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Molecular characterization and phylogenetic analysis of orf virus isolated from goats in Sokoto metropolis, Nigeria

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Aim: The aim of this study was to molecularly characterize orf virus isolated from clinical infections in goats in Sokoto metropolis. **Materials & methods:** Embryonated chicken eggs were used to isolate orf virus according to the established protocol. Viral DNA was extracted and full coding region of *B2L* gene was amplified by polymerase chain reaction, sequenced and blasted for identification and phylogenetically analyzed. **Results and discussion:** The *B2L* gene sequences of the isolate showed slight variability (96–98.7%) with the reference sequences as it clustered within the same clade with Korean, Zambian and Ethiopian strains, signifying a close genetic relationship. Unique amino acid substitutions were noted. This is the first genetic characterization of *B2L* gene of orf virus circulating in Nigeria. **Conclusion:** This study has provided in sight into the genetic diversity of orf virus in the study area.

Lay abstract: Contagious ecthyma has for long been affecting the goat and sheep population in Nigeria leading to huge economic losses to livestock farmers through death and poor market value of the affected animals. Recently, increased death due to this disease has been reported in north-central Nigeria, but the nature of the disease-causing agent has not been reported yet. It is the aim of this study to understand the nature of the virus causing the disease in goats in Sokoto using molecular biology techniques so as to facilitate better vaccine design for the effective control of the disease in Nigeria.

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Keywords: B2L gene • contagious ecthyma • goat • orf virus • phylogenetic analysis

Background

Contagious ecthyma (CE) is a highly contagious viral disease of small ruminants such as sheep and goats [1] that occasionally affects camels [2] and wild ruminants with huge economic impact on the livestock industry [2-6]. Clinically, the disease is associated with skin lesions such as erythema, macules, papules, vesicles, pustules and crusts on the lips, tongue, teat, nose, hooves and other parts of the body [6] especially in young lambs and kids [3,7]. The disease is generally selflimiting, however, secondary bacterial infection may complicate the situation, causing inappetence, severe emaciation and death of the affected animals [1]. Epidemiological evidence indicated morbidity of 60% but the mortality is usually low unless complicated by secondary bacterial infection [8] where it can reach up to 10 and 93% in kids and lambs respectively [9] and even 100% in adult goats [4]. The disease is also of zoonotic significance, causing ulcerative lesions or nodules on the hands of high risk individuals such as veterinarians,





Figure 1. Sample collection from goats with suspected orf virus infection. (A) Sample 1 from male RSG-WAD cross. (B) Sample 2 from female RSG.

butchers and other animal handlers [10]. Except in immunocompromised patients, most human cases of contagious ecthyma are localized and heal spontaneously [11].

The aetiology of CE is orf virus, a member of the genus *Parapoxvirus* in the family *Poxviridae* [12]. The genetic material of the virus is a linear dsDNA [13] of 134–139 kb in size [3,13]. It exhibits high GC content of about 66% [14] and is generally organized into conserved central portion and variable terminal regions [15]. The central portion has a number of genes including the *B2L* gene, that encodes the immunogenic major envelope protein p42K [3,4,16,17]. This gene has been extensively used for molecular detection and diagnosis [18] as well as phylogenic analyses of various orf virus isolates [3,13,19].

Laboratory diagnosis of CE can be achieved using electron microscopy, histopathology and serological tests such as fluorescent antibody technique, virus neutralization test, agar gel immunodiffusion and ELISA [1,3]. Nowadays, confirmation of CE is achieved using polymerase chain reaction (PCR) which has been shown to be highly specific and sensitive [1,13,16,19]. Using PCR, sequencing and phylogenetic analysis, genetic characteristic and diversity of orf virus has been described in many countries around the world including China [5,19,20], Taiwan [13], Malaysia [7], India [12], Uruguay [3] and a few African countries such as Tanzania [21], Ethiopia [17,22], Egypt [23,24], Gabon [25] and Sudan [26,27]. To date, outbreaks of CE in Nigeria are largely reported based on clinical manifestation of the disease and PCR to confirm cases [1,2,4] but no literature on the molecular characterization of the circulating orf virus isolates is currently available in Nigeria as at the time of this study. Therefore, in the present study we reported for the first time the isolation, molecular detection and phylogenetic characterization of orf virus obtained from a flock of goats in Sokoto metropolis, north-west Nigeria.

