Genetic variability of Nigerian sheep breeds at the insulin-like growth factor-1 (IGF-1) gene locus

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Abstract

The genetic variability of some Nigerian breeds of sheep at the IGF 1 locus was investigated. Blood samples from 150 sheep (50 each of Yankasa, Balami and Uda sheep) were collected from the Maiduguri abattoir and analysed at the Biotechnology centre of the University of Maiduguri. A relatively high level of genetic variability was detected in Nigerian sheep; the mean observed heterozygosity (H_o) and expected heterozygosity (H_e) values were 0.433 and 0.476, respectively. Comparison of expected heterozygosity of alleles between the populations showed that Yankasa was the most heterozygous of the sheep populations though the unbiased expected heterozygosity (0.503) for the Yankasa was the same as that of the Balami. All populations were 100% polymorphic at the IGF 1 locus with fixation index ranging from -0.169 to 0.378 and a mean of 0.093, which suggests a general pattern of random mating. Similarly, low mean values for F_{is} (0.093), F (0.089), F_{is} (0.089), F_{it} (0.130) and F_{st} (0.045) supports the assertion that inbreeding was limited due to outcrossing with relatively high level of gene-flow ($Nm = 5.292$). The analysis of molecular variance (AMOVA) revealed that only 1% of the total variations observed in the sampled populations is accounted for by the population differences while individual differences accounted for 85%. The UPGMA phylogenetic tree revealed that the Uda and Yankasa populations are closer to each other than each is to Balami. Thus, it can be concluded that sufficient genetic variation exist in the studied Nigerian sheep breeds and these populations are unlikely to decline due to inbreeding.

Keywords: Variability, Uda, Yankasa, Balami, IGF 1,

Introduction

The population of sheep in Nigeria is currently estimated at 42 million (FAOSTAT, 2017) and are widely distributed in Nigeria where they are a major component of livestock production systems, characterized by small flock sizes. They are found in different agro-ecological zones of Nigeria and occupy a special place in the rural economy where they are not only an emergency source of fund but also contribute significantly to increased animal protein intake for the average Nigerian (RIM, 1992). Local sheep breeds are very important to resource poor farmers because they have been naturally selected to withstand adverse environmental conditions as well as utilize scarce and poor quality feed resources in the localities where they thrive.

The Insulin-Like Growth Factors (IGFs) play a major role in regulating cell proliferation and inhibiting apoptosis. It is a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou *et al.*, 2005). The ovine IGF-1 gene was mapped to chromosome three with identification number 443318 (NCBI, 2015). The predicted amino acid sequence of IGF-1 peptide differs from the human, bovine, and porcine IGF-1s at a single amino acid (at position 66, alanine is substituted for proline) and differs from rat and mouse IGF-1s at positions 4 and 5, respectively. Ovine IGF-I amino-terminal peptides are 1 amino acid longer than other mammalian IGFs due to the presence of an extra amino acid (glutamine) that is present at the

proposed boundary of exon 1 and exon 2 (Wong *et al.*, 1989). Ovine IGF-1 has 5 exon count (NCBI, 2015).

Genetic diversity is the basis for both natural evolutionary changes and artificial selection in breeding populations. Evaluation of the genetic variability is vital to the understanding of underlying genetic structure of sheep genetic resources and for development of optimal strategies for their genetic improvement, conservation and sustainable utilization.

According to FAO (2007), animal genetic diversity allows farmers to select stocks or develop new breeds in response to environmental change, threat of disease, new knowledge of human nutrition requirement, changing market conditions and societal needs. Thus, better understanding of genetic variation holds the key to future utilization through preservation.

The aim of this study was to determine population genetic characteristics of Nigerian sheep based on IGF-1 locus as well as the phylogenetic relationship between the sheep.

Material and methods

Study area

The samples for the study were collected from the Maiduguri abattoir and analysed at the Biotechnology Centre, University of Maiduguri. Maiduguri, the Borno State capital is located on Latitude 11°52N and Longitude 13°14E of the equator. It occupies an area of about 3,000km², and is the largest town in North-eastern Nigeria. Maiduguri Metropolitan Council is bound to the North, West, and East by Mafa, Konduga and Jere Local Government Areas, respectively (Federal Republic of Nigeria, 1991).

