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# GROWTH AND HEAT TOLERANCE TRAITS IN WEST AFRICAN DWARF SHEEP AND VARIATION IN CD14 GENE

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**A B S T R A C T**

Although West African Dwarf Sheep (*Ovis aries)* has for decades played a strategic role in food security and income generation in Nigeria, it is still cultivated by resource-poor farmers and its impact remains mainly in the rural areas. The research investigated growth, heat tolerance traits, and genetic variations in West African Dwarf (WAD) sheep populations from Akwa Ibom State (AKS) and Cross River State (CRS), Nigeria. Forty WAD sheep (20 per population) were sampled, and measurements were taken for growth and heat tolerance traits. Blood samples were collected for genetic analysis of the CD14 gene. Results showed significant differences in body weight (BW) and body length (BL) between AKS and CRS WAD sheep, with higher values observed in AKS. While relative humidity was similar between states, ambient and body temperatures differed significantly. Genetic analysis revealed higher sequence polymorphism in the CD14 gene of AKS WAD sheep compared to CRS, with higher sequence conservation in CRS. Phylogenetic analysis identified distinct clusters, including one exclusively composed of AKS samples. Furthermore, a negative relationship was observed between ambient temperature and CD14 gene sequence variation, suggesting a potential link between genetic factors and heat tolerance in WAD sheep. The findings indicate that environmental factors influence the growth and genetic makeup of WAD sheep populations, highlighting the importance of understanding local adaptation and resilience in livestock farming, particularly in the context of climate change.

## 1. INTRODUCTION

The West African dwarf sheep (*Ovis aries*) is a diminutive breed widely raised across the tropical and subtropical regions of Africa. These resilient animals have played a pivotal role in supporting smallholder farmers in the region, due to their remarkable adaptability to harsh environmental conditions. However, the sustainability and productivity of African dwarf sheep systems face mounting challenges due to the escalating effects of climate change. Rising temperatures, water scarcity, and erratic rainfall patterns are reshaping the landscape, posing significant threats to livestock health and well-being [1].

In Southern Nigeria, the potential for sheep production remains largely untapped, primarily due to the scarcity of high-quality feeds necessary for sustaining livestock growth and the adverse effects of harsh climatic factors on animal management practices [2]. The West African dwarf (WAD) sheep, characterized by its compact stature and diverse coat colors including white, black, brown, or spotted variations, exemplifies the adaptability conferred by genetic factors to varying climatic zones. Coat color, a hereditary trait, significantly influences the performance of livestock in different environments [3].

Heat tolerance, crucial for animals to maintain homeostasis amidst rising temperatures, involves intricate physiological responses orchestrated by temperature-sensitive neurons. However, prolonged exposure to high temperatures, coupled with elevated humidity and prolonged sunlight, induces heat stress, posing a significant challenge to animal production in humid tropical regions [3-4].

Performance traits such as weight and length characteristics directly impact the profitability of animal production enterprises. Consequently, several sheep breeding programs worldwide prioritize the improvement of these traits to enhance economic viability. Understanding the genetic basis underlying such traits, particularly growth rate, aids in selecting breeds with superior adaptability and economic potential [5-6]. Morphological traits and molecular approaches are instrumental in evaluating variations within sheep populations. The Cluster of Differentiation (CD) molecules, crucial for immunophenotyping cells, including CD14, have emerged as candidates for studying disease resistance and heat tolerance in livestock species [7-8]. Therefore, the current research was designed to assess variation in two populations of sheep, focusing on growth and heat tolerance traits, as well as the CD14 gene. By unraveling the genetic and physiological mechanisms underpinning these traits, the study aimed to contribute to the development of strategies for enhancing sheep resilience and productivity in the face of evolving environmental challenges.

## 2. MATERIALS AND METHODS

### Location and sampling

Forty West African Dwarf sheep were sampled from farms in Cross River and Akwa Ibom States, Nigeria. The samples constituted 20 each from the two States giving a total of forty 40 sample size.