Materials & methods

Sample collection, transport & processing

Suspected outbreak of CE was reported in a goat farm located in More area, Sokoto metropolis (13.0059° N, 5.2476° E) in May 2019. On visitation to the farm, a flock of 30 goats consisting of Red Sokoto goat (RSG) and crosses of RSG with West African Dwarf (WAD) was observed (eight males, 22 females). Five of the males were less than a year old, while three were 2–3 years of age. The females on the other hand consisted of ten adults (2–4 years old) and 12 kids (less than a year old). Physical examination of the two affected goats (2-year old male RSG-WAD cross and 3-year old female RSG) revealed scab lesions on the ears, lips and nose (Figure 1). Two samples (1 and 2) involving thick brown scabs were scrapped on clean paper from the two affected goats and immediately transferred in a sterile sample container containing PBS (pH: 7.2–7.4), placed on ice and immediately transported to the Central Veterinary Research Laboratory (Usmanu Danfodiyo University, Sokoto) for analysis. After sample collection, the two animals were isolated from the rest of the flock and were treated with long acting oxytetracycline 20% at 1 ml/20 kg body weight to prevent secondary bacterial infection and the scraped lesions were scrubbed with povidone iodine and sprayed with gentian violet to facilitate wound healing.

The samples were homogenized in PBS using tissue homogenizer and centrifuged at $1000 \times \text{g}$ for 15 min to harvest the supernatants for storage at -20°C until needed for further analysis.

Virus isolation

Nine–11 days old specific antibody free chicken embryonated eggs (CEE) were purchased from the Vaccine Research Division of the National Veterinary Research Institute (Vom, Nigeria). The eggs were candled to ensure their viability and were later inoculated with 500 µl each of the two prepared samples via the chorioallantoic membrane (CAM) route as described by [28], sealed and labeled appropriately. The eggs were incubated and

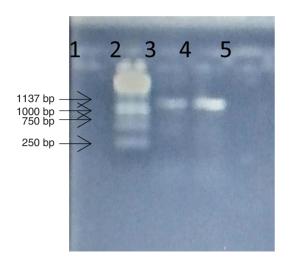


Figure 2. The PCR product showing the 1137bp fragments from sample 1 (well 3) and sample 2 (well 4, More_strain) with the 1kb DNA ladder (well 2) and the negative control (well 5). bp: Base pairs.

observed daily for 5–7 days during which eggs with dead embryos were chilled at 4°C. At the end of the incubation period, those embryos still alive were placed at 4°C overnight. Subsequently, the CAM was harvested and observed for the development of pock lesions before being stored at -20°C until processed.

DNA extraction

Infected CAM harvested from the eggs inoculated with the two samples were used for genomic DNA extraction using DNA Mini kit (QIAGEN, Hilden, Germany). Briefly, about 250 mg of CAM was homogenized and placed in a 1.5 ml micro centrifuge tube. Lysis buffer and proteinase K were added followed by incubation at 56° C in a water bath until complete lysis of the tissues occurs. DNA was then extracted according to the manufacturer's instructions, eluted with 50 μ l elution buffer and stored at -20° C.

