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Preliminary assessment of phenotypic and genotypic parameters associated with resistance to gastrointestinal parasites infestation in Burkina Faso sheep

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ABSTRACT

The purpose of this study was to assess phenotypic parameters associated with resistance to gastrointestinal parasite disease and to identify specific alleles of interferon gamma in two Burkina Faso sheep breed : Djallonké (West African Dwarf) and Sahelian. For this purpose, two experimental flocks (20 individuals of each breed) located in periurban area of Ouagadougou was followed during 60 days. Sampling was focused on young individuals at approximately 3 to 6 months of age. After identification, individuals were dewormed. 28 and 35 days following deworming, phenotypic measurements was carried out including Body weight, FAMACHA score, hematocrit and faecal egg count (FEC). Total DNA was extracted following phenol/chloroform protocol and genotyping have been done with interferon gamma genes reported to be associated to parasitism infection resistance. No variation was found in FAMACHA score which remained constant during the whole experiment in the 2 breeds, 2.7 and 3.2 at day 28 and day 35 in Djallonké and 2.1 and 3 at day 28 and day 35 in Sahelian. However a significant variation has been found between the 2 sampling periods in sahelian breed. FEC values were significantly different between Sahelian and Djallonké 28 days after deworming (214.28±57.44 and 25±17 .078 respectively) but remained constant at day 35 with higher value in Sahelian. A positive correlation was found between FAMACHA score and FEC and negative between these two parameters and hematocrit. The two alleles already described in previous studies were found both in Djallonké and Sahelian and a third one was detected in this study only in Djallonké. This study allowed to note the relative resistance of Djallonké breed and will help to establish and to improve programs for animal identification, to collect phenotypic data and at last to identify genetic markers associated to parasites disease resistance.

Key words : Sheep, gastrointestinal parasites, genetic resistance, phenotype, interferon gamma, Burkina Faso.

INTRODUCTION

Small ruminant (*Capra hircus and Ovis Aries*) population in Burkina Faso numbered 16,738,327 heads (sheep: 6,702,640; goat: 10,035,687) [1] with an annual growth rate of 3%. These species are widely distributed in Burkina Faso providing a full range of useful products to humans including meat, milk, skin and hair that are of major economical importance for the maintenance of rural populations and alleviate the effects of poverty all over the country land. Burkinabé small ruminants can be classified into three major types or breeds [2, 3]: (i) Burkina Sahelian, ii) Mossi and iii) Djallonké (West African Dwarf). Recently, an additional breed named as red goat of Maradi has been introduced from Niger.

The small ruminant production system in Burkina Faso is mostly pastoral and traditional. Parasite infestations have been identified to be one of the main problems affecting productivity [4, 5].

Parasitism and gastro-intestinal parasites in particular is the most serious constraint affecting small ruminants worldwide [6, 7]. Economic losses are caused by decreased production, cost of prevention, cost of treatment and the death of infected animals.

The control of nematode parasites traditionally relies on grazing management, anthelmintic treatment, or both. However, grazing management schemes are often impractical due to expense or to the hardiness of infective larvae on pasture. In addition, the evolution of anthelmintic resistance in nematode populations threatens the success of drug treatment programs [8, 9, 10 11]. Alternative strategies for control of nematode infections are needed.

There is considerable evidence that at least part of the natural variation in resistance to nematode infection is under genetic control [12, 13, 14, 15].
Based on empirical observation, Djallonké breeds (Sheep and goat), but also crossbred individuals between Sahelian and Djallonké have been identified as resistant to parasites diseases.

Parasite resistance is likely to be controlled by several loci and therefore it may receive a strong mutational input which generates genetic variation [16].

From the loci reported to be associated with parasite resistance in sheep that encoding the gamma interferon (INFG) [17] has been widely confirmed across studies [16, 18]. In sheep, IFNG appears to be involved in the immune response to nematode infections [17, 18, 19], which affect the sheep industry worldwide. Sequence variation in the ovine IFNG gene has been reported [17], but only recently this sequence variation has been resolved into alleles [20].

Todate, no information is available on West African sheep breed where gastrointestinal nematodes infestation is one of the main problems causing considerable losses in small ruminants breeding [6].