### Measurement of growth traits

The following growth traits were measured:

**Body weight:** This was measured using a measuring scale in kg.

**Body length:** This was measured from the base of the neck to the end of the pubic bone using measuring tape in (cm).

**Heart girth:** This was measured as the circumference of the body just behind the foreleg.

**Shoulder width:** This was measured as the distance between the right shoulder blade and the left shoulder blade in (cm).

**Head length:** It was measured as the distance between the horn sites and the lower lip in (cm).

**Leg length:** This was measured as the distance between the upper part of the leg and the hooves in (cm).

**Neck circumference:** This was measured as the distance around the mid-neck region in (cm).

**Tail length:** This was measured as the distance from the end of the pubic bone to the tip of the tail in (cm).

**Measurements of heat tolerance traits**

The following heat tolerance traits were measured:

**Pulse rate:** This was measured as the number of heart beats per minute using a stethoscope and stopwatch.

**Respiratory rate:** This was measured as the number of breaths in 60 seconds using a stethoscope and stopwatch.

**Body temperature:** This was measured with the aid of a digital thermometer placed between the forelimbs and the body of the sheep.

**Rectal temperature:** The rectal temperature was measured as the temperature of the rectum by inserting a digital thermometer into the rectum of the sheep.

**Environmental temperature:** Ambient temperature and relative humidity of the sampling location were measured using a thermometer and hygrometer.

**DNA extraction**

About 1 ml of blood was collected from each sheep through jugular venipuncture into Ethylenediamine tetraacetic EDTA bottles for DNA extraction. Genomic DNA extraction was carried out in the Animal Science Molecular Genetics Laboratory, Department of Animal Sciences, University of Port Harcourt, Nigeria using quick - DNA™ miniprep plus kit protocol. Four volumes of genomic lysis buffer were added to the blood sample after vortexing for 4-6 seconds, then let to stand at room temperature for five to 10 minutes and thoroughly centrifuged to remove particulate debris. The mixtures were then transferred to a Zymo-Spin™ column in a collection tube and centrifuged at 10,000g for one minute. The collection tubes were discarded with the flow through. The zymo spin column was transferred to a new collection tube. 200µl of DNA pre-wash buffer was added to the spin column, followed by one-minute centrifugation at 10,000g. 500 µl of g-DNA wash buffer was added to the spin column followed by centrifugation for one minute at 10,000g. After this, the spin column was then transferred to a microcentrifuge tube. 50µl of DNA elution buffer was added to the spin column and incubated for 30 seconds at room temperature. The last step was repeated to elute the DNA. The eluted DNA in the microcentrifuge tube was stored at less than -20˚C pending the amplification.

**PCR amplification**

The region of CD14 of African Dwarf sheep was amplified by the Polymerase Chain Reaction (PCR) technique using specific sets of forward and reverse primers. PCR was carried out in a final reaction volume of 15µl. PCR conditions including the cyclic condition and the concentration of different ingredients of the Polymerase Chain Reaction (PCR) mixture were standardized for the target gene fragment after carrying out PCR using several combinations.

**Sequencing of CD14 gene**

Sequencing of CD14 gene was performed using the same sets of primers for PCR. The sequencing reaction was performed in STABVIDA Laboratory, Quinta de Torre, Portugal with AB13730×L sequencer using 20 µl reaction comprising approximately 20 ng of purified PCR product as template DNA, 8µl of Big Dye Terminator Reaction Mix (dNTPs, ddNTPs, buffer, enzyme and $MgCl_2$), 8µl of deionized water, 2µl of primer programmed as 25 cycles at 96$^{o}$C for 10 seconds, 60$^{o}$C for five seconds and 60$^{o}$C for four minutes.

**Statistical analysis**

Data on growth and heat tolerance traits were analyzed using the student t-test. Analysis of heat tolerance traits was performed using SPSS 20.0 software. Bioedit software version 7.2.5 The method

of [9] was employed to view and edit the sequences. MEGA 7.0 was used for multiple sequence alignment of all the samples excluding all the gaps [10]. The phylogenetic relationship among the sheep was constructed using MEGA 7.0. Genetic variation parameters were examined using DnaSP version 6.12.03 [11]. The relationship between heat traits and variation in the *CD14* gene was evaluated using a multiple correlation model with variations in *CD14* as independent variable (Y) and growth traits as dependent variables ($X_1, X_2, X_3, X_4, X_5 \ldots X_n$).