Polymerase chain reaction

The ORFVB2LF1 5'-TCCCTGAAGCCCTATTATTTTTGTG-3' and ORFVB2LR1 5'-GCTTGCGGGCG TTCGGACCTTC-3' specific forward and reverse primers described by Hosamani *et al.* [29] were used to amplify the complete *B2L* gene of the orf virus (ORFV) with the aid of Toptaq PCR mastermix (QIAGEN) according to the manufacturer's instructions. The mixture was briefly centrifuged and placed in the thermocycler. Amplification was carried out using initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 min. Final extension was performed at 72°C for 5 min. The amplified products were then analyzed by electrophoresis on a 1.5% agarose gel containing 0.5 ng/ml ethidium bromide in Tris-acetate-EDTA (TAE) buffer according to Lawal *et al.* [30]. The amplicons were viewed using a GelDoc imaging system (BioRad, CA, USA).

DNA sequencing, phylogenetic & evolutionary analyses

PCR positive samples were sent to (Inqaba Biotechnical Industries [Pty] Ltd, Pretoria, South Africa) for Sanger sequencing. The sequencing company reported that only one of the two samples passed quality control (QC) for downstream sequencing. Since the two samples were obtained from the same outbreak in the same flock, we asked the company to go ahead and sequence the sample that passed QC as the isolates in the two samples are highly likely to be the same. On receiving the result for the single sample, the Sequence was trimmed and subjected to BLAST similarity search using the BLASTN algorithm of the NCBI database, to confirm the identity of the virus. Subsequently, the obtained sequence was deposited in the GeneBank database with accession no MT272780 (available at https://www.ncbi.nlm.nih.gov/nuccore/MT272780). Reference sequences were downloaded and aligned with the sequence obtained in this study using ClustalW in the MEGA7 software [31]. Phylogenetic tree was constructed using the neighbor-joining method with 2000 bootstrap replicates using MEGA7 [31]. Evolutionary distances were inferred using Tamura–Nei model based on pair-wise sequence comparison with the use of MEGA7 software [31] between the isolates obtained in this study and the reference sequences.

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Figure 3. Nucleotide sequence comparison between More strain and reference sequences downloaded from the GeneBank: NCBI. The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the nucleotide.

Results

Virus isolation

Following the inoculation of the processed two scab materials into the specific antibody free CEE, pathologic changes in form of small grayish white foci (pock lesions) were observed on the harvested CAM membranes. These changes were not observed in the mock inoculated eggs. This signifies the successful presumptive isolation and identification of the virus.

PCR & sequence analysis

The PCR amplification of the CAM homogenate from the two samples yielded a product of the *B2L* gene fragment at the expected band size of 1137 bp when analyzed by gel electrophoresis (Figure 2). The PCR product of sample number 2 from the RSG (well 4) had higher band intensity compared with sample number 1 from the RSG-WAD cross (well 3). The negative control used showed no positive amplification.

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Figure 3. Nucleotide sequence comparison between More strain and reference sequences downloaded from the GeneBank: NCBI (cont.). The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the nucleotide.

When the obtained PCR products were sent for sequencing, only sample 2 from the RSG (product in well 4) passed the quality assurance (QC) test necessary for a successful sequencing services possibly because of the high intensity of the band obtained by PCR amplification (Figure 2) and some inhibitors and impurities that may degrade the PCR product from sample 1. Sample 2 was, therefore, the only product sequenced in both directions using the *B2L* forward and reverse primers. Subsequently, the sequence obtained was subjected to BLAST search in the NCBI database and the identity was confirmed as orf virus which was named 'More_strain'. The sequence was aligned with downloaded orf virus reference sequences at nucleotide (Figure 3) and amino acid levels (Figure 4) to observe for similarities and differences in the nucleotide and amino acid sequences.