According to the 2006 census, Maiduguri has a population of about 1,197,497 million

people (NPC, 2006). The weather condition of the area for most part of the year is hot and dry with short raining season in the months of June to September. The soil of the study area is generally sandy-loam.

Maiduguri lies in the Sahel ecological zone characterized by few trees and vast grassland. The grasses are seasonal and disappear during the dry season. The agricultural activities in the area include arable farming, livestock farming, fishing and hunting. Other major activities in the area include trading and weaving of caps.

Sample collection

Blood samples for DNA extraction were collected from a total of one hundred and fifty (150) adult sheep through the jugular vein, using needle and syringe (5 mL) and preserved in EDTA bottle. Fifty each of Balami, Uda and Yankasa sheep, were randomly sampled. The samples were conveyed to the Biotechnology Centre, University of Maiduguri inside a cooler with ice pack.

DNA extraction

Genomic DNA was extracted from whole blood using standard kits (Promega UK), following manufactures instruction.

Measuring nucleic acid quality and quantity

Nucleic acid concentration was determined spectrophotometrically using NanoDrop spectrophotometer (Thermos scientific).

Polymerase chain reaction (PCR) amplification

Polymerase chain reaction was carried out on 50 µL volume containing approximately 1.0 µL of 0.2 µM each of primer (Table 1), 10 µL of 5X GoTaq colourless buffer, 5 µL of 25 Mmol/MgCl₂, 1.0 µL of 10 µM each of DNtp, 2 µL of 50 µM/µl GoTaq polymerase (Promega USA) and 29.25 µL nuclease free water. PCR condition were as follows; initial denaturation of 95°C for 5 minutes,

followed by 35 cycles of denaturation at 95°C for 60 seconds, annealing for 60°C for 30 seconds, extension at 72°C for 60 seconds, with final extension at 72°C for 5 minutes on Master cycler (Eppendorf AG.2233, Hamburg Germany).

A general master mix was prepared to contain all the reaction components in one cocktail without the template DNA. PCR tubes were labelled according to number of samples, including a negative and positive control. Another tube was labelled as

Master Mix. The reagents were thawed and left on ice. Master mix was prepared according to Table 2 and reagents added in that order. 1 µL of the master mix was transferred into the labeled PCR tubes and 2 µL of the template DNA was added to the master mix. All reactions were done on ice.

Running the thermocycler

PCR tubes were transferred into the heating block of the thermo cycler. Number of cycles (95°C: 5m {95°C:60s; 72°C:1m}72°C:5m) inputted to start amplification.

Table 1: Primers used to amplify sheep genomic DNA for IGF-1 gene analysis

Primer	Product Size AT (°C)	Bp
5' regulatory region F: TGAGGGAGCCAATTACAAAGC R:CCGGGCATGAAGACACACACAT	55	294
Exon 1 F: TCACTGTCACTGCTAAATTCAG R:CTTCAAGAAATCACAAAAGLAGCAC	60	228

Source: He *et al.* (2012)

Table 2: PCR Master Mix

COMPONENTS	FINAL CONC.	RXN VOLUME
F- Primer (10µM)	1.0 µL	0.2 µM
R- Primer (10µM)	1.0 µL	0.2 µM µL
dNTP (10mM)	1.0 µL	0.2 µM µL
Mgcl ₂ (25µM)	5.0 µL	2.5 µM µL
BSA (10mg/ml)	0.5 µL	0.25 mg/mL
TaqPolymerase	0.25 µL	1.25 U/50µL
10X Standard TaqRxnBuffer	10.0 µL	1X
Template	2.0 µL	
DNA Nuclease free water	29.25 µL	
Total	50.0 µL	

Agarose Gel Electrophoresis

1% agarose was prepared by weighing 1g of agarose powder and dissolved in 100 mL of 1X TAE buffer then swirled to ensure thorough mixing. The dissolved agarose was microwaved for about 3-4 minutes and

allowed to cool to about 60°C. 10 µL of ethidium bromide was added and mixed avoiding bubbles. The gel cassette was assembled and the gel was poured in to the gel trough removing all bubble with a needle or pipette tip. The gel comb was

inserted and left to set for about 30 minutes.