## 3. RESULTS

### Variation in growth traits of West African dwarf sheep (WAD)

The group statistics showing the number of samples, mean and standard error of growth traits measured in WAD from Akwa-Ibom state (AKS) and Cross River State (CRS) are presented in Table 1. There was a significant difference in the body weight of WAD from the two states ($p<0.05$) with the highest from AKS (21.95 kg). It was also observed that the body length of WAD from AKS (30.55 cm) was significantly higher than CRS (29.01cm). Similarly, a significant mean heart girth ($p<0.05$) was recorded in WAD from AKS than CRS. This was also similarly observed for tail length. On the contrary, head length, shoulder width, neck circumference, and leg length were statistically similar between WAD from the two locations.

### Heat Tolerant Traits

The results showing variation in the heat tolerance traits measured in the WAD sheep from the two states are presented in Table 2. Relative humidity was similar between the two states ($p>0.05$) while ambient temperature was significantly different ($P<0.05$). Pulse rate, respiratory rate and rectal temperature of WAD sheep were observed to be statistically similar between Akwa Ibom and Cross River State. On the other hand, there was a significantly higher ($P<0.05$) body temperature (38.24 $^0$C) in WAD sheep from AKS than CRS (33.90$^0$C).

### Genetic Variation in CD14 gene of WAD Sheep

Table 3 shows the genetic variation in sequences of CD14 gene obtained from WAD sheep in the study. In both populations, a similar number of polymorphic sites were identified, totaling 30. However, the distribution of these sites differed between AKS and CRS WAD sheep. In AKS, 29 of the polymorphic sites were classified as parsimony variable sites, with only one singleton variable site. In contrast, CRS WAD sheep exhibited a higher number of singleton variable sites (5) compared to AKS, alongside 25 parsimony information sites. The haplotype analysis further showed the genetic diversity within each population. CRS WAD sheep displayed a higher haplotype number (18) compared to AKS (11). This diversity translated into a haplotype diversity of 1.000±0.019 in CRS. Nucleotide diversity was slightly lower in CRS (0.01103±0.00182) compared to AKS (0.01572±0.00188). Sequence conservation was higher in the CD14 gene of WAD sheep from CRS (95.9%) than AKS (94.7%).

### Phylogenetic relationship of WAD Sheep between Akwa Ibom and Cross River States

The phylogenetic relationship among WAD sheep from the two states is presented in Figure 1. There were two major clusters produced from the maximum likelihood tree set at 1000 bootstrap. The first major cluster had only 3 samples of WAD sheep which were all from AKS population. The second major cluster was intermixed with WAD sheep from both populations.

### Single nucleotide polymorphism (SNPs)

The analysis of the CD14 gene in West African Dwarf (WAD) sheep populations from CRS and AKS

revealed significant genetic variation (Table 4). In CRS WAD, a total of 38 single nucleotide polymorphisms (SNPs) were detected, while 50 SNPs were identified in AKS WAD. These SNPs led to numerous amino acid changes, resulting in 44 non-synonymous mutations and 3 synonymous mutations in AKS WAD. In CRS WAD, a total of 33 non-synonymous mutations and 2 synonymous mutations were observed. Furthermore, the ratio of transversion to transition mutations differed between the two populations. In AKS WAD, the ratio was 30:17, while in CRS WAD, it was 23:12. The ambient temperature had a significant negative relationship with sequence variation of WAD sheep CD14 gene from both populations (Table 5).

