.70 % and scarification (traditional medicine; Pict. A11 in Appendix) by 2.24 %. In the West 94.55% of farmers applied at least one trypanosomosis control method, 78.18 % in the South-West. In the North only the chemoprophylaxis/chemotherapy was used to control trypanosomosis by 20.83 % of breeders who used to move to the tsetse challenged areas. Some pure Baoule keepers in the tsetse challenged areas did not use any control method, while most of the farmers combined 2 or 3 methods. Preventive/curative treatments were made in 71.72 % of cases by skilled persons (private or government workers) while 28.28 % of farmers used to treat sick animals themselves. This trend occurred mainly in the West where 45.28 % of farmers treated sick animals themselves compared to the South-West (09.09 %). It was done most of the time when the first treatment made by a skilled person did not work. Farmers who used insecticides tried to control trypanosomosis by fighting against tsetse flies.

Quite a large number of chemicals were used in the field, 13 were recorded . In the North, only Trypamidium was used by 28.83 % of farmers that applied a control method. Farmers of the North were excluded from the analysis below. The drugs were named with trade names by farmers. The most frequently used chemotherapy (Figure 3) was Trypamidium (50.00% of overall respondents) as well as in the South-West (54.70 %) compared to the West (44.21 %). The others were chronologically Berenil (19.77 %) and Diminazene (11.63 %).

We could group the drugs into 4 clusters, the Isometamidium chloride group (Isometamidium, Trypamidium, Securidium), the group of Diminazene aceturate (Diminazene, Berenil, Nozamil, Trypadim, Veriben, Survidim, Diminaveto), the group of Deltamethrin (Butox, Deltamethrin) and the group of Alpha-cypermethrin (Dominex). The first 2 groups being trypanocidal drugs and the last 2 groups were used for spraying in vector control. The Isometamidium chloride group accounted for 53.49 % versus 46.51 % for the Diminazene aceturate group. The most used among the sprays was Dominex (53.33 %). The drugs could be combined or used alone. Among the respondents 28.00 % used only one drug, 57.00 % used 2 drugs, 13.00 % used 3 drugs and 2.00 % used 4 drugs. The sprays have been used in some cases many times within a year. Deltamethrin has been used 4 to 15 times a year in the West, Dominex up to 4 times a year

in West as well. Some trypanocidal drugs such as Trypamidium, Berenil, Diminazene, Survidim, Veriben were also used in curative treatments.

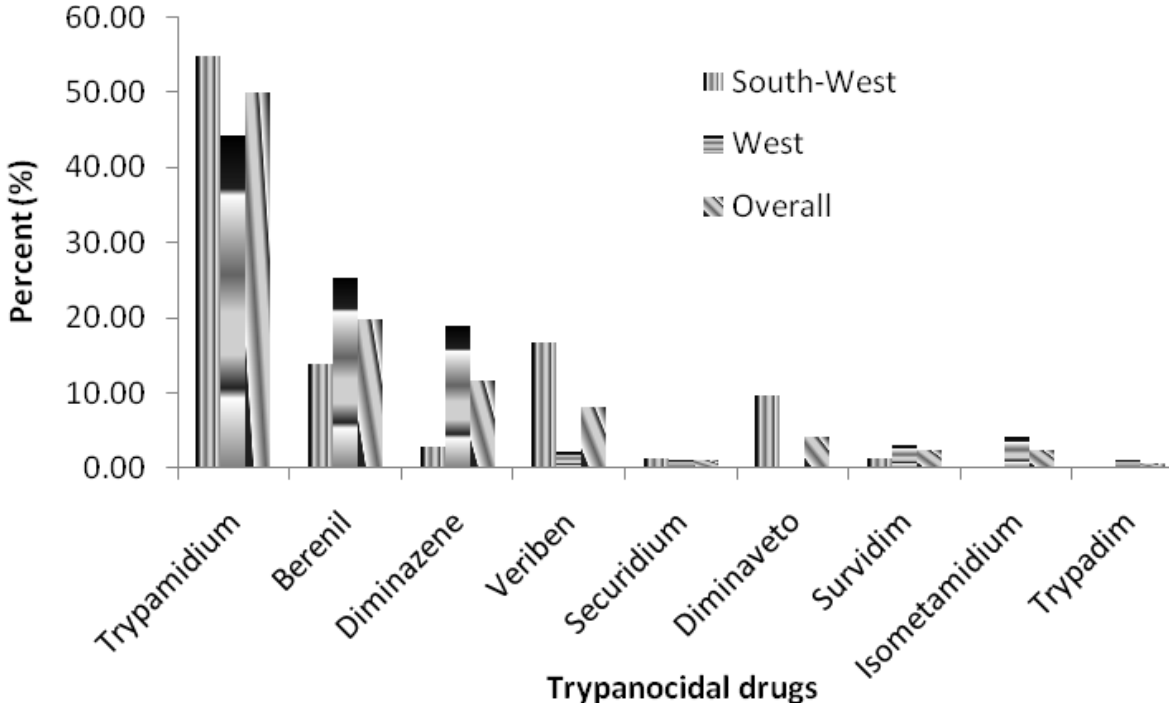

Figure 3: Trypanocidal drugs in use in the field

3.1.4 – Decreased efficacy trypanocidal drugs

The trypanocidal drugs have been used for long in those areas. According to the opinion of many farmers, some of these drugs became less effective over time. Indeed 45.00 % of overall respondents claimed drugs' efficacy has decreased. Depending on the regions, the opinions were logically different. While the majority in the West (59.26 %) maintained the decreasing efficacy of the trypanocidal drugs, only 29.55 % in the South-West shared that opinion. Among the drugs 7 were pointed out as more decreasing in efficacy (Figure 4). The two most appealed drugs were Berenil and Diminazene, with 36.84 % and 26.32 % respectively in the overall area. These drugs were also the most incriminated in the West (38.10 % and 30.95 %). In the South-West it was Veriben (33.33 %). The Isometamidium group accounted only for 14.55 % of drugs with changed efficacy but 85.55 % for Diminazene group. Diverse reasons were guessed for the lower efficacies of the drugs. The main reasons were resistance raised by the trypanosomes (30.77 %)

and the drugs themselves. Indeed 38.46 % of the respondents claimed the drugs were just ineffective and that could be linked to the manufactory. Resistance has been pointed out in the West as the main reason by 42.11 % of respondents while in the South-West the main reason was related to the drug quality (57.14 %). The other reasons were fake drugs (11.54 %), inappropriate dosages (3.85 %), weakness status of the animals before treatments (7.69 %), animals treated not at the beginning of the sickness (3.85 %), and reinfection by tsetse bites (3.85 %). Related to the drugs some reasons could be merged such as drug quality.

The decreased efficacy of drugs may explain the fact that some of them are being used many times within a year. The less effective drugs according to breeders' opinion such as Berenil, Diminazene have been used each 3 times by 5 farmers in the West indeed.

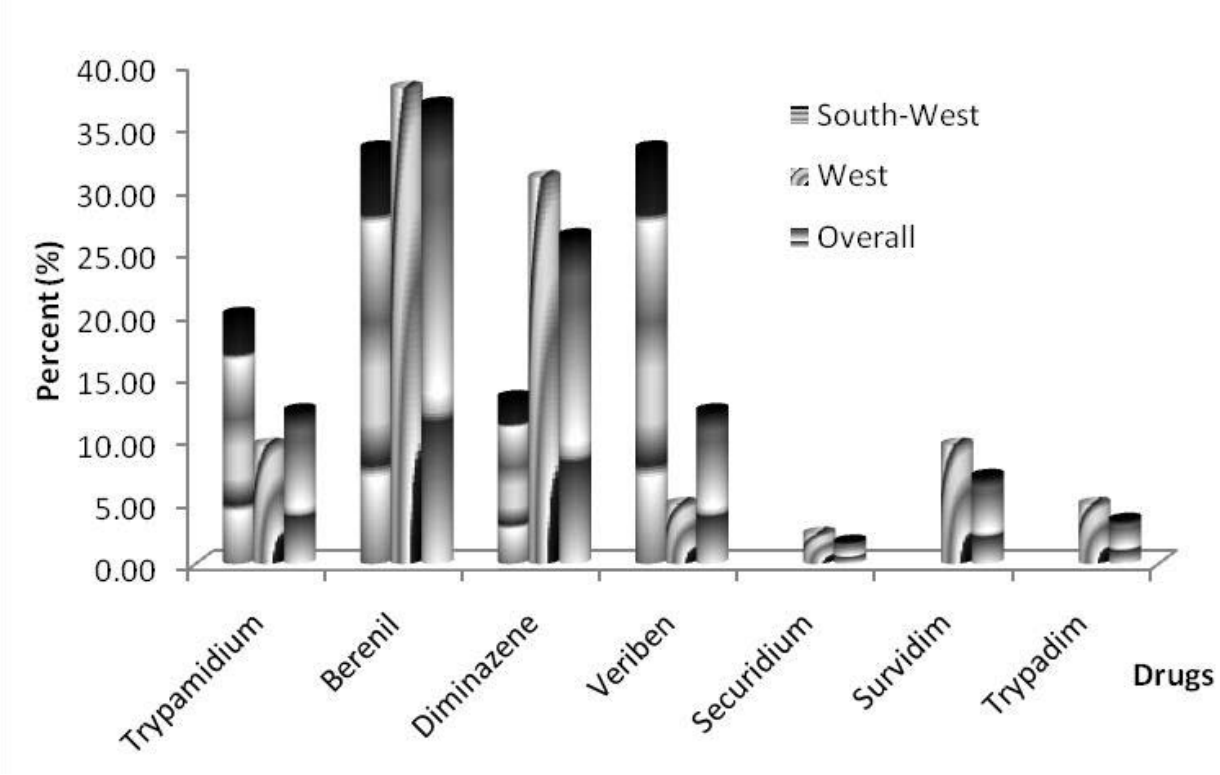

Figure 4: Less effective reported drugs

3.1.5 – Strategies in case of treatment failures

Two main strategies were drawn by farmers in case of failure in trypanosomosis treatment. The first one was to sell the animals suffering from trypanosomosis when the treatment failed to heal them. In overall, 06.72 % (Table 8) of farmers used this strategy but it was more used in the South-West (12.73 %) than in the West (03.64%). The second strategy (39.55 %) comprised 3 others sub-strategies such as - increase the dosages of the drug and treat again - treat again with the same dosages and - same drug and change the drug and treat again. The most used sub-strategy among them was change of drugs. In the South-West, this strategy was used by 21.82 % of farmers and in the West by 23.64 % of respondents.

The sub-strategy use „the same drug and the same dosages and treat again' was also frequently used (18.66 %). Looking at the reasons of treatment, failure the quality of the drugs and the dosages were pointed out by 53.85 % of the respondents. Some of these strategies might have resulted in the resistance mentioned above. For the majority (61.11 % of respondents) the disease was decreasing in frequency while 28.70 % argued the opposite and 10.19 % had no opinion.

Table 8: Strategies used after trypanosomosis treatment failures

Strategies	South-West	West	Overall	
Sell sick animals	12.73	03.64	06.72	06.72
Increase dosages	01.82	18.18	08.21	
Again same dosages and drug	21.82a	23.64a	18.66	
Change drug	14.55a	16.36a	12.69	39.55

Percents with alphabetic letters showed more than 5 observations and hence compared; Percents in the same row showing different values estimated within the disease are different if the letters are different ($P<0.05$).