Loading sample into the gel well

Electrophoresis tank was filled with 1X TAE buffer. The gel comb was removed carefully from the solidified agarose, and the side rubber seal disassembled. The set agarose gel was transferred to the gel tank. The comb grooves were labeled according to the samples to be loaded into the gel to identify the lines on the gel photograph. 2 μ L of loading dye and 5 μ L of PCR amplicons transferred on to a spread sheet of parafilm M then mixed using 10 μ L pipette and the mixture transferred to the gel well. The gel tank was closed and power cords connected. Power pack was switched on and the gel allowed running for 25 minutes at 120 V. The progress of the gel was monitored by reference to the marker dye.

Gel documentation

UV transilluminator (CSL-MICRODUC System Cleaver Scientific UK) fitted with camera (Canon power shot G12 UK) was used to photograph the gel image. The gel image was viewed on a screen for the bands.

Restriction Fragment Length Polymorphism (RFLP)

RFLP analysis was performed by incubating a mixture of 5 μ L of regulatory region of PCR product, 16.3 μ L of distilled water, 2.5 μ L of enzymes buffer and 1.2 μ L of *Hae II/BSP 143 II* (New England Biolabs, Beverly, M.A. USA) at 37°C for 3 hours inside a water bath, allowing the restriction enzymes to cut, at its restriction sites. Gel were visualized using a 2.0% Agarose gel that contained ethidium bromide, photographed and visualized using UV trans-illumination (CSL-MICRODUC System Cleaver Scientific UK) fitted with camera (Canon power shot G12 UK) to visualize the size of the fragments and assess whether or not the DNA was cleaved by the enzymes.

Data collection

PCR was performed on sheep genomic DNA and the PCR products were digested using restriction enzyme *Hae II/BSP 143 II*. PCR-RFLP genotyping was done at the ovine locus for insulin-like growth factor 1. The PCR products and gel images were then read to obtain data (bands formed by the images) which was analyzed.

Statistical analysis

Allele frequencies at the locus for each breed were calculated using a computer program FSTAT (version 2.9.3) (Goudet, 1995). The FSTAT program was also used to calculate F-statistics that include three indices FIT, FST and FIS. FIT is the inbreeding coefficient of an individual (I) relative to the total (T) population, FIS is the inbreeding coefficient of an individual (I) relative to the subpopulation (S) and FST is the effect of subpopulation (S) compared to the total population (T). Weir and Cockerham (1984) estimation of FIT, FIS and FST were performed for IGF-1 locus over all breeds. Per pair estimator of FST (measure of differentiation among populations) were also calculated between all pairs of the tested breeds. GENPOP version 3.1 (Raymond and Rousset, 1995) was used for calculating the observed and expected heterozygosity overall loci in each of the three breeds (Levene, 1949; Nei, 1978). The genetic distance (DA) was estimated according to the method of Nei (1978) and the unweight pair group method with arithmetic mean (UPGMA) was used for dendrogram construction using the same program.

Results

The population genetic characteristics of Nigerian sheep are presented in Table 3. The number of alleles per locus was two for all populations with an effective number of alleles of 1.906, 1.849 and 1.908,

respectively for Balami, Uda and Yankasa sheep populations. Heterozygosity is an important measurement of gene diversity (Slatkin and Barton, 1989). In this study, a relatively high level of genetic diversity was detected in Nigerian sheep; the mean H_o and H_e values were 0.433 and 0.476, respectively. The observed heterozygosity ranged from 0.286 (Uda) to 0.556 (Balami) while expected heterozygosity was between 0.459 (Uda) and 0.492 (Yankasa). Similar magnitudes of genetic variation (H_o and H_e) were reported for afrikaner sheep in south Africa and Tibetan sheep in India (Qwabe *et al.*, 2015; Sharma *et al.*, 2016). The authors observed that in assessing genetic variability, values from different studies should be compared with caution as different authors may use different methods. The heterozygosity recorded in this study supports a very high heterozygosity for classical genetic markers (Nei, 1987). The comparison of expected heterozygosity of alleles between the populations showed that Yankasa was the most heterozygous of the sheep populations though the unbiased expected heterozygosity (0.503) for the Yankasa was the same as that of the Balami. All populations were 100% polymorphic at