## 4. DISCUSSION

The results of this study reveal significant differences in growth traits among West African Dwarf (WAD) sheep populations from Akwa Ibom State (AKS) and Cross River State (CRS), Nigeria. AKS sheep exhibited higher mean values for body weight (BW), body length (BL), hip girth (HG), and tail length (TL), while CRS sheep had the highest mean for shoulder width (SW). These findings suggest that various intrinsic and extrinsic factors may influence growth traits in WAD sheep. Intrinsic factors such as genetic type, age, sex, birth rank, litter size, and birth weight, as well as extrinsic factors like agro-ecological area, seasons, diets, production systems, and ovine pathologies, contribute to the observed differences in growth performance [12]. The higher body weight observed in AKS WAD sheep may be attributed to environmental peculiarities unique to their region. Favorable nutrition, influenced by seasonal variations such as the rainy season, positively impacts animal productivity [12]. The environmental peculiarities of AKS may contribute to the increased body weight observed in WAD sheep from this region. However, exposure to high environmental temperatures, relative humidity, and prolonged sunlight induces heat stress in sheep, leading to physiological changes that could negatively impact growth performance. Additionally, deficiencies in feed quality and quantity, harsh environmental conditions, and inadequate housing contribute to stressful conditions that directly or indirectly affect sheep growth, development, and reproductive ability.

However, environmental factors such as high ambient temperature, relative humidity, and prolonged sunlight exposure induce heat stress in sheep, leading to increased rectal temperature and physiological changes that negatively impact growth performance [13]. Additionally, deficiencies in feed quality and quantity, coupled with harsh environmental conditions and inadequate housing, create stressful conditions for sheep management, directly affecting their growth, development, and reproductive ability. The results further highlight the adverse effects of relative humidity and ambient temperature on heat stress in WAD sheep populations, with similar humidity levels but different ambient and body temperatures between AKS and CRS sheep. These findings suggest the importance of considering environmental factors and implementing appropriate management practices to mitigate heat stress and promote optimal growth and productivity in WAD sheep. The complex interplay between genetic, environmental, and management factors in shaping growth traits and highlights the need for targeted interventions to enhance the resilience and performance of WAD sheep under varying environmental conditions

The Cluster of Differentiation CD14 gene plays a multifaceted role in immune response and disease resistance, serving as a key component of innate immunity against bacterial pathogens [14]. Our analysis of genetic variation within the CD14 gene revealed similar levels of polymorphic sites in WAD sheep populations from both Akwa Ibom State (AKS) and Cross River State (CRS), Nigeria.

Interestingly, we observed a negative relationship between ambient temperature and sequence

variation in the CD14 gene of WAD sheep from both populations. However, physiological indices such as pulse rate, respiratory rate, and body temperature did not exhibit significant associations with CD14 gene variation. Coat characteristics were found to significantly affect physiological indices and heat tolerance in WAD sheep, as reported in previous studies (Okoruwa, 2015).

Phylogenetic clustering of WAD sheep from AKS and CRS highlighted distinct genetic lineages within AKS WAD sheep, suggesting potential regional genetic differentiation. Nevertheless, the overall genetic structure and relatedness of WAD sheep populations from both states demonstrated some level of similarity thereby indicating the importance of considering regional genetic diversity and population dynamics in conservation and breeding programs aimed at enhancing the resilience and productivity of WAD sheep in Nigeria.

## 5. CONCLUSION

There were significant differences and patterns between the two populations of West African Dwarf sheep through the different analyses conducted. Despite a similar number of polymorphic sites, AKS and CRS WAD sheep exhibit distinct distributions of these sites, with CRS displaying a higher haplotype number and slightly lower nucleotide diversity compared to AKS. The presence of singleton variable sites and parsimony information sites further underscores the genetic complexity within each population. Notably, sequence conservation is higher in CRS WAD sheep, suggesting a greater degree of genetic stability within this population. These findings shed light on the genetic composition, variability, and conservation status of WAD sheep populations, providing crucial information for conservation and breeding programs aimed at preserving the genetic integrity and resilience of this valuable breed.