Comparison of the obtained sequence with reference sequences from different countries using Tamura–Nei model revealed nucleotide similarities range of 96.7–99.0% among the sequences. Similarly, at the amino acid level, the percentage homologies ranged from 95.7 to 98.9% between the Nigerian orf virus isolate and the reference sequences. At the amino acid level, several mutations were observed in the More strain compared with the New Zealand NZ-2 strain used as a reference guide. There was a W2G at position 2 that was unique

U06671.1/orfv/NZ-2/B2L gene/Ne MT272780.1/More_strain_ORFV/ MN422332.1/Orfv_isolate_IRGG2 MH790955.1/isolate_ORFV-Myso MH790954.1/isolate ORFV-Jalan MH790953.1/isolate OREV-Oriss MH790947.1/isolate_ORFV-Ludhi MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/201 KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD KX129982.1/ORFV/Goat/India/IC KX029228.1/Orfv/strain GY-AHF KU851936.1/Orfv/isolate_Chalin KT438530.1/Orfv/Gondar_zuria/C KT438526 1/Orfv/Debre zeit/O02 KT438524.1 Orfv/ATARC/O02/20 KT438520.1 Orfv/Amba_Giorgis/C KT438517.1 Orfv/Adet/O05/2012 JQ349520.1 Orfv/strain PA11/B2 JN846834.1/isolate_ORFV_Assar JN565696.1/strain_ORFV/ShanXi JN565694.1/strain OREV/XinJiar JN088053.1 Orfv/isolate A/goat GU320351.1/strain_ORFV/HuB/20 GU139356.1 ORFV Mukteswar/0 GQ328006.1/ORFV/2009/Korea/E AY278209.1/Orfv/vaccine strain

U06671.1/orfv/NZ-2/B2L gene/Ne MT272780.1/More_strain_ORFV/ MN422332.1/Orfv_isolate_IRGG2 MH790955.1/isolate_ORFV-Mysc MH790954.1/isolate_ORFV-Jalar MH790953.1/isolate ORFV-Oriss MH790947.1/isolate_ORFV-Ludh MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/201 KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD KX129982.1/ORFV/Goat/India/IC KX029228 1/Orfv/strain GY-AHE KU851936.1/Orfv/isolate_Chalin KT438530.1/Orfv/Gondar_zuria/C KT438526.1/Orfv/Debre_zeit/O02 KT438524.1 Orfv/ATARC/O02/20 KT438520.1 Orfv/Amba_Giorgis/C KT438517.1 Orfv/Adet/O05/2012 JQ349520.1 Orfv/strain PA11/B2 JN846834.1/isolate_ORFV_Assa JN565696.1/strain_ORFV/ShanX JN565694.1/strain ORFV/XinJiar JN088053.1 Orfv/isolate A/goat GU320351.1/strain_ORFV/HuB/2 GU139356.1 ORFV Mukteswar/ GQ328006.1/ORFV/2009/Korea/E AY278209.1/Orfv/vaccine_strain

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//01	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA	CTCCGGGGGGC
//01 a24	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA ••••• A•••• ••••• A••••	CTCCGGGGGC
//01 24 sore	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A A	CTCCGGGGGGC
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//01 a24 sore and ssa hia	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A A A A A	CTCCGGGGGC
//01 a24 sore and ssa	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A A A A A A	CTCCGGGGGC
//01 G24 Gore and Ssa hia hia //2	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC G G G	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA	CTCCGGGGGC
//01 a24 aore and asa hia //2 015/ 5 D/2	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A A A A A A A A A A A A	CTCCGGGGGC
//01 624 sore ind ssa hia //2 015/ 5 D/2 CA	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC 	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 a24 aore and asa hia //2 015/ 5 D/2	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A A A A A A A A A A A	CTCCGGGGGC
//01 a24 sore and ssa hia //2 015/ D/2 CA F1	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 a24 sore and ssa hia //2 015/ D/2 CA F1 O	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC G. G. G. G. G. G. 	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 624 sore ind isa hia isa hia /2 015/ 2015/ CA F1 CA F1 00 2	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A.	CTCCGGGGGC
//01 624 sore und ssa hia //2 015/ CA F1 D/2 CA F1 00 2010	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 624 sore ind isa hia isa hia /2 015/ 2015/ CA F1 CA F1 00 2	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC . G	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 624 sore sa hia /2 015/ D/2 CA F1 O 22 010 /0	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC G. G. G. G. G. G. G. G. 	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 624 sore sa hia /2 015/ D/2 CA F1 O 22 010 /0	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC . G	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 224 324 30re 329 329 329 329 329 329 329 329	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC . G. . G.	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 224 sore ind isa hia //2 D/2 CA F1 O 02 010 //O 22 22 am/	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A.	CTCCGGGGGC
//01 324 sore ind issa hia /2 D15/ D15/ CA F1 O2 CA F1 O2 CA F1 O2 CA S0 2 010 /0 2 2 2 am/ Xi/ CA	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC . G	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 i24 sore sore sore sore sore ind isa hia /2 D/2 CA F1 CO 2010 /0 22 aam/ Xi/ and 200	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC . G	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC
//01 i24 sore ind isa hia hia hia hia hia bia bia bia bia bia bia bia b	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A.	CTCCGGGGGC
//01 i24 sore sore sore sore sore ind isa hia /2 D/2 CA F1 CO 02 010 /0 22 aam/ Xi/ and 22 200	CTACTCCATG	ATCGTGGAGC	CGAAGGTGCC	GTTCACGCGG	CTCTGCTGCG	CCATCGTCAC . G	GCCCACGGCC	ACGAACTTCC	ACCTCGACCA A	CTCCGGGGGC