the IGF 1 locus with fixation index ranging from -0.169 to 0.378 and a mean of 0.093, suggesting a general pattern of random mating. This is expected as locus heterozygosity is related to the polymorphic nature of the locus. In this study, the average heterozygosity did not deviate much from the expected and there is no indication of inbreeding among the sampled populations as the two measures ($F_{is} = 0.093$ and $F = 0.089$) of inbreeding estimated were approaching zero. In a consistent trend, low mean values for F_{is} (0.089), F_{it} (0.130), F_{st} (0.045), and a relatively high value for N_m (5.292) supports the assertion that inbreeding was limited due to outcrossing with high level of gene-flow. Qing *et al.* (2009), made a similar observation in their study with Chinese sheep breeds. Gene flow between populations is typically measured as the migration rate N_m , which acts as a measure of movement of alleles from one population to another. This represents the number of migrations per generation. Gene flow between the populations was moderate (5.292). When N_m values are greater than 1, it means there is gene flow across populations at sufficient levels to prevent genetic drift.

Table 3: Population genetic characteristics of some Nigerian sheep breeds

Pop	Locus	Na	Ne	I	Ho	He	uHe	F	%P
Balami	IGF-1	2	1.906	0.668	0.556	0.475	0.503	-0.169	100
Uda	IGF-1	2	1.849	0.652	0.286	0.459	0.476	0.378	100
Yankasa	IGF-1	2	1.969	0.685	0.458	0.492	0.503	0.069	100
Total	Mean	2	1.908	0.668	0.433	0.476	0.494	0.093	100
	SE	0	0.035	0.01	0.079	0.01	0.009	0.158	0

F-Statistics and Estimates of N_m over All Pops for each Locus

All Pops.	Locus	Fit	Fst	Fis	Nm
	IGF-1	0.13	0.045	0.089	5.292

Na = No. of Different Alleles

Ne = No. of Effective Alleles = $1 / (\sum p_i^2)$

I = Shannon's Information Index = $-1 * \sum (p_i * \ln(p_i))$

H_o = Observed Heterozygosity = No. of Hets / N

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H_e = Expected Heterozygosity = $1 - \sum p_i^2$

uH_e = Unbiased Expected Heterozygosity = $(2N / (2N-1)) * H_e$

F = Fixation Index = $(H_e - H_o) / H_e = 1 - (H_o / H_e)$

F_{is} = $(\text{Mean } H_e - \text{Mean } H_o) / \text{Mean } H_e$, F_{it} = $(H_t - \text{Mean } H_o) / H_t$, F_{st} = $(H_t - \text{Mean } H_e) / H_t$, N_m = $[(1 / F_{st}) - 1] / 4$

Key: Mean H_e = Average H_e across the populations. Mean H_o = Average H_o across the populations.

H_t = Total Expected Heterozygosity = $1 - \sum t p_i^2$ where $t p_i$ is the frequency of the i th allele for the total & $\sum t p_i^2$ is the sum of the squared total allele frequencies.

Table 4: Analysis of molecular variance

Source	Df	SS	MS	Est. Var.	%
Among Pops	2	0.715	0.358	0.003	1%
Among Indiv	44	12.519	0.285	0.036	14%
Within Indiv	47	10.000	0.213	0.213	85%
Total	93	23.234		0.251	100%

The analysis of molecular variance (AMOVA) in Table 4 revealed that only 1% of the total variations observed in the sampled populations is accounted for by the population differences. The bulk (85%) of these variations came from the individual differences rather than population differences (Chart 1). This is similar to the findings of Agaviezor *et al.* (2012) who observed that 0.23% of the variation is found among populations compared with 99.77% variation found within populations. A higher variation within than among Nigerian sheep breeds suggest high levels of female mediated gene flow (Tserenbataa *et al.*, 2004). In addition, Wang *et al.* (2006) in their report observed that AMOVA indicated that some breeds are differentiated relative to a random

collection of genotypes and reflect differences in the spatial distribution of genetic variance. Pariset *et al.* (2011) also reported that the major mitochondrial variation was distributed within breeds (95.04), while it was lower among regions (0.90%) and among breeds within regions (4.06%)

According to Gizaw *et al.* (2011), an important characteristic to note in genetic diversity in livestock populations is that the variation within a population is much larger than that between the populations. This is well exemplified in the study of Ethiopian sheep where the diversity between Ethiopian sheep populations accounted for only 4.6 percent of the overall genetic diversity, the rest being accounted for by within-population variation.