The overall objective of this study is to establish phenotypic data related to the resistance / sensibility to gastrointestinal parasite disease in two Burkina Faso sheep breed (Djallonké and Sahelian) and to check possible connexion with interferon gamma specific alleles.

MATERIALS AND METHODS

Study location

This study has been carried out in periurban area of Ouagadougou (figure 1) in sudan-sahel area during the rainy season where the parasite challenge is high.

The Sudan-Sahel domain is a transitional zone with regards to rainfall and temperature, covering the central part of Burkina Faso (roughly from latitude 11° 3′ N to 13° 5′ N), with a short rainy season from June to September and very variable rainfall with average of 750 mm per year, temperatures varying between 20°C and 42°C, and vegetation varying from North to South with better hydric conditions, from the Sahel to the Sudan savannah to tend eventually toward a clear forest in the Southwestern extreme of the domain [21].

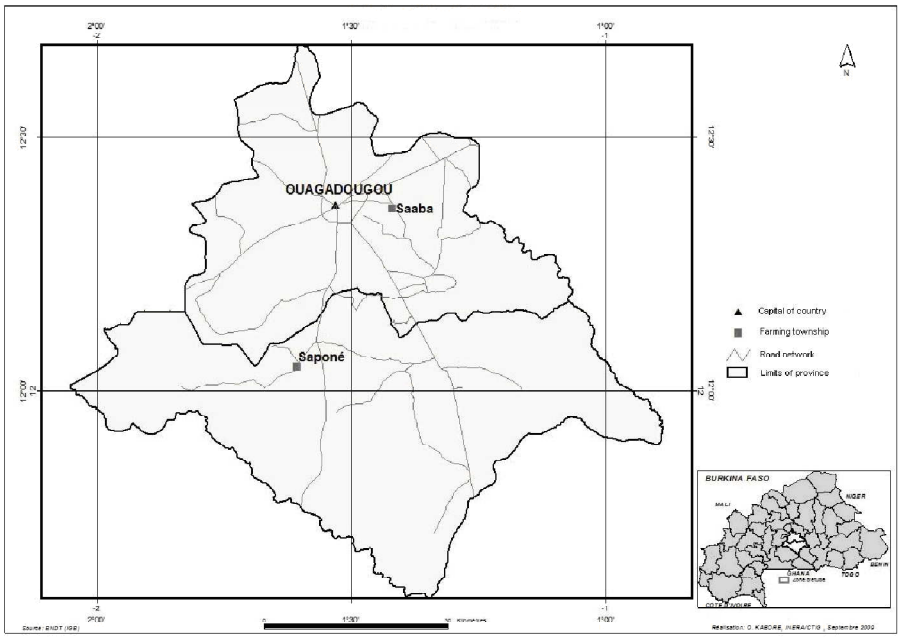


Figure 1: Map showing periurban area of Ouagadougou where the study have been carried out

Animal, sampling and phenotypic measurements

The experiment was conducted on two (2) groups of twenty (20) individuals each, with age ranged from 3 to 6 months. The first group comprised 20 Djallonkés sheep breed and the second group comprised 20 Sahelian breed maintained in their flock of origin and conducted by traditional breeding system.

Each individual were identified with ear tags and conducted to the same grazing area everyday from 9 AM to 3 PM. Feed complementation was given to animal after grazing including concentrate feed. Distribution of water was done *ad-libitum*.

Body weigh were noted using a weighing scale of 100 kg (precision 50 g).

Fecal samples were collected from each animal directly from the rectum in small polythene bags and labeled for further process.

FAMACHA score have been carried using FAMACHA card [22].

Blood samples have been collected from jugular punction using EDTA tubes for hematocrit measurement and DNA extraction.