33.2 – Results of autosomal microsatellites genotyping

3.2.1 – Polymorphism

In this study, 22 out of 30 autosomal chromosomes in cattle have been surveyed. Autosomes that have not been surveyed are chromosomes 6, 13, 24, 25, 27 to 29. Chromosomes 5, 11, 21 have been surveyed at 2 loci each. 6 populations have been considered in the analysis (Table 9), Zebu of the challenged areas have been merged (Other Zebu) due to low number (4) of Zebu sampled in the West. Baoule×Zebu population in the West was the biggest sample out of the 6

populations. It resulted from crossbreeding the 2 main breeds (Zebu and Baoule) to control trypanosomosis disease in the tsetse challenged areas of Burkina Faso. Blood from a total of 1045 animals was sampled, 450 of these were genotyped for autosomal microsatellites.

In total, 311 alleles were detected in the 25 loci surveyed, giving a mean number of 12.44 ± 4.31 observed alleles per locus while the mean of effective number of alleles was 4.67 ± 1.48 alleles per locus (Table 10). The number of alleles ranged from 3 at CSSM066 to 22 at TGLA122. The lowest number of effective allele number was observed at CSSM066 (1.57) and the highest number at TGLA53 (7.65). The total number of alleles per population ranged from 176 to 270 alleles respectively for Baoule in the West and Baoule×Zebu in the West (Table 9). The lowest number of alleles in Baoule West population may be due to the small number of the Baoule samples (21) from this region. The total number of alleles in taurine was somewhat higher than in Zebuine, potentially because most of the primers have been designed based on taurine samples.

Some 7% of the total alleles have been detected as breed-specific alleles using Convert package, 12 private alleles of Zebu at 11 loci and 10 private alleles of Baoule at 9 loci.

Among the loci in the study, 14 deviated from HWE ($P<0.0001$ using the probability exact test of Fisher) as shown in Table 3; markers displaying a highly significant deviation from HWE are in italics. Furthermore, deviations from HWE were statistically significant ($P<0.0001$) for 26 locus-population combinations. Out of the 25 loci analyzed in each population, 1 to 8 deviated significantly from HWE. All populations also deviated from HWE ($P<0.0001$), probably because of inbreeding.

Table 9: Allele numbers, heterozygosity, deviation from HWE, intra-population diversity and proportion of membership of the 6 populations.

Breed/Population	N	TNA	MNa	MNe	*Hob*	*Hexp*	HWE	F_{IS}	§Membership (%) and ranges	
									Zebu	Baoule
Zebuine	***117***	***229***	***9.56***	***4.22***	***0.66***	***0.774***		***0.102***		
			(3.03)	***(1.19)***	***(0.11)***	***(0.11)***				
Zebu North	66	218	8.72	4.20	0.65	0.74	4	0.116	98	02
			(2.96)	(1.15)	(0.13)	(0.13)			83-100	00 - 17
Other Zebu	51	205	8.20	4.02	0.67	0.73	2	0.084	92	08
			(2.40)	(1.29)	(0.11)	(0.11)			81 - 96	04 - 19
Taurine	***145***	***284***	***10.04***	***3.80***	***0.60***	***0.70***		***0.121***		
			(3.45)	***(1.46)***	***(0.13)***	***(0.13)***				
Baoule South-West	124	239	9.56	3.56	0.60	0.68	6	0.122	15	85
			(3.44)	(1.31)	(0.14)	(0.14)			01 - 57	43 - 99
Baoule West	21	176	7.04	4.03	0.65	0.74	1	0.120	76	24
			(2.09)	(1.40)	(0.15)	(0.11)			61 - 85	15 - 39
Crosses	***158***	***364***	***11.20***	***4.69***	***0.67***	***0.77***		***0.122***		
			(3.96)	***(1.39)***	***(0.11)***	***(0.09)***				
Baoule×Zebu South-West	35	205	8.20	4.28	0.63	0.75	5	0.157	66	34
			(2.87)	(1.26)	(0.17)	(0.12)			25 - 95	05 - 75
Baoule×Zebu West	153	270	10.80	4.70	0.68	0.77	8	0.114	80	20
			(3.82)	(1.45)	(0.10)	(0.08)			62 - 89	11 - 38

N: sample size, TNA: total number of alleles, MNA: mean number of alleles observed, MNE: mean number of effective alleles, *Hob*: observed heterozygosity, *Hexp*: expected heterozygosity, (2.96): standard deviation, HWE: locus-population deviation ($P<0.0001$), F_{IS}: intra-population heterozygosity deficiency. §: proportion obtained using STRUCTURE program.

3.2.2 – Breed diversity

Expected heterozygosity has been generally higher than observed heterozygosity not only at marker level but also at population level. This difference is due to the deviation from HWE. At population level, the most diversified population was the Baoule×Zebu in the West with the highest observed and expected heterozygosities (*Hob* = 0.68±0.10, *Hexp* = 0.77±0.08) and the

least variable the Baoule from the South-West (*Hob* = 0.60±0.14, *Hexp* = 0.68±0.14). In the overall population the crosses as expected are more diverse (*Hexp* = 0.77±0.09) than the pure breeds (Zebu and Baoule) followed by Zebu (*Hexp* = 0.74±0.11). Observed heterozygosity ranged from 0.34 (CSSM066) to 0.76 (INRA032 and TGLA227). The most variable marker in this study was TGLA53 (*Hexp* = 0.87) compared to CSSM066 (*Hexp* = 0.36). Markers which contributed much to the variability were INRA032 and HEL 9 (Ave. Het. = 0.82). The mean number of migrants per generation for all loci estimated based on the formula Nm = 0.25(1-F_{ST})/F_{ST} as implemented in POPGENE was 6.06.

The mean estimates of *F*-Statistics obtained by jackknifing over loci were: F_{IS} = 0.117±0.019 (within population heterozygote deficiency or inbreeding; in 99% confidence interval), F_{IT} = 0.158±0.019 (total inbreeding estimate), F_{ST} = 0.047±0.005 (estimate of population differentiation) are shown in Table 11. The mean estimates F_{IT} and F_{ST} were both in the confidence interval of 95%. The most inbred locus was HEL1 (0.417), the least inbred being ETH3. HEL1 contributed logically to the total inbreeding coefficient, F_{IT} = 0.466. The Baoule×Zebu population from the South-West surprisingly showed the highest value of F_{IS} (0.157), i.e. was the potentially most inbred population in this study. Two markers in this population contributed to the high value of F_{IS} Hel (0.695) and ILSTS033 (0.545) (data not shown). This is most probably due to null alleles present in these two markers. The F_{IS} values within group (obtained using the permutation method (10^4 permutations)) ranged from 0.102 (Zebuine), to 0.122 (Crosses) (Table 9). Comparisons of F_{IS} of the 3 groups were not statistically significant as well as the comparisons of the F_{ST} values.

Table 10: alleles number per locus, observed, expected, average heterozygosity and *P*-values

Loci	NA	NE	*Hob*	*Hexp*	Ave. Het.	*P*-value§
BM1824	9	3.45	0.65	0.71	0.69	0.0476
BM2113	*17*	*6.19*	*0.67*	*0.84*	*0.80*	*0.0000*
INRA023	*11*	*4.52*	*0.70*	*0.78*	*0.73*	*0.0000*
MGTG4B	*15*	*5.12*	*0.73*	*0.81*	*0.79*	*0.0000*
AGLA293	15	5.84	0.74	0.83	0.79	0.0090
ETH10	*10*	*4.75*	*0.65*	*0.79*	*0.73*	*0.0000*
ILSTS006	*9*	*3.47*	*0.52*	*0.71*	*0.67*	*0.0000*
HEL9	*11*	*6.18*	*0.75*	*0.84*	*0.82*	*0.0000*
ETH225	13	3.96	0.65	0.75	0.67	0.0020
ILSTS005	9	3.01	0.60	0.67	0.65	0.0004
INRA032	17	5.96	0.76	0.83	0.82	0.0086
HEL13	7	3.01	0.58	0.67	0.63	0.0167
ILSTS033	*12*	*3.19*	*0.54*	*0.69*	*0.64*	*0.0000*
CSSM066	3	1.57	0.34	0.36	0.33	0.0181
HEL1	*8*	*5.04*	*0.44*	*0.80*	*0.76*	*0.0000*
TGLA53	*19*	*7.65*	*0.60*	*0.87*	*0.81*	*0.0000*
ETH185	*13*	*3.85*	*0.63*	*0.74*	*0.72*	*0.0000*
TGLA227	*16*	*6.07*	*0.76*	*0.84*	*0.78*	*0.0000*
ETH3	11	3.36	0.69	0.70	0.68	0.7610
TGLA126	9	4.40	0.75	0.77	0.75	0.0722
HEL5	*12*	*5.41*	*0.67*	*0.82*	*0.79*	*0.0000*
TGLA122	*22*	*3.30*	*0.65*	*0.70*	*0.69*	*0.0000*
HAUT24	*19*	*6.90*	*0.71*	*0.86*	*0.81*	*0.0000*
BM1818	12	6.33	0.74	0.84	0.81	0.0068
HAUT27	12	4.32	0.65	0.77	0.75	0.0003
Total	311					
Mean	12.44	4.67	0.65	0.76	0.73	
Std. Dev.	4.30	1.48	0.10	0.10	0.10	

NA: observed number of alleles, NE: effective number of alleles, *Hob*: observed heterozygosity, *Hexp*: expected heterozygosity, Ave. Het: average heterozygosity, §Fisher's probability exact test across all populations with deviations from HWE (P<0.0001)

The locus ETH10 (0.121) contributed the most to the population differentiation. But the overall F_{ST} being < 0.15 the population differentiation seems to be moderate. Assuming that F_{ST} is

closely related to a genetic distance, comparisons made based on population F_{ST} (15000 permutations, P<0.0001) in Table 12 revealed that the Baoule population from the West was not significantly different from the crosses (0.00653 and 0.00487). This may mean that there was not really pure Baoule anymore in the West.