## 6. ACKNOWLEDGMENTS

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## 7. CONFLICT OF INTEREST

The authors declare that there is no existing conflict of interest

## 8. FUNDING

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**TABLE 1**

**Variation in growth traits of WAD from Akwa Ibom and Cross River**

| Growth traits | Locations | Mean ± SE | $t_{cal}$ | P-value |
|---|---|---|---|---|
| Body weight | AKS | 21.95±0.87[a] | 3.627 | 0.001 |
| | CRS | 16.3±1.29[b] | | |
| Body length | AKS | 30.55±0.50[a] | 1.767 | 0.086 |
| | CRS | 29.01±0.71[b] | | |
| Heart girth | AKS | 17.33±0.45[a] | 5.482 | 0.000 |
| | CRS | 13.06±0.63[b] | | |
| Shoulder width | AKS | 7.71±0.26[a] | -1.218 | 0.231 |
| | CRS | 12.99±4.33a | | |
| Head length | AKS | 7.46±0.23[a] | | |
| | CRS | 7.66±0.25[a] | -0.587 | 0.561 |
| Neck circumference | AKS | 7.13±0.27[a] | | |
| | CRS | 7.23±0.45[a] | -0.190 | 0.850 |
| Tail length | AKS | 7.10±0.17[a] | | |
| | CRS | 6.55±0.30[b] | 1.597 | 0.121 |
| Leg length | AKS | 16.33±0.78 [a] | | |
| | CRS | 16.57±0.51[a] | -0.256 | 0.799 |

Mean values with different superscripts within each measured trait are significantly different ($p<0.05$)

AKS= Akwa Ibom State

CRS= Cross River

WAD= West African dwarf sheep

SE= Standard error

**TABLE 2**

**Variation in heat tolerance traits of WAD sheep from two locations**

| Heat tolerance traits | Location | Mean ± SE | $t_{cal}$ | P-value |
|---|---|---|---|---|
| Relative Humidity ($g/m^3$) | AKS | 64.05±1.80[a] | -3.323 | 0.002 |
| | CRS | 70.80±0.95[a] | | |
| Ambient temperature ($^oC$) | AKS | 35.94±0.92[a] | 4.519 | 0.000 |
| | CRS | 31.50±0.34[b] | | |

| | | | | |
|---|---|---|---|---|
| Pulse rate (BPM) | AKS | 92.20±4.64[a] | 0.460 | 0.648 |
| | CRS | 88.80±5.76[a] | | |
| Respiratory rate (bpm) | AKS | 49.85±1.89[a] | 0.012 | 0.990 |
| | CRS | 49.82±2.15[a] | | |
| Body temperature (°C) | AKS | 38.24±1.88[a] | 2.240 | 0.036 |
| | CRS | 33.90±0.45[b] | | |
| Rectal temperature (°C) | AKS | 36.44±2.70[a] | 0.387 | 0.701 |
| | CRS | 35.37±0.54[a] | | |

Mean values with different superscripts within each measured trait are significantly different ($p<0.05$)
AKS= Akwa Ibom State
CRS= Cross River
WAD= West African dwarf
SE= Standard error
BPM (beats per minute)
Bpm (breath per minute)

**TABLE 3**
**Genetic variation in CD14 of *Ovis aries* from Akwa Ibom and Cross River states**

| Polymorphism indices | AKS | CRS |
|---|---|---|
| Number of sequences (NSQ) | 17 | 18 |
| Number of sites (NS) | 864 | 875 |
| Monomorphic sites (MNS) | 834 | 845 |
| Polymorphic sites (PS) | 30 | 30 |
| Singleton variable sites (SVS) | 1 | 5 |
| Parsimony information sites (PIS) | 29 | 25 |
| Number of haplotype (NH) | 11 | 18 |
| Haplotype (gene) diversity (Hd) | 0.941±0.036 | 1.000±0.019 |
| Nucleotide diversity (Nu) | 0.01572±0.00188 | 0.01103±0.00182 |
| Average number of nucleotide difference (ANND) | 13.581 | 9.654 |
| Sequence conservation (SC) | 0.947 (94.7%) | 0.959 (95.9%) |
| Minimum number of recombination (MNR) | 3 | 3 |