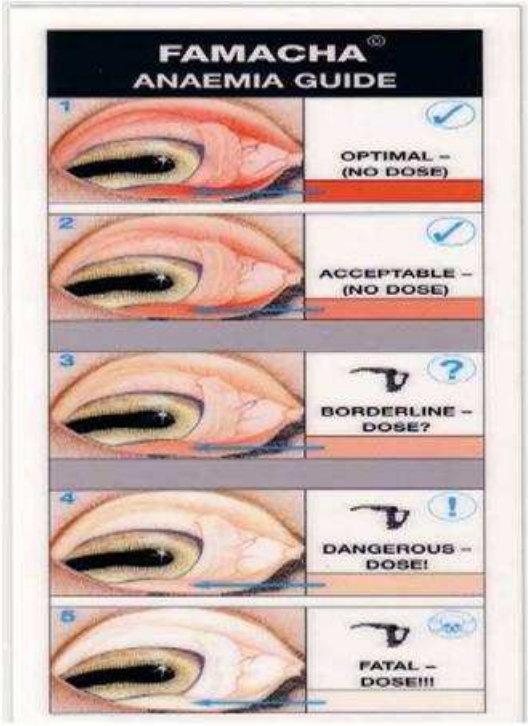
Methodologies

The two experimental flocks have been conducted and followed during 60 days for phenotypic parameters assessment linked to parasite resistance/sensibility (Body weight, FEC, FAMACHA score and hematocrit) and blood sampling for DNA extraction at the first sampling time.

Animal of each breed have been identified using ear tags and dewormed. Fecal egg counts (FEC) from 10 lambs per breed have been collected 10 days after deworming to determine efficacy of the dewormer.

Four weeks after final deworming, body weight, FEC, PCV, and FAMACHA scores have been taken twice, one week apart (days 28 and 35). A blood sample for DNA extraction has been collected at the first sampling time.
Counting of Eggs was made by Modified McMaster Technique [23] using a saturated sodium chloride solution (sg1.20) with a sensitivity of 50 for an egg.

FAMACHA score was taken using FAMACHA card as described by Van Wyk and Bath [22] (Figure 2). Color of ocular mucous membranes of each animal was classified into five categories according to the FAMACHA eye color chart:



1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, mildly anemic; 4 = pink-white, anemic; 5 = white, severely anemic.
Figure 2 : FAMACHA card

Hematocrit measurement was obtained after centrifugation of the whole blood at 8500g. Hematocrit values were determined by the procedure outlined by McGovern et al. [24].

DNA extraction and genotyping
DNA was extracted from the whole blood following standard phenol-chloroform technique of Sambrook et al. [25] and stocked at 4^{o}C prior PCR amplification.

PCR have been carried out using the interferon gamma sequence located within intron 1 with the following primers: 5'-TTGTGACTGTTAGCTAGATGTGTT-3' (forward) and 5'-ATACACATATTATGCCCATCTTTT-3' (reverse).

About 100 ng of genomic DNA have been amplified using 0.25 unit of *Taq* DNA polymerase in the recommended buffer provided by the manufacturer (Biotools, SA) for 35 cycles (2.5mM MgC1; 1 min denaturation at 94°C, 1 min annealing at 56^{o}C ; 2 min extension at 72^{o}C). Final extension has been done at 72^{o}C during 45 min.

Genotyping have been carried on 34 samples (15 Sahelian and 19 Djallonkés) using a semi-automated ALFexpress sequencer (Amersham Biosciences, Barcelona).

Data processing and analyses
Data collected have been processed on Excel 4.0 and subjected to one-way analysis of variance using Statview 4.57 for multiple means comparison test on phenotypic parameters.

FEC data were first log transformed before applying a one-way ANOVA analysis. The overall relationship between phenotypic parameters was assessed by Pearson's correlation analysis.

Alleles were determined according to their size as previously described by Schmidt et al. [26] : 125 pb for the short allele et 129 pb for the long allele. Allelic frequencies have been determined using the software Genepop of Raymond et Rousset [27].

RESULTS

Between breed phenotypic parameters variation according to sampling date
Means values for phenotypic parameters according to breed and sampling date are given in table 1.

Table 1: Means Values for Body weight, FAMACHA, hematocrit and FEC per breed at days 28 and 35 after deworming.

Sampling date	Breed	Body weight	FAMACHA score	Hematocrit	FEC
D28	Djallonké	16.550 ± 0.540[a]	2.700 ± 0.153[a]	29.600 ± 1.318[a]	25.000 ± 17.078[a]
	Sahelian	15.714 ±0.918[a]	2.143 ±0.261[a]	31.000 ± 2.225[a]	214.286 ± 57.440[b]
D35	Djallonké	17.100 ± 0.623[a]	3.200 ±0.249[a]	31.500 ± 0.898[a]	160.000 ± 37.118[a]
	Sahelian	16.250 ± 2.314[a]	3.000 ± 0.000[a]	27.750 ± 1.250[b]	275.000 ± 77.728[a]

([a, b]) Different letters as superscripts mean significant differences (p < 0.05).