Table 11: *F*-Statistics (Weir & Cockerhan, 1984), standard errors for each locus across populations

Loci	F_{IS}	F_{IT}	F_{ST}
BM1824	0.059(0.045)	0.098(0.036)	0.042(0.033)
BM2113	0.173(0.046)	0.227(0.083)	0.063(0.051)
INRA023	0.056(0.035)	0.114(0.072)	0.060(0.046)
MGTG4B	0.084(0.017)	0.095(0.017)	0.012(0.007)
AGLA293	0.074(0.014)	0.124(0.022)	0.055(0.037)
ETH10	0.099(0.037)	0.211(0.112)	0.121(0.096)
ILSTS006	0.266(0.050)	0.292(0.038)	0.036(0.028)
HEL9	0.085(0.036)	0.114(0.050)	0.032(0.028)
ETH225	0.064(0.029)	0.148(0.048)	0.092(0.072)
ILSTS005	0.076(0.025)	0.109(0.047)	0.035(0.032)
INRA032	0.068(0.010)	0.094(0.023)	0.029(0.019)
HEL13	0.061(0.025)	0.141(0.072)	0.085(0.061)
ILSTS033	0.153(0.076)	0.241(0.125)	0.100(0.076)
CSSM066	0.051(0.134)	0.159(0.169)	0.106(0.045)
HEL1	0.417(0.039)	0.466(0.063)	0.082(0.058)
TGLA53	0.278(0.025)	0.321(0.041)	0.059(0.043)
ETH185	0.148(0.034)	0.163(0.028)	0.018(0.011)
TGLA227	0.055(0.007)	0.107(0.042)	0.055(0.041)
ETH3	-0.005(0.011)	0.028(0.027)	0.033(0.025)
TGLA126	0.023(0.019)	0.044(0.024)	0.021(0.016)
HEL5	0.153(0.034)	0.168(0.038)	0.018(0.011)
TGLA122	0.061(0.042)	0.083(0.057)	0.022(0.017)
HAUT24	0.133(0.047)	0.177(0.033)	0.052(0.017)
BM1818	0.087(0.028)	0.133(0.023)	0.050 0.033)
HAUT27	0.127(0.037)	0.163(0.031)	0.042(0.017)
Overall	0.117 (0.019)**	0.158(0.019)*	0.047(0.005)*

* : 95 % confidence interval ; ** 99 % confidence interval

Table 12: *P*-values of the different population for F_{ST} comparisons

	Other Zebu	Baoule South-West	Baoule West	Baoule×Zebu South-West	Baoule×Zebu West
Zebu North	0.00007	0.00007	0.00007	0.00007	0.00007
Other Zebu		0.00007	0.00007	0.00020**	0.00007
Baoule South-West			0.00007	0.00007	0.00007
Baoule West				0.00653^{NS}	0.00487^{NS}
Baoule×Zebu South-West					0.00007

**: P<0.001, NS: not significant

Table 13: Nei's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal)

	Zebu North	Other Zebu	Baoule South-West	Baoule West	Baoule×Zebu South-West	Baoule×Zebu West
Zebu North		0.9500	0.7125	0.8868	0.9425	0.9362
Other Zebu	0.0513		0.7469	0.8974	0.9575	0.9384
Baoule South-West	0.3390	0.2918		0.8562	0.8456	0.8272
Baoule West	0.1202	0.1083	0.1552		0.9209	0.9569
Baoule×Zebu South-West	0.0592	0.0434	0.1677	0.0824		0.9548
Baoule×Zebu West	0.0659	0.0636	0.1897	0.0441	0.0462	

3.2.3 – Structure of the populations

A neighbor-joining dendogram constructed based on unbiased genetic distances showed 2 main clusters, one cluster composed of Baoule South-West and the second being composed of the remaining populations (Figure 5). In the second cluster the populations clustered further into 3 genetic groups; the first group had Baoule×Zebu South-West and Other Zebu. Those groups had the smallest genetic distance (D_A = 0.0434 in Table 13) and the highest genetic identity (0.9575). The last group had only Zebu North. The unbiased genetic distance between Baoule South-West and Zebu North was the longest one (D_A = 0.3390). Figure 5 confirmed once more that Baoule

West was not different from crosses. A phylogenetic tree (Figure A1 in Appendix) confirmed the dendogram in Figure 5. The bootstraps showed the Baoule South-West segregating from the other populations with 100 % of replicates.

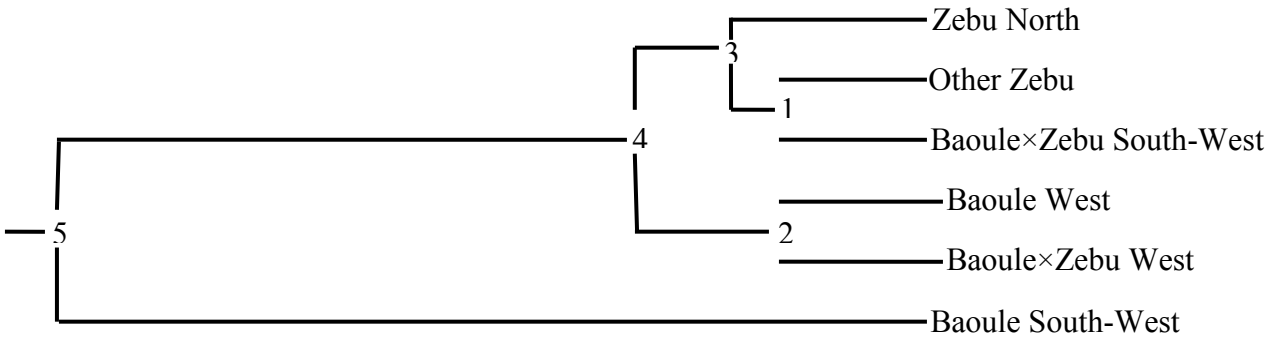

Figure 5: Dendogram based Nei (1978) genetic distance of the six cattle populations

Graphical displays of the results from the Structure analysis are presented in Fig. X6. Using STRUCTURE, the most likely K is that where lnPr(G/K) is maximized. The maximum value of ln Pr(G/K) was obtained at K = 2, that provided an explanation of the genetic structure and levels of admixture for the populations. This assumption has been supported by farmers' assumption as well about clusters on the field. At K = 2, all the studied population showed an admixture pattern between the 2 different clusters. That was more reliable than the pattern in K = 3. Two admixture proportions were designated q_t (Zebu, *Bos indicus*), and q_i (Baoule, B. taurus), with $q_t + q_i$ = 100. Zebu North had the highest proportion of members assigned to Zebu breed (98%, ranged from 83 % - 100% in Table 9) as expected, Baoule South-West being assigned at 85% (47% - 99%) to Baoule breed as we could presume, while the crosses had the lowest proportion of members assigned to Zebu (66% and 80%) or Baoule breed (20% and 34%). The clusters shown in Figure 6 have been confirmed using BAPS program.

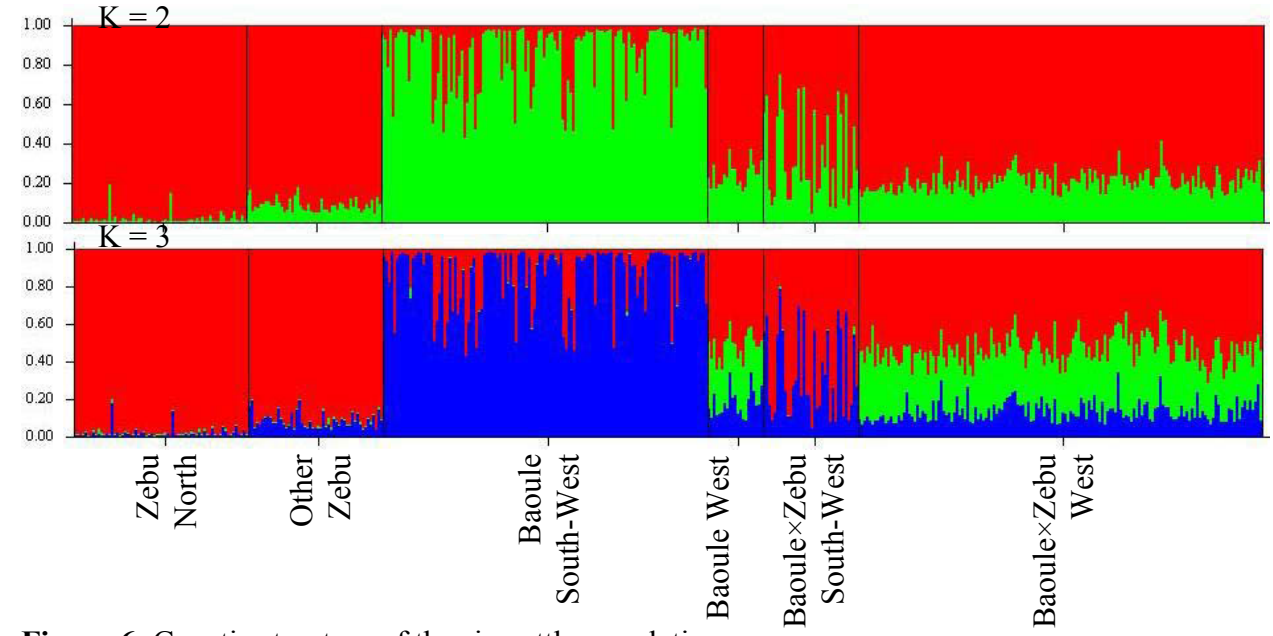

Figure 6: Genetic structure of the six cattle populations

Each individual animal is represented by a single vertical line divided into K colours, where K is the number of clusters assumed and the length of the coloured segment represents the individual's ancestry proportion of membership to a particular cluster estimate. Black lines separate individual populations whose names are indicated below the diagram.

3.3 – Results of sex - chromosome microsatellites genotyping

3. 3.1 – Polymorphism

A total of 300 male animals were genotyped for 26 loci on BTAX and 232 alleles were detected. The loci are presented in the genetic map order. Computed average spacing between adjacent markers was 6.02 ± 6.23 cM (range: 0.80 – 30.60 cM). The mean number of observed alleles per locus was 8.92 ± 3.59 while the mean of effective alleles' number was 4.46 ± 2.64 alleles per locus (Table 14). The number of observed alleles ranged from 3 at INRA30 to 17 at AGLA257. The lowest number of effective allele number was observed at INRA30 (1.29) and the highest number at BMS903 (11.57). Observing the number of effective and observed alleles per locus, it seemed like the loci above the centromere (nearby loci are XBM7 and XBM111) are more polymorphic. However, as usual the observed number of alleles is somehow different from the effective number of alleles. The discrepancy may be due to genotyping errors or null alleles. Average gene diversity was 0.69 ± 0.18, the range was 0.22 to 0.91. The least and most diversified loci were INRA30 and BMS903, respectively.

The number of populations considered in the analysis was 5. Zebu of tsetse challenged areas have been merged (Other Zebu) due to low number of Zebu individuals from the West in the sample (2 males). Baoule individuals from the West (7 males) have been merged to the Baoule population from South-West (Baoule) as well (Table 15). The Baoule×Zebu population in the West was the biggest sample (127 individuals) compared to the other populations, and crosses group was the biggest in overall, more than 50 % of the total sample size.

Table 14: Loci physical positions, polymorphism and gene diversity across populations

Loci	Physical position (cM)	N	Na	Ne	H
BMS631	00.0	299	05	02.68	0.63
BM6017	04.7	292	12	04.31	0.77
BMS903	12.1	284	15	11.57	0.91
BMS811	42.7	291	08	02.75	0.64
AGLA257	44.7	285	17	10.09	0.90
BMS2227	52.8	296	15	09.15	0.89
BMS960	53.6	295	08	05.99	0.83
XBM7	56.3	297	09	05.12	0.80
XBM111	60.4	296	04	01.86	0.46
BMS417	69.9	288	10	04.72	0.79
RM350	71.7	291	09	04.13	0.76
XBM19	73.5	300	11	06.01	0.83
BM4604	76.5	285	09	03.92	0.75
XBM11	78.8	300	06	02.67	0.63
BMS2592	80.0	295	12	05.73	0.83
BMC6021	91.3	298	07	02.75	0.64
BM9208	99.9	287	05	02.83	0.65
BMS2798	104.3	300	05	01.78	0.44
XBM16	119.5	297	10	02.82	0.65
XBM25	126.2	288	11	06.65	0.85
INRA120	128.5	297	10	03.70	0.73
BMS2576	136.2	295	12	04.99	0.80
XBM24	139.1	300	05	01.74	0.43
TGLA325	143.9	293	07	05.04	0.80
INRA30	148.6	289	03	01.29	0.22
XBM451	150.5	297	07	01.68	0.41
Average loci spacing	6.02 ± 6.23 *(00.80 – 30.60)				
Total			232		
Mean ± Std. Dev.		294	08.92 ± 03.59	04.46 ± 02.64	0.69 ± 0.18

*: minimum and maximum loci spacing; N: sample size; Na: observed number of alleles, Ne: expected number of alleles, H: Nei's 1973 gene diversity

The total number of alleles per population ranged from 150 to 223 alleles respectively for Baoule×Zebu in the South-West and Baoule×Zebu in the West (Table 15). The lowest number of alleles in Baoule×Zebu South-West population is certainly due to the small number of the representatives of this population (24) in the sample. One would expect a higher number of alleles in crossbred population than in the pure populations. This was confirmed when looking at the total number of alleles per breed, 194, 198 and 227 respectively for Baoule breed, Zebuine and Crossbreed. The same logic was observed in the mean number of observed alleles per breed (7.46 ± 3.27; 7.62 ± 3.13; 8.73 ± 3.63), the expected number of alleles per breed (3.64 ± 2.54; 4.00 ± 2.04; 4.41 ± 2.47) and the gene diversity (0.59 ± 0.24; 0.69 ± 0.15; 0.70 ± 0.17) in the same breed order. The gene diversity of the crossbreeds was somehow similar to the gene diversity of the Zebuine. The highest mean number of observed and expected alleles and diversified population was the Baoule×Zebu of the West, 8.58 ± 3.43; 4.38 ± 2.42 and 0.70 ± 0.17 respectively.