**TABLE 4**

**Single nucleotide polymorphism in *CD14* of *O. aries* from Akwa Ibom and Cross River State**

| AKS | | | | | CRS | | | |
|---|---|---|---|---|---|---|---|---|
| **SN** | **SNP** | **Amino acid** | **dS/dN** | **Mutation** | **SNP** | **Amino acid** | **dS/dN** | **Mutation** |
| 1 | 1T>A | Cys1Ser | Non-synonymous | Transversion | 1T>A | Cys1Ser | Non-synonymous | Transversion |
| 2 | 2G>C | Cys1Ser | Non-synonymous | Transversion | 2G>C | Cys1Pro | Non-synonymous | Transversion |
| 3 | 3C>T | Cys1Ser | Non-synonymous | Transition | 3C>T | Cys1Pro | Non-synonymous | Transition |
| 4 | 4C>T | Gln2Leu | Non-synonymous | Transition | 4C>T | Gln2Leu | Non-synonymous | Transition |
| 5 | 5A>T | Gln2Leu | Non-synonymous | Transversion | 5A>T | Gln2Leu | Non-synonymous | Transversion |
| 6 | 7T>A | Trp3Arg | Non-synonymous | Transversion | 9G>T | Trp3Cys | Non-synonymous | Transversion |
| 7 | 8G>A | Grp3Glu | Non-synonymous | Transition | 10T>A | STP4Arg | Non-synonymous | Transversion |
| 8 | 9G>T | Trp3Cys | Non-synonymous | Transversion | 13T>C | Phe5Leu | Non-synonymous | Transition |
| 9 | 10T>A | STP4Arg | Non-synonymous | Transversion | 13T>C | Phe5Pro | Non-synonymous | Transition |
| 10 | 11G>A | STP4Lys | Non-synonymous | Transition | 15T>C | Phe5Pro | Non-synonymous | Transition |
| 11 | 13T>C | Phe5Leu | Non-synonymous | Transition | 16C>T | Pro6Ser | Non-synonymous | Transition |
| 12 | 14T>C | Phe5Thr | Non-synonymous | Transition | 21T>A | Ser7Ser | Synonymous | Transversion |
| 13 | 16C>T | Pro6Ser | Non-synonymous | Transition | 22G>C | Val8Arg | Non-synonymous | Transversion |
| 14 | 17C>G | Pro6Gly | Non-synonymous | Transversion | 23T>G | Val8Arg | Non-synonymous | Transversion |
| 15 | 18T>C | Pro6Gly | Non-synonymous | Transition | 25C>G | Leu9Gly | Non-synonymous | Transversion |
| 16 | 19T>C | Ser7Pro | Non-synonymous | Transition | 26T>G | Leu9Gly | Non-synonymous | Transversion |