Figure 3. Nucleotide sequence comparison between More strain and reference sequences downloaded from the GeneBank: NCBI (cont.). The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the nucleotide.

to the isolate studied, P3A present only in Ankara strain (MF997468), L9V present in almost all the other reference sequences, A41T which More strain share with five other reference sequences (MN422332; MH790954; KT438524; JN846834 and CQ328006), E98A found in four other reference sequences (LC208799; KX951407; KU851936 and KT438524) and A126T found also in nine other reference sequences (MN422332; LC208799; KX951407; KX029228; KT438524; JN846838; JN565696; GU320351 and GQ328006). Other substitution mutations found were at amino acid positions D196N present in More_strain and all other reference sequences except few (MF997468; JQ349520; JN088053 and the vaccine strain AY278209), S249G, Q256R, N267D and finally I352V (Figure 4).

Phylogenetic tree analysis based on the *B2L* gene showed that More_strain was closely related to the Ethiopian isolate (KT438524) which explained the high degree of nucleotide and amino acid similarities of 99.0 and 98.9% respectively that exist between them. They form a distinct cluster together with the isolates from Iran (MN422332), Korea (GQ328006) and Zambia (LC208799) (Figure 5). The Iranian strain was the most recent common ancestor to the Korean, Zambian, Ethiopian and More_strains. Other isolates from diverse geographical locations (Asia,

Molecular characterization & phylogenetic analysis of orf virus isolated from goats in Sokoto Metropolis, Nigeria Research Article