Table 15: Populations, allele numbers and diversity

Breed/Population	N	TNA	MNa	MNe	H
Zebuine	***66***	***198***	***7.62 ± 3.13***	***4.00 ± 2.04***	***0.69 ± 0.15***
Zebu North	33	164	6.31 ± 2.62	3.85 ± 2.21	0.66 ± 0.18
Other Zebu	33	163	6.27 ± 2.15	3.68 ± 1.55	0.68 ± 0.14
Taurine	***83***	***194***	***7.46 ± 3.27***	***3.64 ± 2.54***	***0.59 ± 0.24***
Baoule	83	194	7.64 ± 3.27	3.64 ± 2.54	0.59 ± 0.24
Crosses	***151***	***227***	***8.73 ± 3.63***	***4.41 ± 2.47***	***0.70 ± 0.17***
Baoule×Zebu South-West	24	150	5.77 ± 2.01	3.66 ± 1.78	0.65 ± 0.19
Baoule×Zebu West	127	223	8.58 ± 3.43	4.38 ± 2.42	0.70 ± 0.17

N: sample size; TNA: total number of alleles; MNa: mean nunmber of observed alleles, Ne: mean number of expected alleles, H: Nei's 1973 gene diversity

3.3.2 – Structure of the populations and admixture time estimate

The 3 breeds (Zebu, Baoule, Baoule×Zebu) were admixed at different levels. To better estimate the proportion membership of each breed, the Fst and the values of switching rate *r,* an indicator of time since admixture*,* we considered 3 different situations.

Situation A didn't segregate the crosses according to their parents' genetic status (No distinction), all sampled crosses were then included in the analysis (151 in Table 6).

Situation B took only into account individuals (56) with at least one parent being pure breed (At least 1 pure parent).

In situation C, 95 crossed individuals considered whose parents were both crossbreds (Both parents crosses). The situations have been drawn based on the background information on each sampled individual from the field.

The proportion of Zebu individuals assigned to Zebu breed was better in situations A and C (Table 16), 84 and 86 % respectively than in B (75 %).The individual ancestry assignment was also better in A (52 – 96) and C (54 – 96) than in B (51 - 80). The same observation was true for both populations and individual ancestry assignments to Baoule breed. 85 % of Baoule population assigned to Baoule cluster both in A and C while in B it was only 74%. The Baoule×Zebu population was assigned 58 % to Zebu and 42 % to Baoule cluster in A, half-half in B; 65% to Zebu and 35% to Baoule in C. The rate of drift from the ancestral Zebu was the same in all 3 situations (6 %) while the drift from ancestral Baoule decreased in B (11 %).

The mean estimates of *r* were 0.1430, 0.0542 and 0.1342 for A, B and C respectively. When restriction was made on the proportion of individual ancestry inferred (all individuals with < 85% affiliation with Zebu or Baoule group excluded) the value of F_{ST} changed for Baoule individuals while it increased in B (0.07) but decreased in C (0.05) compared to previous values (0.06). The overall average of Zebu 0.06 ± 0.01 was more than 2 times less the average of Baoule 0.13 ± 0.02. With that restriction, most of the animals with crossbred ancestors displayed a membership proportion to Zebu or Baoule of more than 80 % (Figure 7B) while in the other 2 cases Figure 7A and 7C) the majority of animals with crossbred ancestors displayed almost half – half proportion membership to Zebu and Baoule. These figures show how important the admixture impacted the BTAX pattern in A and C.

The trend remained the same when restriction was made on the proportion of ancestral membership of the presumed pure Zebu and Baoule individuals for *r* values. The value is even very low in this case (0.0018) while the other values increased importantly from 0.1430 to 0.1860 and 0.1342 to 0.1694 respectively for the situation where no distinction was made on the ancestors of the crosses and when both ancestors were crossbreds. The overall average of switching rate is 0.1148 ± 0.0716. That means the first admixture event took place 11.48 ± 7.16 generations back in the past. The standard deviation was high due to the difference between the values of *r* for both A and C compared with B. If we consider a generation length of 6 years in cattle then one could estimate the age of admixture of the sampled animals' mothers at 69 ± 43 years from 2007 when samples were taken.

Table 16: Breeds levels of admixture, differentiation and switching values

	No distinction §Breed membership and *inferred ancestry ranges* (%)			**At least 1 pure parent** §Breed membership and *inferred ancestry ranges* (%)			**Both parents crosses** §Breed membership and *inferred ancestry ranges* (%)		
Breed	**N**	**Zebu**	**Baoule**	**N**	**Zebu**	**Baoule**	**N**	**Zebu**	**Baoule**
Zebu	66	84 *52 - 96*	16 *04 – 48*	66	75 *51 - 80*	25 *20 - 49*	66	86 *54 - 96*	14 *04 – 46*
Baoule	83	15 *04 - 96*	85 *04 – 96*	83	26 *20 - 80*	74 *20 - 80*	83	15 *04 - 97*	85 *03 – 96*
Baoule ×Zebu	151	58 *05 - 96*	42 *04 – 95*	56	50 *20 - 80*	50 *20 - 80*	95	65 *05 -96*	35 *04 – 95*
F_{ST}		0.06	0.14		0.06	0.11		0.06	0.14
* F_{ST}		0.06	0.14		0.07	0.11		0.05	0.14
Overall average of F_{ST}		F_{ST}_Zebu = 0.06 ± 0.01				F_{ST}_Baoule = 0.13 ± 0.02			
Mean *r*		0.1430			0.0542			0.1342	
*Mean *r*		0.1860			0.0018			0.1694	
Overall average of *r*					0.1148 ± 0.0716				

§Proportion of membership of each pre-defined population in each 2 clusters and *ranges of inferred ancestry of individuals*. Fst: drift away from the ancestral population at a different rate (Fk)

*All individuals with < 85% affiliation with Zebu or Baoule breed were excluded from the analyses. Final set included 41 Zebu and 67 Baoule, the size of crosses remained the same.

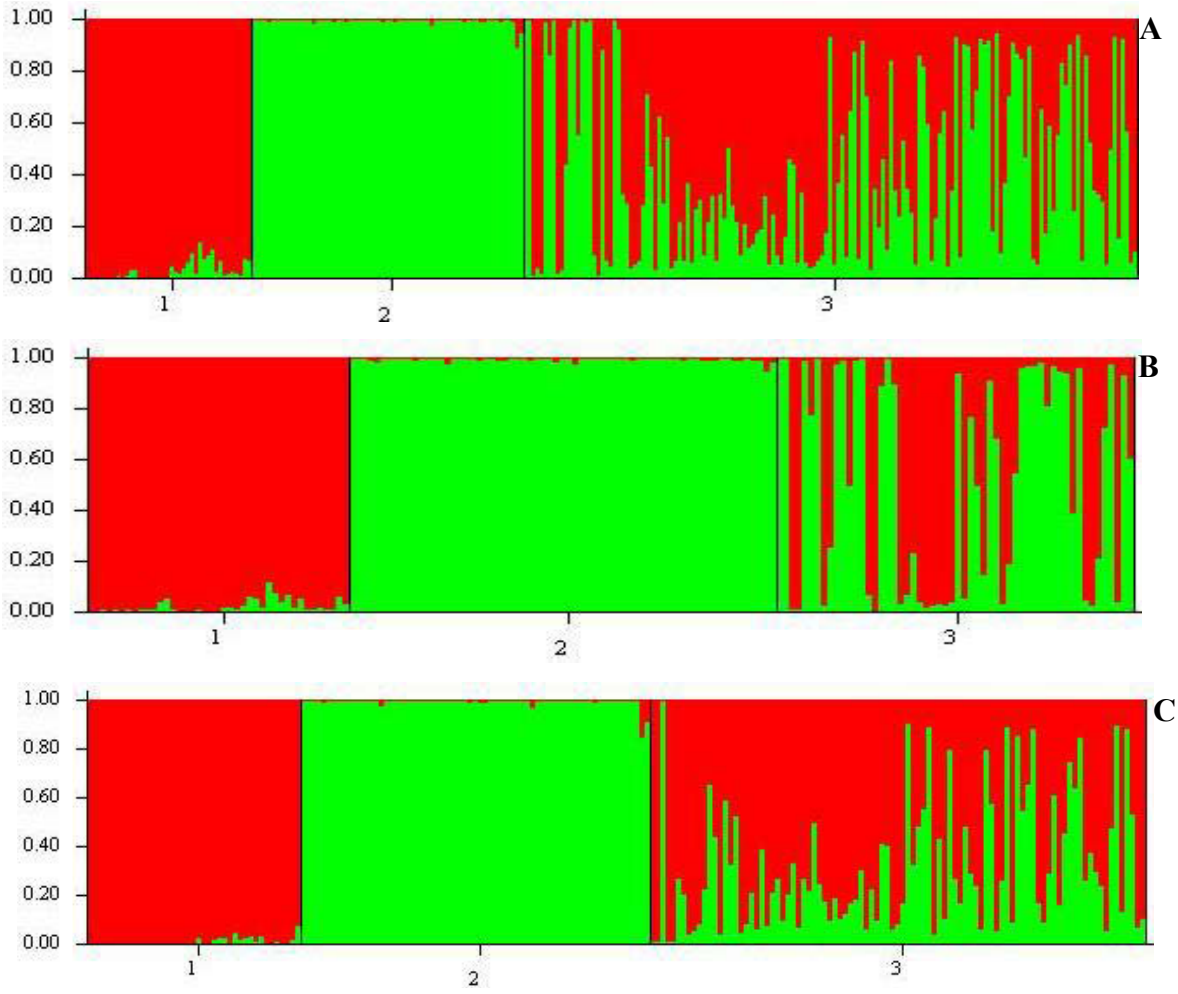

Figure 7: Genetic structure of the 3 main cattle populations

Each individual animal is represented by a single vertical line divided into 2 colours, where 2 is the number of assumed clusters and the length of the coloured segment represents the individual's ancestry proportion of membership to a particular cluster estimate. Black lines separate individual populations whose numbers (1 = Zebu; 2 = Baoule; 3 = Baoule×Zebu) are indicated below the diagram. **B**: at least one parent of the crossbred individuals is pure bred; **C**: both parents are crossbred; **A: B + C**

3.3.3 – Assessment of population admixture, x-markers vs. autosomal markers

A comparison has been made between the admixture in males using the X chromosome and the autosomal markers. For that comparison the individuals involved were the same, 300 males in total belonging to the 3 main populations (Zebu, Baoule and Baoule×Zebu). We used the results generated from STRUCTURE with same parameters but the phase information was different. While with the X chromosome the data were phased (genotypes derived only from the dam), with the autosomes the data were not phased (genotypes derived from both sire and dam). The

results are shown in Table 17. They showed that the proportion of individuals of Zebu population assigned to Zebu breed was 21% higher with autosomal markers than the x-markers (96% vs 75%). Further, the autosomal markers displayed always a larger proportion of Zebu individuals being assigned to Zebu breed than the x-markers. The difference decreased from 11% to 9% respectively for both parents being crosses and no distinction. However the percent of assignment of the presumed Baoule population individuals to Baoule breed is higher only when both parent were considered being crosses (Baoule $_{\text{x-markers}}$ = 85% vs. Baoule $_{\text{Autosomal-markers}}$ = 77%) or when no distinction was made on the parents' status (No distinction). Half crossbred individuals were assigned to one or the other breed in case of x-markers if one parent at least was pure bred but the majority (65% vs 58%) was assigned to Zebu breed. Still the majority was assigned by the autosomal markers to Zebu breed and the percent was higher in the situation where both parents are crosses (84%) compared to the other 2 situations (69% and 79%).