Figure 1: Source of Variation for AMOVA

The unweight Pair Group Method with Arithmetic Mean (UPGMA) phylogenetic tree (Figure 2) revealed that the Uda and Yankasa populations are closer to each other than they are to Balami. Genetic distance and tree construction methods give insight into the genetic uniqueness of breeds under investigation (Thaon d'Arnoldi *et al.*, 1998). Hence from the UPGMA phylogenetic tree method thus utilized, Balami with value of 0.027 units and Uda and Yankasa (0.027units) breeds

can be said to be close. Despite the small sample size, the similarities found in these analyses are large enough to be apparent.

The tree showed that all the breeds originated from a common source. Furthermore, Balami diverged first, followed by Uda and Yankasa which though separated are more genetically related. However, Adebambo *et al.* (1999) showed that the breeds were clearly separated from each other. The authors observed that these native breeds stood out as three distinct groups on the evolutionary scale.

Figure 2: Phylogenetic Tree (UPGMA)

Furthermore, the phylogenetic tree (Neighbour-Joining method) on Figure 3 showed that the breeds stood out as two distinct groups on the phylogenetic Tree. The Uda and Yankasa were clustered together with a 0.032 occurrence bootstrap value, while the Balami stands out alone. This report contrasts the findings of Adebambo *et al.* (1999) who observed that, Uda and Balami which are basically northern breeds are clustered together with a 65% occurrence bootstrap value, far from the West African dwarf (WAD), whilst the Yankasa stood between the extremes of the WAD (a southern humid zone breed) and the Uda and Balami (northern arid Zone breeds).

Genetic distances of 0.032 and 0.054 were found between the Uda and Yankassa and the Uda and Balami, respectively, showing that they might have diverged relatively early during evolution. The efficiency of construction of phylogenetic trees by means of genetic distance is found to depend on linear relationships with time and the sampling error of the distance measures used (Nei *et al.*, 1983; Goldstein and Pollock, 1994; Tajima and Takezaki, 1994). The Genetic distance estimates (derived from allele frequencies) between each pair of breeds and the dendrogram generated based on the genetic distances showed Uda to be more closely related to Yankassa compared to Balami sheep.

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Figure 3: Phylogenetic Tree (Neighbour-Joining)

Plate 4.1: Gel Electrophoresis of PCR amplicon for Yankasa Sheep Breed

Plate 4.2: Gel Electrophoresis of PCR Amplicon for Uda Sheep Breed

Plate 4.3: Gel Electrophoresis of PCR Amplicon for Balami Sheep Breed

Conclusion

The study showed that sufficient genetic variation exist in the studied Nigerian sheep breeds and these populations are unlikely to decline due to inbreeding. However, there may be gradual erosion of genetic diversity due to gene flow among these sheep breeds which are majorly caused by uncontrolled breeding systems in Nigeria. This trend should be checked urgently to prevent a complete loss of our pure breeds.