	610	620	630	640 • • • • • • •	650	660	670	680	690	700
U06671.1/orfv/NZ-2/B2L gene/Ne				TTCCTAGGCT						
MT272780.1/More_strain_ORFV/01				G						
MN422332.1/Orfv_isolate_IRGG24				•••••G••••						
MH790955.1/isolate_ORFV-Mysore				•••••G••••						
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MH790953.1/isolate_ORFV-Orissa				•••••G••••						
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KX029228.1/Orfv/strain_GY-AHF1				•••••G••••						
KU851936.1/Orfv/isolate_Chalin				•••••G••••						
KT438530.1/Orfv/Gondar_zuria/O										
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JN846834.1/isolate_ORFV_Assam/				•••••G••••						
JN565696.1/strain_ORFV/ShanXi/				•••••						
JN565694.1/strain_ORFV/XinJian				•••••						
JN088053.1 Orfv/isolate A/goat				•••• T •G••A•						
GU320351.1/strain_ORFV/HuB/200 GU139356.1 ORFV Mukteswar/09 c				G						
GQ328006.1/ORFV/2009/Korea/B2L				G						
AY278209.1/Orfv/vaccine strain										
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U06671.1/orfv/NZ-2/B2L gene/Ne	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC	GTGGAGTACT	GGCCGCAGAT	CATTGACGCG	CTGCTGCGCG	CGGCCATCAA
MT272780.1/More_strain_ORFV/01	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC	GTGGAGTACT	GGCCGCAGAT	CATTGACGCG	CTGCTGCGCG	CGGCCATCAA
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G	GTGGAGTACT	GGCCGCAGAT G	CATTGACGCG A	CTGCTGCGCG	CGGCCATCAA G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G	GTGGAGTACT	GGCCGCAGAT G G	CATTGACGCG A A	CTGCTGCGCG	GGGCCATCAA G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G G	GTGGAGTACT	GGCCGCAGAT G G	CATTGACGCG A A A	CTGCTGCGCG	CGGCCATCAA G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G G	GTGGAGTACT	GGCCGCAGAT G G G	CATTGACGCG A A A A	CTGCTGCGCG	CGGCCATCAA G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA 	CGCCAGCGCC G G	GTGGAGTACT	GGCCGCAGAT G G A.	CATTGACGCG A A A A A A	CTGCTGCGCG	CGGCCATCAA G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G G	GTGGAGTACT	GGCCGCAGAT	CATTGACGCG A A A A C A	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G	GTGGAGTACT	GGCCGCAGAT	CATTGACGCG A A A A C A	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/istrain_ORFV/AH-FD/2	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA ···G····· ···G····· ···G····· ···T····	CGCCAGCGCC G G G G G	GTGGAGTACT	GGCCGCAGAT	CATTGACGCG A A A A A A A A	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790943.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA <th>CGCCAGCGCC G G G. G. G.</th> <th>GTGGAGTACT</th> <th>GGCCGCAGAT G G G G G G G G</th> <th>CATTGACGCG A A A A A A A A A A</th> <th>CTGCTGCGCG</th> <th>CGGCCATCAA G. G. G. G. G. G. G. G.</th>	CGCCAGCGCC G G G. G. G.	GTGGAGTACT	GGCCGCAGAT G G G G G G G G	CATTGACGCG A A A A A A A A A A	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/strain_GY-AHF1	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA ···G····· ···G····· ···G····· ···T····	CGCCAGCGCC G G G G G G	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G	CATTGACGCG A	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790943.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G G G G G G G G	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX9551407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/istrain_GY-AHF1 KU851936.1/Orfv/isolate_Chalin	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA 	CGCCAGCGCC G G G G G G G	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G G G	CATTGACGCG A	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/istrain_GY-AHF1 KU851936.1/Orfv/isolate_Chalin KT438526.1/Orfv/Debre_zeit/O02 KT438526.1/Orfv/Debre_zeit/O02	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G G G	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Ofr/_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia MF907468.1 Ofr//TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Ofr/v/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX029228.1/ORFV/Goat/India/ICA KX052928.1/Orfv/strain_GY-AHF1 KU851936.1/Orfv/isolate_Chalin KT438520.1 Orfv/Gondar_zuria/O KT438520.1 Orfv/ATARC/O02/2010 KT438520.1 Ofr//Amba_Giorgis/O	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Ofrv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF907468.1 Ofr//TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Ofr/v/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX092228.1/Ofr/v/strain_GY-AHF1 KU851936.1/Ofrv/isolate_Chalin KT438530.1/Ofrv/Condar_zuria/O KT438526.1/Ofrv/Debre_zeit/O02 KT438520.1 Ofrv/AtaRC/O02/2010 KT43851.1 Ofrv/Adet/O05/2012	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA G G 	CGCCAGCGCC G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G G G G G	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790955.