From the results, it can clearly be noted that the outcomes are different depending on which type of microsatellites have been used. The range of discrepancy is larger within Zebu population (9% – 21%) than within Baoule population (7 – 9%) using the 2 types of markers. The x-markers narrowed the inference of populations to the breeds than the autosomal markers.

Table 17: Populations structure using sex-chromosomes vs. autosomal markers

	At least 1 pure parent Breed membership (%)			**Both parents crosses** Breed membership (%)			**No distinction** Breed membership (%)		
Populations	***N***	**Zebu**	**Baoule**	***N***	**Zebu**	**Baoule**	***N***	**Zebu**	**Baoule**
Zebu $_{\text{x-markers}}$	*66*	**75**	25	*66*	**86**	14	*66*	**84**	16
Zebu $_{\text{Autosomal-markers}}$	*66*	**96**	04	*66*	**95**	05	*66*	**95**	05
Baoule $_{\text{x-markers}}$	*83*	26	**74**	*83*	15	**85**	*83*	15	**85**
Baoule $_{\text{Autosomal-markers}}$	*83*	23	**77**	*83*	23	**77**	*83*	24	**76**
Baoule×Zebu $_{\text{x-markers}}$	*151*	**50**	**50**	*56*	**65**	**35**	*95*	**58**	**42**
Baoule×Zebu $_{\text{Autosomal-markers}}$	*151*	**69**	**31**	*56*	**84**	**16**	*95*	**79**	**21**

N: number of individuals within population

3.3.3 – SNP genotypes from trypanotolerance candidate regions

Two analyses were made for 246 animals from 5 different populations, considering a total of 32 SNP genotyped in 9 trypanotolerance regions. The Zebu population from the North (45 samples), the Baoule (58 samples) and crosses (29) from the South-West, the Baoule (12) and crosses (102) from the West have been considered. The same individuals have been used in STRUCTURE runs both for autosomal and SNPs markers analysis. One analysis involved Zebu and the 2 populations from the South-West (Figure 8) and the other one involved the same Zebu population and the 2 populations from the West (results not shown).

Figure 8A provides a STRUCTURE graph for the autosomal microsatellites of these animals. Individuals of Zebu (1) and Baoule (2) in predicted populations looked pure, but not all of them. This might be due to wrong pedigree information, genotyping errors or simply noise from the software. The highest allele frequency of Zebu in the Baoule population was 0.15. In the crosses the allele frequencies of Zebu varied from 0.24 to 0.96. Taking this background information as baseline and having the Baoule as a reference we could analyze the 9 blocks. From there it could be seen in block 1 to 3 that the SNP of crosses in candidate regions derived mostly from Baoule and somehow in block 4 and 9 as well. The other blocks did not show a very clear pattern of crosses being derived from Baoule. This assessment was confirmed by computing the allelic deficiency in those blocks (results shown in Table 19). The highest deficiency was registered in block 3 (-0.34) and the difference was highly significant $P < 0.0001$). The lowest was located in block 8 (-0.15) with a *P-value* of 0.0238. The blocks 5 to 7 as stated previously presented excess of Zebu alleles into crosses.

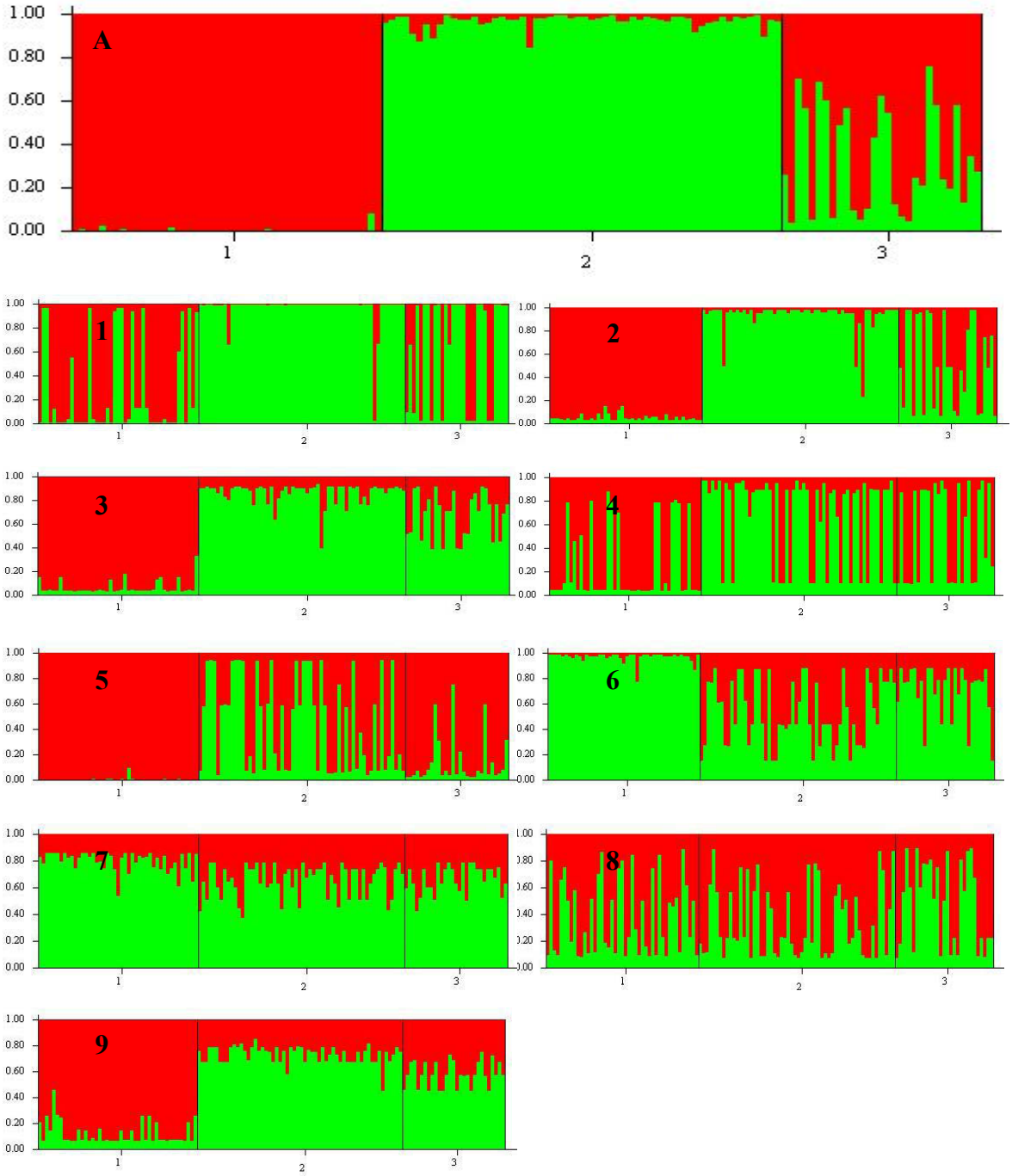

Figure 8: Genetic structure of 3 cattle populations

Each individual animal is represented by a single vertical line divided into 2 colours, where 2 is the number of assumed clusters and the length of the coloured segment represents the individual's ancestry proportion of membership to a particular cluster estimate. Black lines separate individual populations whose numbers (1 = Zebu; 2 = Baoule South-West; 3 = Crosses South-West) are indicated below the diagram. **A**: structured using autosomal microsatellites. **1** to **9:** SNP markers in candidate regions **1 to 9.**

Table 18: Allelic means of the blocs, differences and *P-values*

Block	Allelic Mean	Std. Dev.	Difference	*P-value*
1	0.36	0.45	-0.31	0.0004
2	0.48	0.38	-0.19	0.0003
3	0.33	0.19	-0.34	<0.0001
4	0.47	0.40	-0.19	0.0354
5	0.86	0.19	0.19	0.0002
6	0.71	0.21	0.04	0.7060
7	0.67	0.10	0.00	0.9735
8	0.51	0.32	-0.15	0.0238
9	0.42	0.10	-0,25	<0.0001

Allelic frequency in autosomal markers 0.67 ± 0.24. Sample size: 29 for each block as well as for autosomal markers.

4 – Discussion

4.1– Survey

The occurrence of the diseases depended on the climate of the different regions even if some diseases were common. Indeed the North is dry and therefore less favourable for some diseases, except the bacterial (CBP, hemorrhagic septicaemia, gas gangrene infection) and viral diseases (FMD). The South-West and the West are more wet (1000 – 1400 mm rainfall/year) the forests more dense and diversified and ground water is more frequent. This is suitable for the development of most diseases. Such conditions favour especially the life of tsetse flies that are the main vector of trypanosomosis. Sow *et al.* (2008) also reported that the annual rainfall in the North was weak (575 – 825 mm) and not favourable to tsetse flies. Despite that, some cases may be detected and treated because other vectors like *Liprosia spp.*, *Stomoxys spp.,* tabanids may transmit trypanosomosis and various infection mechanisms do exist (Desquesnes & Dia, 2003a; Desquesnes & Dia, 2003b, Desquesnes & Dia, 2004; Dia *et al.*, 2008; Desquesnes *et al.*, 2009; Grace *et al.*,2009). In our sample, in the North none reported trypanosomosis as an important disease even the 5 farmers in Yako (Passoré province) who used to go for transhumance. However, those transhumant farmers used to prevent using trypanocidal drug before the transhumance. Transhumance is important in the North indeed, as herdsmen may go from 28 – 82 km away from their home place (20 – 82 km in overall study area, 43.01 ± 19.19 km in average) and stay from 2.50 – 3.50 months (0.75 – 7.50 in overall study area, 3.01 ± 1.85 months in average; Table A1 in Appendix). Among breeders 17.61 % were practising transhumance. The distances and durations of transhumance are higher than those reported by Affognon (2007), probably because his study was restricted to one region of Burkina Faso one of Mali and one of Guinea while in our study the surveyed area of Burkina Faso was larger and not restricted only to tsetse challenged area.

Ranking disease made trypanosomosis the first ranked disease in overall, above all in the tsetse challenged area. Affoghon (2007) reported also that in Kénédougou Province (Burkina Faso), trypanosomosis was considered by 90.22 % as a priority disease and 84.52 % considered so in Mali. In Burkina Faso once more, Soudre *et al.* (2009) reported more that more than 50 % of farmers considered it a priority disease. Further, Grace (2005) and Grace *et al.* (2009) reported respectively that 84.00 % and 89.1 % of farmers in West Africa assigned trypanosomosis first

place. These proportions are higher than ours may be because in our study we considered many locations (24 villages in tsetse challenged area). These villages had different experiences with trypanosomosis but also some of the interviewees owned trypanotolerant animals. The owners of trypanotolerant animals have a different opinion about the disease compared to those who owned crossbred and trypanosusceptible cattle. When looking at the proportion within region, in the West it was higher (70.91 %) than the overall area. The Kénédougou province in Grace's (2005) study as well as in Affognon's (2007) was one the 3 provinces sampled in the West. All these results indicate that trypanosomosis is the major disease in cattle of the region.