References

- Adebambo, O., Williams, J. I., Blott, S. and Urquhart, B. 1999.** Genetic relationships between native sheep breeds in Nigeria based on microsatellite DNA polymorphisms. *Nigerian Journal of Animal Production* 27(1):1-8
- Agaviezor, B. O., Gunn, H. H., Amusan, S. A. and Imunorin, I. G. 2013.** Gene flow between Nigerian sheep breeds as revealed by microsatellite DNA markers. *Journal of Animal Production Advances* 3(2): 35-39
- Agaviezor, B. O., Adefenwa, M. A., Peters, S. O., Yakubu, A., Adebambo, O. A., Ozoje, M. O., Ikeobi, C. O. N., Ilori, B. M., Wheto, M., Ajayi, O. O., Amusan, S. A., Okpeku, M., De Donato, M. and Imumorin, I. G. 2012.** Genetic diversity analysis of the mitochondrial D-loop of Nigerian indigenous sheep. *Animal Genetic Resources* 50: 13-20
- FAOSTAT, 2017.** Food and Agriculture Organization of the United Nations <http://faostat.fao.org/default.aspx>. Retrieved on 12 November, 2017
- Federal Republic of Nigeria 1991.** Gazettes of place names fourth edition, published by Federal Ministry of works and Housing Lagos Nigeria.
- Gizaw, S., Komen, H., Hanote, O., van Arendonk, J. A. M., Kemp, S., Aynalem, Haile, Mwai, O. and Dessie, T. 2011.** Characterization and conservation of indigenous sheep genetic resources: a practical framework for developing countries. ILRI Research Report No. 27. Nairobi, Kenya, ILRI.
- Goudet, J. 1995.** FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* 86: 485-486.
- He, J. N., Zhang, B. Y., Chu, M. X., Wang, P. Q., Feng, T., Cao, G. L., Di, R. Fang, L. Huang, D. W., Tang, Q. Q and Li, N. 2012.** Polymorphism of insulin-like growth factor 1 gene and its association with litter size in Small Tail Han sheep. *Molecular Biology Reports* 39 (10):9801-9807
- Levene, H. 1949.** On a matching problem in genetics. *Annals of Mathematical Statistics* 20:91-94.
- NCBI 2015.** National Centre for Biotechnology Information www.ncbi.nlm.gov/
- Nei, M. 1978.** Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590
- Nei, M. 1987.** Molecular evolutionary genetics. Columbia University Press, New York.
- NPC 2006.** National Population Commission Census 1991, Federal Ministry of Internal Affairs, Nigeria

- Pariset, L., Mariotti, M., Gargani, M., Joost, S., Negrini, R., Perez, T., Bruford, M., Marsan, P.A. and Valentini, A. 2011. Genetic diversity of sheep breeds from Albania, Greece, and Italy assessed by mitochondrial D N A and nuclear polymorphisms (SNPs). *The Scientific World Journal* 11: 1641–1659.
- Qwabe, S. O., Marle-Köster, E. V. and Visser, C. 2015. Genetic diversity and population structure of the endangered Namaqua Afrikaner sheep. *Journal of Animal Breeding and Genetics*. doi: 10.1111/jbg.12160.
- Qing, G. R., Ping, W. L., Hong, C. and Tsunodak, K. 2009. Genetic differentiation among four Chinese sheep breeds. *Journal of Animal and Veterinary Advance* 8(7):1381–1384.
- RIM 1992. National Livestock Resource Survey (Vol. 4). Report by Resource Inventory and Management Limited (RIM) to FDL & PCS, Abuja, Nigeria 287pp
- Sharma, R., Kumar, B., Arora, R., Ahlawat, S., Mishra, A.K. and Tandia, M.S. 2016. Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. *Meta Gene* 8:14–20.
- Slatkin, M. and Barton, N. H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43(7):1349–1368.
- Tajima, F. and Takezaki, N. 1994. Estimation of evolutionary distance for reconstructing molecular phylogenetic trees. *Molecular Biology and Evolution* 11:278–286.
- Thaon d'Arnoldi, C., Foulley, J.L. and Ollivier, L. 1998. An overview of the Weitzman approach to diversity. *Genetics, Selection and Evolution* 30: 149–161.
- Tserenbataa, T., Ramey, R.R., Ryder, O.A., Quinn, T.W. & Reading, R.P. 2004. A molecular population genetic comparison of argali sheep (*Ovis ammon*) in Mongolia using the ND5 gene of mitochondrial DNA: implications for conservation. *Molecular Ecology* 13:1333–1339
- Wang, Y., Price, S. E. and Jiang, H. 2006. Cloning and characterization of the bovine class 1 and class 2 insulin-like growth factor-1 mRNAs. *Domestic Animal Endocrinology* 25:315–328.
- Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

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