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790948.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX12982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/isolate_Chalin KT438530.1/Orfv/Isolate_Chalin KT438526.1/Orfv/Debre_zeit/O02 KT438526.1 Orfv/ADE/O2/2010 KT438520.1 Orfv/ATARC/O02/2010 KT438520.1 Orfv/ArtARC/O02/2012 JQ349520.1 Orfv/Atrain_PA11/B2	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G G G G	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Urissa MH790974.1/isolate_ORFV-Urissa MH790974.1/isolate_ORFV-Uridhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/Strain_Erzinsk KX951407.1/istrain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX0529228.1/Orfv/Isolate_Chalin KT438530.1/Orfv/Gondar_zuria/O KT438520.1 Orfv/Abrer_zeit/OO2 KT438520.1 Orfv/Abrer_zeit/OO2 KT438520.1 Orfv/Amba_Giorgis/O KT438521.1 Orfv/Arba_Giorgis/O KT43851.1 Orfv/Ater/Oo5/2012 JQ349520.1 Orfv/strain_PA11/B2 JN846834.1/isolate_ORFV_Assam/	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA G G 	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G 	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790955.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790948.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX12982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/isolate_Chalin KT438530.1/Orfv/Isolate_Chalin KT438526.1/Orfv/Debre_zeit/O02 KT438526.1 Orfv/ADE/O2/2010 KT438520.1 Orfv/ATARC/O02/2010 KT438520.1 Orfv/ArtARC/O02/2012 JQ349520.1 Orfv/Atrain_PA11/B2	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G 	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G. G. G.
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MT272780.1/More_strain_ORFV/01 MN422332.1/Ofr/_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF907468.1 Ofr//TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Ofr/v/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX052228.1/ORFV/Goat/India/ICA KX0529228.1/Ofr/v/strain_GY-AHF1 KU851936.1/Orfv//solate_Chalin KT438520.1 Orfv//Bohrz_zuria/O KT438520.1 Orfv/ATARC/002/2010 KT438520.1 Orfv/Amba_Giorgis/O KT438520.1 Orfv/Amba_Giorgis/O KT43851.1 Orfv/Atan_ORFV/ShanXi/ JN565696.1/strain_ORFV/ShanXi/ JN565694.1/strain_ORFV/ShanXi/ JN565694.1/strain_ORFV/XinJian JN088053.1 Orfv/isolate A/goat GU320351.1/strain_ORFV/IuB/200	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G 	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/O/fv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF907468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX092228.1/Orfv/isolate_Chalin KT438530.1/Orfv/isolate_Chalin KT438530.1/Orfv/Isolate_Chalin KT438520.1 Orfv/ATARC/O2/2010 KT438526.1/Orfv/AtaRC/O2/2010 KT438520.1 Orfv/AtaRC/O2/2012 J3849520.1 Orfv/Atarin_ORFV/Asam// JN565696.1/strain_ORFV/ShanXi/ JN565694.1/strain_ORFV/SinJian JN088053.1 Offv/isolate A/goat GU320351.1/strain_ORFV/IhuB/200 GU139356.1_ORFV_Mukteswar/09_c	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G 	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G. G. G. G. G. G.
MT272780.1/More_strain_ORFV/01 MN422332.1/Ofr/_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF907468.1 Ofr//TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Ofr/v/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX052228.1/ORFV/Goat/India/ICA KX0529228.1/Ofr/v/strain_GY-AHF1 KU851936.1/Orfv//solate_Chalin KT438520.1 Orfv//Bohrz_zuria/O KT438520.1 Orfv/ATARC/002/2010 KT438520.1 Orfv/Amba_Giorgis/O KT438520.1 Orfv/Amba_Giorgis/O KT43851.1 Orfv/Atan_ORFV/ShanXi/ JN565696.1/strain_ORFV/ShanXi/ JN565694.1/strain_ORFV/ShanXi/ JN565694.1/strain_ORFV/XinJian JN088053.1 Orfv/isolate A/goat GU320351.1/strain_ORFV/IuB/200	TCGACCTCTC	GCTGCTCTCG	ATGGTGCCGG	TGATCAAGCA	CGCCAGCGCC G. G. G. G. G. G. G. G. G. G. G. G. G. G.	GTGGAGTACT	GGCCGCAGAT G G G G G G G G G G G G G G	CATTGACGCG 	CTGCTGCGCG	CGGCCATCAA G. G. G. G. G. G. G. G. G. G. G. G. G. G. G.