The assignment of the disease seems to be somehow linked to the composition of the herd along the regions. In the South-West where more herds are pure Baoule (15/55 herds) compared to the West (no pure Baoule herd) trypanosomosis was first ranked at a lower proportion (but not significantly different) compared to the West. The herds in the West were mostly crosses (Baoule×Zebu) as shown in Table 4. All of the interviewees in the tsetse challenged area believed that Baoule is more resistant than Baoule×Zebu which is also more resistant than pure Zebu. Mwangi *et al.* (1998) also reported different susceptibility of breeds exposed to trypanosome infection in Kenya.

Trypanosomosis control was via trypanocidal drugs by the majority of farmers interviewed. In Kenya Ohaga *et al.*, (2007) reported 92 % of farmers using trypanocides while Grace *et al.*, (2009) reported 49.7 % of farmers in West African cotton zone. Both authors also reported the indirect control method farmers used such as use of insecticides. Our result in this case was somewhat higher than the 4.5 % reported by Grace *et al.*, (2009). Scarification was also mentioned by the last authors in Mali, Burkina Faso and Guinea. A different control method that was not explicitly reported by farmers but in use in the tsetse belt area was cross-breeding trypanotolerant cattle with trypanosusceptible ones. In Figure 9, it is shown that one of the reasons for keeping crossbred is trypanotolerance. This was mentioned by 12.73 % of the farmers in the South-West and 25.50 % in the West but the difference was not significant ($P = 0.09$). If it is considered that adaptation and growth can only be possible if the breed is trypanotolerant considering trypanosomosis as a priority disease then the proportion could be higher (29.09 % and 83.64 % respectively for the South-West and the West) than what is presented in the Figure 3. This strategy was as well reported by Talaki (2008) in Burkina Faso and Grace *et al.* (2008) in Mali and Clausen *et al.* (2010) in West African cotton zone. Instead of

4 strategies Grace *et al.* (2009) reported 17 strategies known by the farmers in West African countries from which they drawn 5 categories which included the 3 methods of our results. The categories reported were modern medicines, traditional medicines, control of tsetse by insecticides or traps/screens (communal vector control), avoidance of tsetse, and general good husbandry. From these, farmers applied in average 3.5 strategies per farmer in Mali and in the West African cotton zone as well (Grace *et al.*, 2008; Grace *et al.*, 2009). Achukwi *et al.* (2009) reported that farmers owning the Doayo/Namchi breed in Cameroon did not use any trypanocide. That is in concordance with our findings as well. It should be noticed that in some cases, some practices are obvious from breeders' understandings that they even don't mention them. That is the case of avoiding high risk areas as reported by Grace *et al.* (2008), Grace *et al.* (2009).

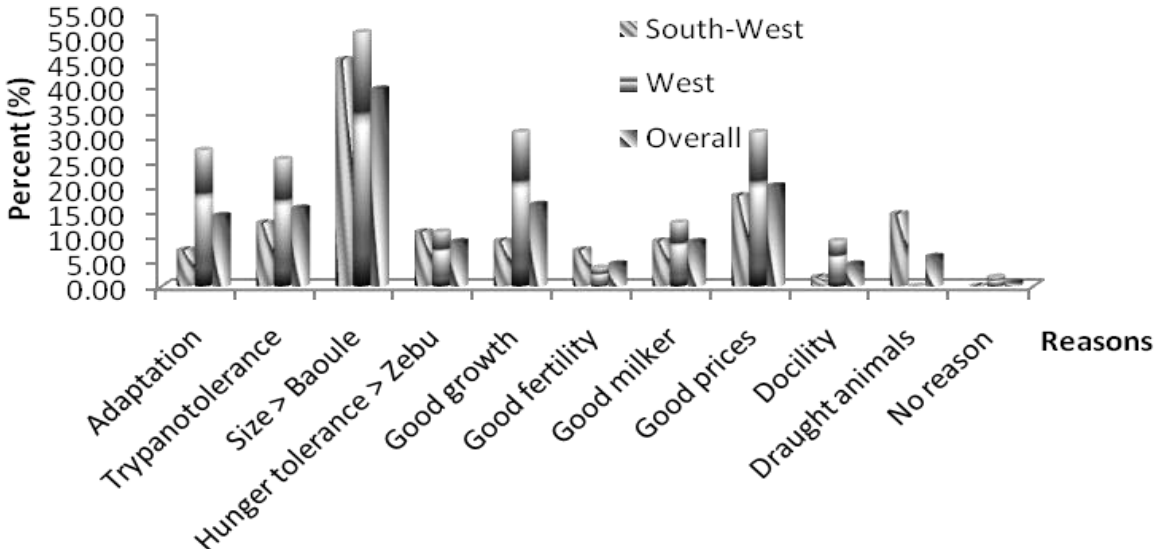

Figure 9: Reasons of keeping crossbreds in tsetse challenged areas

Trypanocides were widely used in the field but its utilization varied from region to regions.I Isometamidium was largely used by the breeders and diminazene was a second choice drug. This result is inconsistent with Grace *et al.* (2009) results that made isometamidium a second choice drug and diminazene the first one. Breeders appreciated drugs differently. In the South-West many farmers claimed that there were many fake drugs and unofficial veterinarians. That region is not far from the borders of Ghana and Côte d'Ivoire where many drugs were supposed to be imported from and many unofficial veterinarians came from. In that South-West there were less

cross animals than in the West and also less treated animals, hence less experience with resistance. For resistance, many others reasons attempted to justify treatment failures and those given by breeders in our study are in agreement with Gu *et al.* (1999).

Resistance was reported by many previous studies (Peregrine *et al.*, 1991; Clausen *et al.*, 1992; Mulugeta *et al.*, 1997; Gu *et al.*, 1999; McDermott *et al.*, 2003; Dia el al., 2008; Grace *et al.*, 2008; Murik *et al.*, 2008; Bouyer *et al.*, 2009; Grace *et al.* 2009). Further Talaki (2008) stated that the resistance occurrence has been reported in more than 13 countries in West Africa. However Mahama *et al.* (2004) could not report any resistance in Ghana.

The importance of resistance to trypanocidal drugs may be due to the prolonged use of them (Bauer *et al.*, 1999). Ouédraogo *et al.* (2006) stated that trypanocidal drugs have been in treatments in Burkina Faso more than 40 years ago and resistance cases were revealed more than 15 years back (Clausen *et al.*, 1992). The under-dosage (Geerts Holness, 1998) incorrect injection technique (Gu *et al.*, 1999; Grace *et al.*, 2009) but also the use of fake drugs as raised by farmers in our study are part of several reasons that favour drug resistance in trypanosomosis. Farmers developed strategies to overcome trypanocidal drug failures. Increasing dosage is one option and according to Grace *et al.* (2009) that seems to work. But in our opinion cross-breeding trypanotolerant animals with the susceptible one as already implemented by breeders in tsetse belt areas and keeping the pure lines may be more sustainable and less costly. In addition for the majority of interviewees, the disease is decreasing. This is supported by the finding of Rayaisse *et al.* (2009) that reported a decreasing density of tsetse flies in Burkina Faso especially the *G. morsitans* group which now restricted to the protected areas (Courtin *et al.*, 2010). Furthermore Courtin *et al.* (2010) found that the Northern limit line of the flies shifted around 150 km to South making areas in the North free of tsetse. However Courtin *et al.* (2010) highlighted that the decrease of the tsetse population does stand for a long term decrease in trypanosomosis prevalence because small tsetse populations do remain vectors of trypanosomosis. But also *G. palpalis* group can survive to very high human density levels and they have been shown to increasingly transmit trypanosomes when they are submitted to environmental stress.

4.2 – Autosomal microsatellites genotyping

The genetic diversity of Burkina Faso cattle populations sampled from different regions across the country was assessed. The mean number of observed alleles was similar to the 11.4 alleles per locus reported by Loftus *et al.* (1999) but considerably higher than the 8.4 reported by MacHugh *et al.* (1997), 9.7 reported by Thévenon *et al.* (2007) in the Southern-West of Burkina Faso, 4.59 and 4.37 reported in Pakistan breeds by Rehman & Khan (2009). This difference may reflect a bias in the selection of the loci that have been selected for polymorphism but also the absence of selection pressure in cattle in Burkina Faso. Compared to Thévenon *et al.* (2007) the results were similar when we only consider mean number of alleles per locus from tsetse challenged area from where they took their samples as well.

Deviation from HWE might result from inbreeding but technical problems or null alleles with the 14 markers need to be considered as well. It may also result from population substructure specifically in Zebu breed. In Burkina Faso, the breed is known to consist of Fulani zebu, M'Bororo zebu, Azawak zebu originated from Niger, and a few years ago Gudali zebu which was formally from Nigeria. Individuals from these zebu types are thought to be included. The deviation in Baoule may result from misclassifying N'Dama type or their cross bred in Baoule type. N'Dama cattle were present in the South-West in the International Centre for Research and Development in Animal Husbandry in Subhumid Zones (CIRDES) research farm for scientific experiences and some individuals have been introduced in farmers' herds. One more reason of departure from HWE could be the admixture linkage disequilibrium, the correlations that arise between linked markers in admixed populations, as described by Falush *et al.* (2003).

The observed and expected heterozygosity across populations were similar or comparable to those reported by Moazami-Goudarzi *et al.* (1997); Ibeagha-Awemu *et al.* (2004) in West and Central African cattle populations, Sodhi *et al.*, (2005) in Indian cattle populations; Zerabruk *et al.* (2007) and Dadi *et al.* (2008) in Ethiopian indigenous cattle populations. But lightly different from Martin-Burriel *et al.*, (2007) may be because the populations in the study were endangered. The average heterozygosity was within the range (0.3 – 0.8) as suggested by Takezaki & Nei (1996) to be useful for measuring genetic variation. The overall F_{ST} revealed a moderate level of genetic differentiation among the populations in the study. The overall value of F_{ST} observed is

similar to that observed in Ankole cattle in Uganda (Kugonza *et al.*, 2010), lower than that reported in 2 Indian cattle populations (Sodhi *et al.*, 2005) greater than that observed in Ethiopian populations (Dadi *et al.* 2008), in Ankole cattle in the African Great lakes region (Ndumu *et al.*, 2008). The moderate genetic differentiation could be a result of gene flow of other populations. Populations in the present study have been sampled across regions with different breeding system and different purposes. In the Northern part the animals are reared without any trypanosomosis pressure therefore there is less or not crossing with the taurine breed. But in the tsetse challenged regions where trypanosomosis is the most important disease in cattle (Soudre *et al.*, 2009) crossbreeding is frequent, especially when farmers with Zebu cattle decide to settle. In addition, the pastoral production systems, long distance migrations within and across countries, utilization of communal pastures, exchange of breeding animals, uncontrolled mating facilitate constant gene flow. That situation may result in the no significant differentiation between the Baoule West and the crosses.