Body weight and FAMACHA score between Djallonké and Sahelian do not differ significantly according to sampling date.

Hematocrit values are equal for the two studied breeds at only day 28 after deworming. However, these values in Djallonkés are more high than those reported in Sahelian (P = 0.040).

FEC values are statiscally different between the two breeds with higher values in Sahelian at day 28 (P < 0.0001). No difference was found at day 35.

Within-breed phenotypic parameters variation according to sampling date

Djallonkés breed

Within Djallonké phenotypic parameters have been assessed and the results are reported in table 2.

Table 2 : Within phenotypic parameters variation in Djallonké according to sampling date.

Sampling date	Body weight	FAMACHA score	Hematocrit	FEC
D28	16.550 ± 0.540[a]	2.700 ± 0.153[a]	29.600 ±1.318[a]	25.000 ± 17.078[a]
D35	17.100 ± 0.623[a]	3.200 ± 0.249[a]	31.500 ± 0.898[a]	160.000 ± 37.118[b]

Different letters as superscripts mean significant differences (p < 0.05).

The whole assessed phenotypic parameters in Djallonké breed remained constant during the experiment at days 28 and 35 excepted for FEC where a significant variation were noted between the two sampling dates (25±17.08 and 160±37.11 respectively) (P=0.005).

Sahelian breed

The same phenotypic parameters than those of Djallonké have been assessed in Sahelian and the main results are given in table 3.

Table 3: Within phenotypic parameters variation in Sahelian according to sampling date.

Sampling date	Body weight	FAMACHA score	Hematocrit	FEC
D28	15.714 ± 0.918[a]	2.143 ± 0.261[a]	31.000 ± 2.225[a]	214.286 ± 57.440[a]
D35	16.250 ± 2.314[a]	3.000 ± 0.000[b]	27.750 ± 1.250[a]	275.000 ± 77.728[a]

Different letters as superscripts mean significant differences (p < 0.05).

As found in Djallonkés, the assessed phenotypic parameters remained constant between the two sampling dates excepted for FAMACHA score where a significant variation was found (2.143±0.261 and 3 respectively at days 28 and 35).

Correlations between phenotypic parameters

Pearson's correlation test has been carried out between FAMACHA score, hematocrit and FEC and the results are given in table 4.

Table 4: Correlations between FAMACHA score, hematocrit and FEC

	FAMACHA score	Hematocrit	FEC
FAMACHA score	1	-0.052	0.231
Hematocrit		1	0.366
FEC			1

FAMACHA score and FEC are positively linked and these two measures are negatively correlated with hematocrit.

Genotyping

Genotyping done on 34 individuals (15 Sahelian and 19 Djallonkés) allowed detecting a total of 3 alleles: 2 and 3 respectively in Sahelian and Djallonké as noted in figure 3.