**TABLE 4 CONTD**

**Single nucleotide polymorphism in *CD14* of *O. aries* from Akwa Ibom and Cross River State**

| AKS | | | | | CRS | | | |
|---|---|---|---|---|---|---|---|---|
| **SN** | **SNP** | **Amino acid** | **dS/dN** | **Mutation** | **SNP** | **Amino acid** | **dS/dN** | **mutation** |
| 17 | 21T>A | Ser7Ser | Synonymous | Transversion | 28T>A | Phe10MET | Non-synonymous | Transversion |
| 18 | 22G>C | Val8Arg | Non-synonymous | Transversion | 29 | 29delTTCAGAATT | | |
| 19 | 23T>G | Val8Arg | Non-synonymous | Transversion | 30T>G | Phe10MET | Non-synonymous | Transversion |
| 20 | 24T>G | Val8Thr | Non-synonymous | Transversion | 31 | Not Mutated | Not Mutated | Not Mutated |
| 21 | 25C>G | Leu9Gly | Non-synonymous | Transversion | 32A>C | Gln11Pro | Non-synonymous | Transversion |
| 22 | 26T>G | Leu9Gly | Non-synonymous | Transversion | 33G>C | Gln11Pro | Non-synonymous | Transversion |
| 23 | 28T>C | Phe10del | Non-synonymous | Transition | 34 | Not Mutated | Not Mutated | Not Mutated |
| 24 | 29 | 29delTTCAGAATT | | | 35A>G | Asn12Ser | Non-synonymous | Transition |
| 25 | 30T>G | Phe10del | Non-synonymous | Transversion | 36T>A | Asn12Lys | Non-synonymous | Transversion |
| 26 | 31C>A | Gln11Thr | Non-synonymous | Transversion | 37T>C | Xaa13Leu | Non-synonymous | Transition |
| 27 | 32A>C | Gln11Thr | Non-synonymous | Transversion | 38T>A | Xaa13Tyr | Non-synonymous | Transversion |
| 28 | 33G>C | Gln11Pro | Non-synonymous | Transversion | 39C>A | Xaa13Leu | Non-synonymous | Transversion |
| 29 | 34A>C | Asn12Pro | Non-synonymous | Transversion | 40C>A | Xaa14Gly | Non-synonymous | Transversion |
| 30 | 35A>C | Asn12Pro | Non-synonymous | Transversion | 41A>G | Xaa14Gly | Non-synonymous | Transition |
| 31 | 36T>C | Asn12Asn | Synonymous | Transition | 42C>A | Xaa14Gln | Non-synonymous | Transversion |
| 32 | 37T>C | Tyr13Leu | Non-synonymous | Transition | 43T>C | STP15Leu | Non-synonymous | Transition |
| 33 | 38A>T | Tyr13Leu | Non-synonymous | Transversion | 44A>T | STP15Leu | Non-synonymous | Transversion |
| 34 | 39C>G | Tyr13Leu | Non-synonymous | Transversion | 722C>A | Pro24His | Non-synonymous | Transversion |

**TABLE 4 CONTD**

**Single nucleotide polymorphism in *CD14* of *O. aries* from Akwa Ibom and Cross River State**

| AKS | | | | | CRS | | | |
|---|---|---|---|---|---|---|---|---|
| **SN** | **SNP** | **Amino acid** | **dS/dN** | **Mutation** | **SNP** | **Amino acid** | **dS/dN** | **Mutation** |
| 35 | 40C>G | His14Gly | Non-synonymous | Transversion | 734T>G | Leu245Arg | Non-synonymous | Transversion |
| 36 | 41A>G | His14Gly | Non-synonymous | Transition | 835T>C | STP279 Arg | Non-synonymous | Transition |
| 37 | 42C>A | His14Gln | Non-synonymous | Transversion | 836A>G | STP279 Arg | Non-synonymous | Transition |
| 38 | 43T>C | STP15Leu | Non-synonymous | Transition | 884T>G | Xaa295Xaa | synonymous | Transversion |
| 39 | 44A>T | STP15Leu | Non-synonymous | Transversion | | | | |
| 40 | 45G>C | STP15Tyr | Non-synonymous | Transversion | | | | |
| 41 | 46 | Not Mutated | Not Mutated | Not Mutated | | | | |
| 42 | 245T>C | lle82Thr | Non-synonymous | Transition | | | | |
| 43 | 254G>C | STP85Ser | Non-synonymous | Transversion | | | | |
| 44 | 272A>C | Asn91Thr | Non-synonymous | Transversion | | | | |
| 45 | 722C>A | Pro241His | Non-synonymous | Transversion | | | | |
| 46 | 734T>G | Leu245Arg | Non-synonymous | Transversion | | | | |
| 47 | 834del CT | | | | | | | |
| 48 | 835T>C | STP279Arg | Non-synonymous | Transition | | | | |
| 49 | 836A>G | STP279Arg | Non-synonymous | Transition | | | | |
| 50 | 884T>G | Xaa295Xaa | Synonymous | Transversion | | | | |

**TABLE 5: Relationship between variation in *CD14* and heat tolerance traits in WAD from Akwa Ibom and Cross River States**

| Heat tolerance traits | SNP-AKS | SNP-CRS |
|---|---|---|
| Relative Humidity | 0.173 | 0.360* |
| Ambient Temperature | -0.358* | -0.440** |
| Pulse rate | -0.050 | -0.083 |
| Respiratory rate | 0.046 | -0.122 |
| body temperature | -0.152 | -0.220 |
| Rectal temperature | 0.066 | -0.075 |