The proportion of admixture of the populations were different from those reported in African zebus (50% to 83%) by MacHugh *et al.* (1997) and Hanotte *et al.* (2002); 45% in African taurines (Hanotte *et al.*, 2002). The general point of view is that the admixture pattern in the present study is a consequence of the initial admixture of *Bos indicus* and *Bos taurus* cattle that formed the founders of the present African cattle. In the time course the cattle in the tsetse free areas became more pure than those in tsetse challenged areas because of the migration and settlement of the farmers with zebu cattle herds despite the sensitivity of zebu breed to trypanosomosis. That may result on the clustering of the Zebu North, the crosses and Baoule West as intermediate groups between Baoule South-West and Zebu North. Baoule West with a mean proportion of 76 % Zebu ancestry may not be a pure population as called by the famers but a stabilized crossbreed or breed with many back crossed individuals.

4.3 – Sex-chromosome microsatellites genotyping

Out of 475 microsatellites found so far on BTAX on NCBI database (Btau_4.0; http://www.ncbi.nlm.nih.gov/ consulted on 08/11/2010), 26 were surveyed in the current study. The BTA1 (153 cM) and BTAX (151 cM) reported by Kappes *et al.*, (1997) and 155.8 cM for BTAX by Sonstegard *et al.*, (2001) are both the longest chromosomes on the bovine genetic map. The BTAX is submetacentric (arms' length are unequal) as reported by Sonstegard *et al.*

(2001). For the chromosomes in general previous studies reported a tendency of higher recombination rates on short arms in various species (Morton, 1991; Rohrer *et al.*, 1996; Sondar *et al.*, 2006). So, the selected markers for our study were distributed on both arms.

The 26 markers held for the study were all polymorphic as shown by the results. Some of them were already tested by previous studies (Bishop *et al.*, 1994; Ponce De Leon *et al.*, 1996; Yeh *et al.*, 1996; Kapes *et al.*, 1997; Smith *et al.*, 1997; Sonstegard *et al.*, 1997; Freeman *et al.*, 2006; Sandor *et al.*, 2006; Nguyen *et al.*, 2007) that gave a proof of their polymorphism. The average spacing over the BTAX was 6.02 cM which was higher than the one reported by Freeman *et al.* (2006) using 5 markers witin 10 cM, 1.97 cM in the study by Harmegnies *et al.* (2006), 2.5 cM in the study by Thevenon *et al.* (2007). But it was smaller than 13.4 cM in study by Farnir *et al.*, (2000) and 20 cM by McRae *et al.*, (2002). The spacing would have been smaller and more regular if the 31 markers selected from the beginning were all polymorphic.

The range of observed alleles was higher than 3 – 7 reported by Smith *et al.* (1997) using 4 BTAX markers, 3 – 15 by Yeh *et al.* (1996) with 15 markers but similar to 4 – 17 reported by Sondar *et al.* (2006) with 25 markers in Holstein-Friesian. The heterozygosity found in our study was somewhat similar to 0.61 ± 0.05 found by Sondar *et al.* (2006) and the mean number of alleles (8.0 ± 1.1) as well. The comparisons made here show that the range of observed alleles may depend on the number of markers used in the study. More markers used tend to give a larger range of observed alleles. But the markers seem to be more polymorphic and diverse on autosomes than on BTAX. Indeed using 25 microsatellites markers on the same breeds, we found 311 microsatellite genotypes, a mean of 12.44 ± 4.30 alleles per locus and heterozygosity of 0.76 ± 0.10 (Soudre *et al.*, in preparation) versus 232 observed alleles, mean of 8.92 ± 3.59 alleles per locus and heterozygosity of 0.69 ± 0.18 with 26 markers in this study; the same was reported by Kappes *et al.* (1997) in cattle, Schaffner (2004), Altshuler *et al.* (2005) in humans. The discrepancy is may be due to the larger possible crossover events in autosomes than in sex-chromosomes and sex-specific difference in recombination rate (Beever *et al.*, 1996; Sandor *et al.*, 2006). Also, despite the higher number of loci on BTAX, Kapes *et al.* (1997) found a relatively small number of recombination events on BTAX compared to the majority of autosomes due probably to the higher LD in chromosome X. However, Sandor *et al.* (2006) found more polymorphic markers on BTAX.

Our result revealed that the gene diversity and the mean number of observed and effective alleles were lower in the taurine breed than the other 2 populations. The differences may denote the selection pressure on the taurine which made Baoule a trypanotolerant cattle breed. Indeed, trypanosomosis was present in the rearing area of Baoule breed for very long and the survival of the breed is unmistakably due to resistance developed against the disease. As Sandor *et al.* (2006) have shown that contrary to previous results there was evidence that BTAX harbored several quantitative trait loci (QTL) affecting traits of importance to the dairy breeding industry, perhaps there are trypanotolerance trait loci on BTAX. We also noticed the same difference of heterozygosity when using autosomal loci markers to study diversity in the populations (Soudre *et al.*, in preparation). The discrepancy may also result from the reducing population size of pure Baoule breed due to admixture that is a consequence of crossing Zebu individuals to Baoule individuals.

Except for the crossbred population all Zebu and Baoule populations were inferred to the ancestral breed at a proportion of at least 84 percent. This picture may reflect the real situation given that the markers were all located on BTAX linked group. The linked markers typed on BTAX, as mentioned previously, displayed lower heterozygosity and higher LD than those on the autosomes and therefore more conservative. It could be then be deduced that the populations were better inferred to the breed of origin (of the mother) than using autosomes markers data. Additionally, it has been reported by studies in different species (Kaback *et al.* 1992; Kappes *et al.*, 1997) that longest chromosomes like BTAX display lower recombination rates than shorter ones.

The admixture levels displayed using BTAX were above what has been reported on African zebus (50% to 83%) by MacHugh *et al.* (1997) and Hanotte *et al.* (2002); 45% in African taurines (Hanotte *et al.*, 2002). Regarding the proportion in the Baoule population it is in the interval of 81% to 100% of the N'Dama in the study by MacHugh *et al.* (1997), also in the same as other African taurine breeds in the study by Freeman *et al.* (2004), but higher than the proportion of the N'Dama in the study by Loftus *et al.* (1999). From these comparisons it can be deduced that the zebu populations from our study areas are among the purest zebu populations of the West African continent (Freeman *et al.*, 2004). The discrepancy with previous studies may result from the distinction made in our study between presumed pure individuals with crosses in the analyses.

The crossbreeding took place according to our results, 11.48 ± 7.16 generations ago in the study areas. This mean is the range of 4.27 ± 2.70 for Mbororo to 13.00 ± 5.01 for Kuri (West African hybrids), 7.99 ± 4.92 – 15.45 ± 7.23 of East African hybrids 8.87 ± 4.28 – 21.30 ± 4.78 of Southern African but of the range of 13.13 ± 5.05 – 17.76 ± 4.98 of Near Eastern hybrids, all results in the study by Freeman *et al.* (2006). By excluding individual with less than 85 % affiliation, power was given to the analyses. Therefore the visualization of the graphical displays of the recent admixture (Situation B) showed largely intact ancestral haplotypes (Wiehe *et al.* 2000).

The number of recombinations observed on a chromosome of post-admixture populations depends on the number of markers and the marker density used on a given chromosome. Even if the interval size in our study was not regular the results were comparable to the findings of Freeman *et al.* (2006) whose study was carried out using 10 markers within 10 cM on BTAX. One could notice that with our microsatellite markers panel, we had 2 to 5 markers within some 10 cM regions and 1 to 4 markers within other 10 cM regions. So, marker density is likely to be one of the limiting factors in our study to assess the age of admixture of the crosses. Also we observed greater spacing between adjacent markers located on the BTAXp arm where the occurrence of recombination events is greater (Morton, 1991; Sondar *et al.*, 2006).

Choosing 6 years as a generation length in cattle (Mahadevan 1955; Amadou-N'Diaye *et al.*, 2003) among different lengths suggested by previous studies (Franklin *et al.*, 1976; Koch *et al.*, 1974; Martinez *et al.*, 2008), the age of admixture of the crosses in our study was 69 ± 43 years. The age is consistence with Grace *et al.*, (2005) findings where farmers' responses lead to situate crossbreeding after the introduction of draft cattle early in the 20s - 30s of the 20th century.

From the results of the marker type comparison, the autosomal markers did not work similarly to the x-markers. This was expected because the autosomal markers have been applied on many chromosomes (22) within each individual while 26 markers have been used on a single chromosome (X chromosome) within each individual. The chromosome regions covered were then different. The x-markers covered a larger region of the X chromosome than the autosomal markers on the autosomes. That led to better accuracy in admixture information gained with x-markers in our studies. Looking at the proportion of each breed in the crossbred, the x-markers seemed to give more realistic information than autosomal markers as well. Indeed the proportion of Zebu breed in crosses ranged from 69 – 84% (Table 17) what is almost close to pure bred

individuals compared to the range (50 – 65%) with x-markers. Furthermore, there is only a single source of variation added to other sources in the case of x-markers (maternal inheritance) compared to the 2 sources of the autosomal markers (paternal and maternal inheritance).

4.3 – SNP genotypes from trypanotolerance candidate regions

The primary hypothesis of the approach used in this study was that strong selection for trypanotolerance in tsetse infested areas can lead to a deviation of breed composition of crossbred animals in these regions from background breed composition of an animal. This type of selection signature will be in effect even after a relatively short period of admixture (Tang *et al.*, 2007, Olesyk *et al.*, 2010).

The crosses sampled in the tsetse challenged regions were those that survived to trypanosomosis. Indeed animals aged more than 10 years were encountered in tsetse regions (Olesyk *et al.*, 2010). Even those calves that were sampled could be considered as survived because they wouldn't survive without inheriting resistance genes from their parents or being treated. As mentioned in the background infertility of males and females and abortion are sequels of the disease. Therefore, born alive crossed calves may have inherited the resistance genes of Baoule origin knowing that trypanosomosis is an endemic disease in those areas (Kamuanga *et al.*, 2001; Desquesnes & Dia, 2003; Hanotte *et al*., 2003; Grace *et al*., 2007). Inheritance of those genes is made within blocks in admixture populations (Gautier *et al*., 2007; Tang *et al.*, 2007). The 5 blocks and 4 chromosomes surveyed confirmed that those regions are trypanotolerance candidate regions (Kemp *et al.*, 1998; Thévenon *et al.*, 2007; Dayo *et al.*, 2009; Stella *et al.*, 2010). They are also located within QTL regions as reported by previous studies (Kemp *et al*., 1998; Hanotte *et al.*, 2003; Dayo *et al.*, 2009; Stella *et al.*, 2010) that affect trypanotolerance. Indeed trypanosomosis is a very complex disease involving control of many symptoms like anemia, parasitemia, body lost so that many loci on many chromosomes do contribute. Therefore the SNPs identified in the present study are part of those which really control the disease in the trypanotolerant animals.

Conclusion

From this study it can be unmistakably maintained that trypanosomosis is one of the worst diseases of cattle in Burkina Faso in general but particularly in tsetse belt zone. The raising drug resistance will be a big issue in the coming years even if the tsetse fly population is decreasing. This is particularly important when it is realised that the production of trypanocids is not always guaranteed but in addition marginal vectors such tabanids are more and more involved in trypanosomosis occurrence. However, the present use of trypanotolerant cattle as an integrated approach is giving confidence and could be sustainable. Indeed rearing crossbreds seems to be a strategy for some autochthonous and many migrants. Such a strategy should be taken into account for any improvement of cattle production. Also, the future development of the disease may be uncertain given the current climate change and the possible impact on trypanosomosis development.