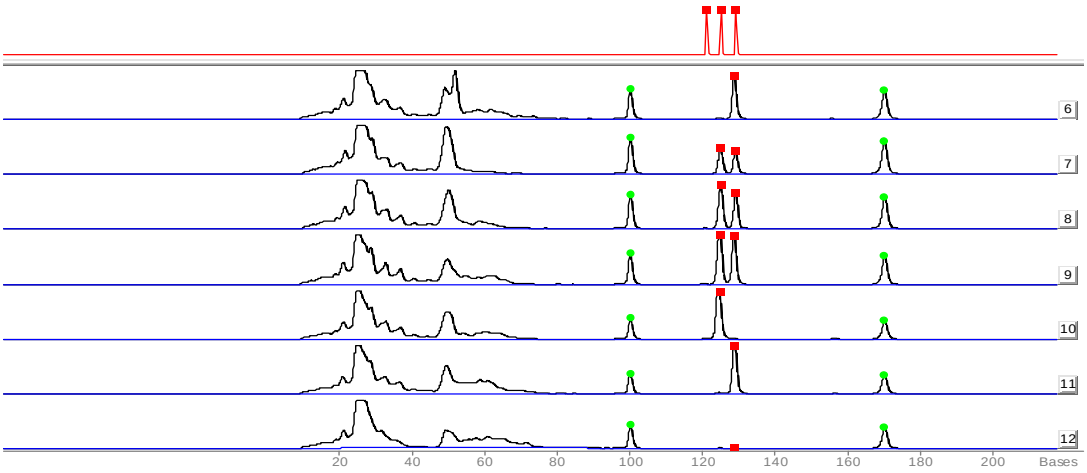


Figure 3: Genotyping of interferon gamma gene

Allelic frequencies are shown in table 5.

Table 5: Allelic frequencies of interferon gamma gene in the two study breeds

	Frequencies (%)		
Breeds	Allele A' (121 pb)	Allele A (125 pb)	Allele B (129 pb)
Djallonké	21.1	26.3	52.6
Sahelian	0	26.7	73.3

Besides the short allele (125 pb) and the long allele (129 pb) previously described by Schmidt et al. [26], a third allele (121 pb) has been identified and present only in Djallonké individuals.

DISCUSSION

Body weights measured in both Djallonké and Sahelian in this study do not significantly differ with the sampling even if values are higher in Djallonké breeds. However, in the literature, the body weight at young age of Sahelian breed is significantly higher than that of Djallonké. The paradox found in this experiment might be due to the non homogeneity of ages in the two groups with an average of 5.5 months in Djallonké and 4.8 months in Sahelian.

FAMACHA score remained constant during the whole experiment both in Djallonkés and Sahelian breeds during the two sampling periods: 2.7 and 3.2 respectively at days 28 and 35 in Djallonké and 2.1 and 3 respectively at days 28 and 35 in Sahelian. These values remained in normal admitted limits [22]. However, FAMACHA score varied significantly in Sahelian between the two sampling dates which mean a relative sensibility in gastrointestinal parasites of Sahelian breed than Djallonké.

FEC values are also significantly higher in Sahelian 28 days after deworming (25±17.078 and 214.28±57.44) but remained constant at day 35. This observation consolidates that of FAMACHA score regard to gastrointestinal parasite resistance between the two studied breeds. However, FEC is not considered as a good indicator in the assessment of resistance to gastrointestinal parasite [28, 29].

Pearson correlations test showed a positive correlation between FAMACHA score FEC and negative with hematocrit. These results confirm those of other authors on sheep breed [29, 30].
Genotyping carried out with the interferon gamma gene allowed to detect 3 loci with specific one in Djallonké. A similar study conducted in Romney, Perendale, Coopworth, Merino, Texel, Finnish Landrace breed and the Scottish Blackface [26, 31] allowed to detect the two common alleles (short and long) with frequencies of 41 % frequencies for the short allele and 59 % for the long allele.

The short allele's frequencies in our study are below that of Schmidt et al. [26]: 26.3 and 26.7 respectively for Djallonké and Sahelian. In contrast, the long allele's frequencies are higher in Djallonké and Sahelian (52.6 % and 73.3 % respectively). The third has a frequency of 21.1 % in Djallonké. Before an anticipated conclusion on the presence of this specific allele, this study is still ongoing including more individuals to allow carrying out an association study between phenotypic parameters with molecular information.

CONCLUSION

This study was conducted with the main achievement to assess phenotypic and molecular parameters associated with resistance to gastrointestinal parasites in two Burkina Faso sheep breeds. The assessed parameters include Body weight, hematocrit, FAMACHA score and FEC.

Body weight and hematocrit did not undergo strong variation during the whole experiment according to sampling dates after deworming. The main variations concerned specifically FAMACHA score and FEC which are the main parameters used in the assessment of resistance or resilience of individual animals gastrointestinal parasites. The mean values of both parameters remained higher in Sahelian, suggesting the great sensibility of this breed compared to Djallonké.

At the molecular level, genotyping of interferon gamma gene revealed a specific allele in Djallonké which need to be confirming with a large sample.

In perspectives, it is suggested to go ahead with this study in experimental condition on the three identified Burkina Faso sheep breeds described in previous study, considering only *Hæmonchus contortus*, the main parasites affecting sheep in our country.

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