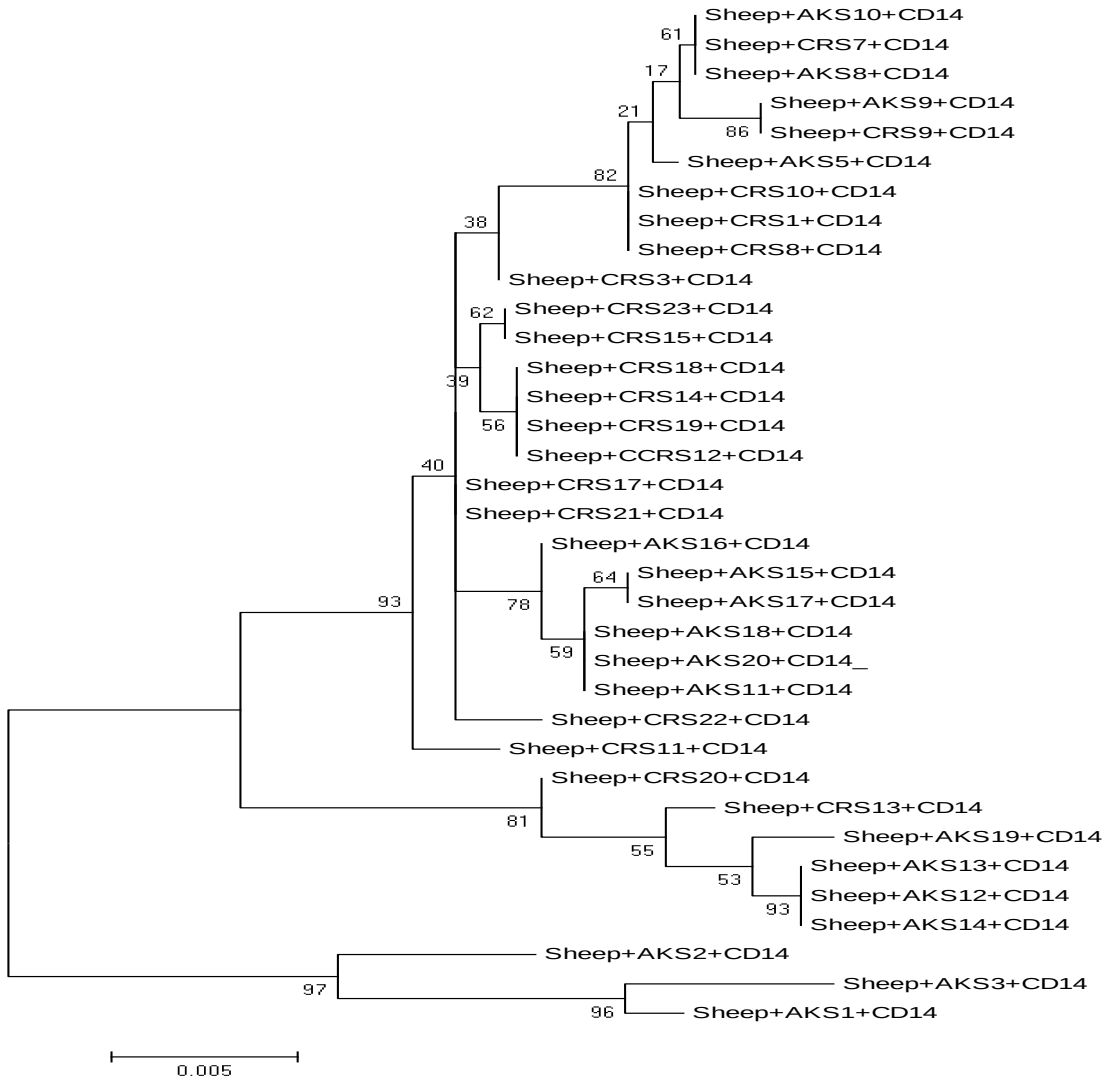


**FIG. 1: Phylogenetic relationship between *Ovis aries* in Akwa Ibom and Cross River State**