The existence of a fairly moderate genetic differentiation among indigenous cattle populations in Burkina Faso across the loci makes it possible to improve these breeds by selection for production and conservation and diversity in general. Regarding trypanosomosis, it may help to improve the tolerance of the crosses to trypanosomosis in the tsetse infested regions. Trypanotolerance of crosses and composites investigations identified 16 SNPs within 5 blocks that are candidate regions that could potentially be used in selection.

Little is known about the genetic diversity, degree and age of admixture of Burkina Faso cattle populations. That supports the statement of Hanotte and Jianli (2005), that knowledge of both the global diversity of the breeds and admixture events will be needed in order to be able to make sound priority decisions.

With respect to diversity, actions should be drawn to conserve and improve the Baoule breed that is a reservoir of trypanotolerant cattle. As revealed by the results the Baoule breed is impacted by the introgression of Zebu breed to its biotope and pure Baoule seems to be confined to the South-West with very few exceptions in the West. Smart strategies need to be devised to best cope with the challenge of combining trypanotolerance and larger body size of animals desired for draft purposes. Government action for maintaining the pure Baoule cattle in a particular region is one option, farmer support in crossbreeding strategies, including a desired level of crossing, is another. The introgression of Zebu in the Southern areas of Burkina Faso will be perhaps more important with the climate change. More research is needed to learn about the best level of

crossbreeding, i.e. genomic proportions of Baoule and Zebu in the crosses. For this, a combination of on farm research and use of a small set of ancestry informative SNP markers for prediction of breed composition are the way forward. Such a small SNP chip should include markers in trypanotolerance regions as found in this thesis.

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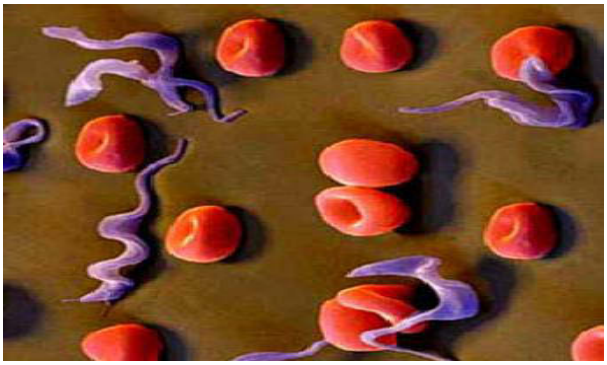
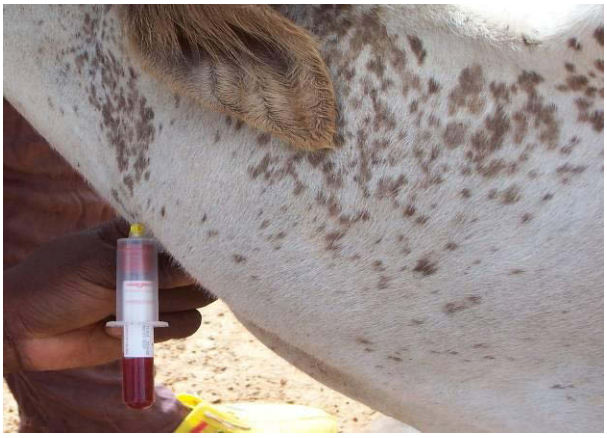
Appendix



Picture A1: Trypanosomes and red blood cells

Picture A2: Tsetse fly, a vector of trypanosomosis

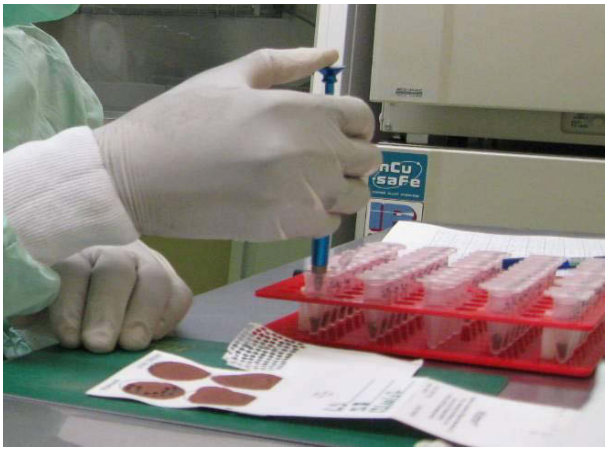


Picture A3: Blood sampling from jugular vein

Picture A4: Baoule cow from the South-West

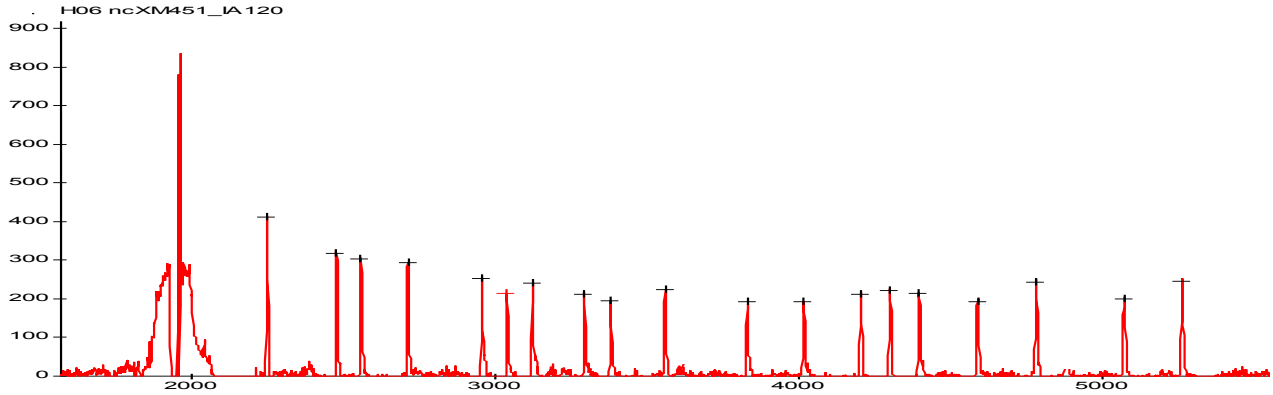
Picture A5: Zebu cow from the North

Picture A6: Baoule×Zebu (crossbred) cow from the West

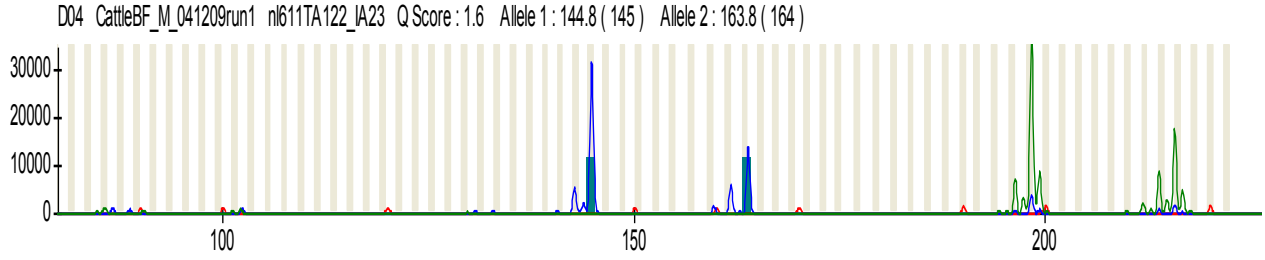


Picture A7: Taking discs with Harris punch from spotted FTA card

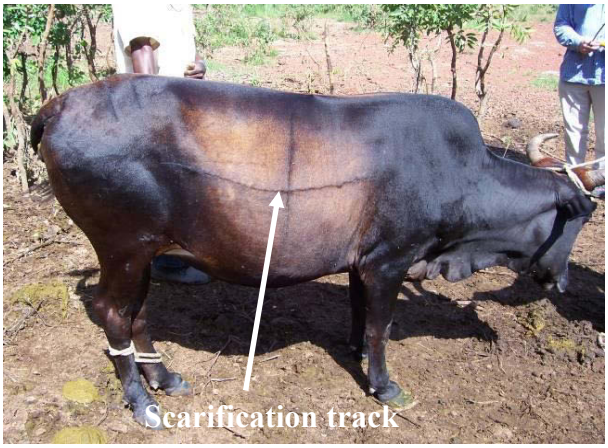
Picture A8: Spotted blood on labelled FTA card with punching tracks



Picture A9: Displaying of ET Rox 400 banding. Peaks with crosses on the top are standard positions ranged from 60bp up to 400bp.



Picture A10: Fam (blue dye) and Tet (green dye) alleles called and scored using Genetic profiler software. X axis displays the length of the products and Y the signal intensity



Picture A11: A scarification made to control trypanosomosis

Table A1: Transhumance durations and distances per region

		Freq	Mean	Std. Dev.	Min	Max
North	Duration (mth)	6	02.92	00.38	02.50	03.50
	Distance (km)	6	53.50	18.00	28.00	82.00
South-West	Duration (mth)	7	02.86	02.14	01.00	07.50
	Distance (km)	7	44.43	25.52	20.00	79.00
West	Duration (mth)	10	03.18	02.30	00.75	07.00
	Distance (km)	10	35.80	13.85	23.00	68.00
Study area	Duration (mth)	23	03.01	01.86	00.75	07.50
	Distance (km)	23	43.04	19.59	20.00	82.00

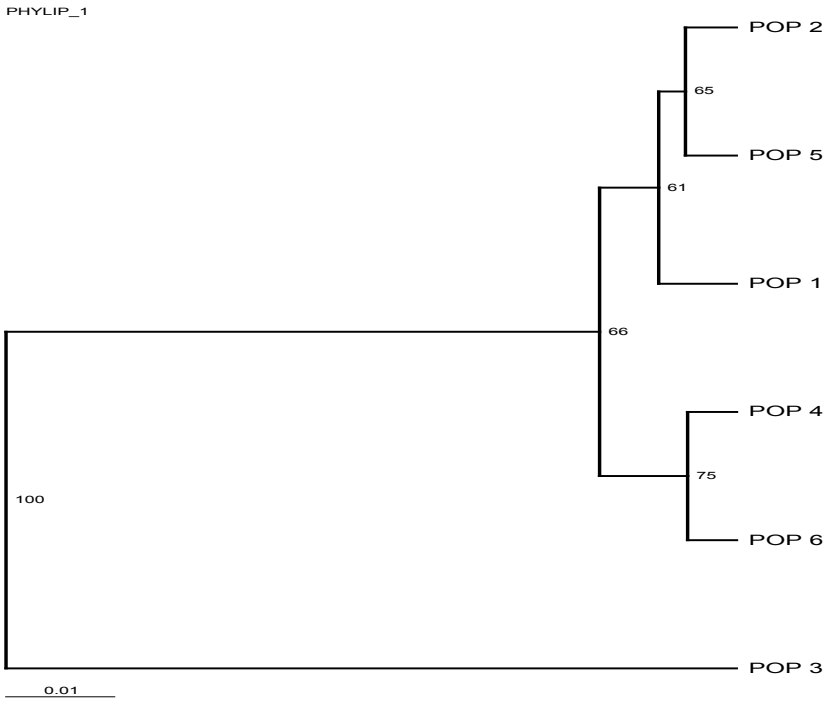


Figure A1: Neighbor joining tree summarizing genetic distances among 6 cattle populations

(P1: Zebu North; P2: Other Zebu; P3: Baoule South-West; P4: Baoule West; P5:Baoule×Zebu South-West; P6: Baoule×Zebu West). Bootstrap values indicating the degree of support for each branch point are shown beside the node as the percentage of replicates in which the cluster to the right **of** the node was recovered.