Figure 3. Nucleotide sequence comparison between More strain and reference sequences downloaded from the GeneBank: NCBI (cont.). The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the nucleotide.

Africa, Europe, America and New Zealand) formed separate clusters and subclusters indicating a wide range of genetic diversity that exist in orf viruses.

Discussion

Orf or contagious ecthyma is endemic in Nigeria and causes huge economic losses in the national livestock industry [4] just as are other bacterial diseases such as brucellosis [32], yersiniosis [33] and parasitic infections such as hydatidosis [34] and gastrointestinal nematodes [35,36]. Effective control of the disease, therefore, demands early detection and identification of the causative agent. In Nigeria, outbreaks of CE are largely documented based on clinical signs which unfortunately may be confused with infection resulting from other viral disease agents such as sheep poxvirus, foot and mouth disease virus, bovine herpes virus type-2 and bluetongue virus. Thus, reliable estimate of the true prevalence of the disease in the country is currently lacking. In recent times, different techniques such as physical examination for clinical signs and PCR have been used to confirm contagious ecthyma outbreaks in Nigeria [1,2,4]. Furthermore, previous study conducted in 2018 involving three flocks of goats in northern Nigeria

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U06671.1/orfv/NZ-2/B2L g MT272780.1/More_strain_ MN422332.1/Orfv_isolate_ MH790955.1/isolate_ORF MH790954.1/isolate ORF\ MH790953.1/isolate ORF MH790947.1/isolate_ORF\ MF997468.1 Orfv/TR-Anka LC208799.1 B2L gene OR KY652170.1/Orfv/strain_E KX951407.1/strain_ORFV/ KX129982.1/ORFV/Goat/Ir KX029228 1/Orfv/strain G KU851936.1/Orfv/isolate_C KT438530.1/Orfv/Gondar_ KT438526.1/Orfv/Debre z KT438524.1 Orfv/ATARC/0 KT438520.1 Orfv/Amba_G KT438517.1 Orfv/Adet/O05 JQ349520.1 Orfv/strain PA JN846834.1/isolate_ORFV JN565696.1/strain_ORFV/ JN565694.1/strain OREV/ JN088053.1 Orfv/isolate A GU320351.1/strain_ORFV GU139356.1 OREV Mukt GQ328006.1/ORFV/2009/h AY278209.1/Orfv/vaccine strain

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India/ICA			C			G				
GY-AHF1			•••••C•••			G				
_Chalin			C			G		A		
_zuria/O	A		C			••••G•••••				
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//ShanXi/										
//XinJian										
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V/HuB/200										
/Korea/B2L										
strain										
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gene/Ne	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA		ACGACGCCGC	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
_ORFV/01	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
_ORFV/01 e_IRGG24	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
_ORFV/01 e_IRGG24 FV-Mysore	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
ORFV/01 e_IRGG24 FV-Mysore FV-Jaland	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	cccggccggg	ACGACGCCGC T T.	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
ORFV/01 9_IRGG24 FV-Mysore FV-Jaland FV-Orissa	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC T. T.	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
ORFV/01 -IRGG24 -V-Mysore -V-Jaland -V-Orissa -V-Ludhia	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	cccggccggg	ACGACGCCGC T. T.	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
ORFV/01 e_IRGG24 FV-Mysore FV-Jaland FV-Orissa FV-Ludhia kara/12/2	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC T. T.	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
ORFV/01 PIRGG24 V-Mysore V-Jaland V-Orissa V-Orissa V-Ludhia (ara/12/2 RFV/2015/	GTGGACATGT	СССТЕСССАА	GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC T. T.	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACEG
ORFV/01 PIRGG24 V-Mysore V-Jaland V-Orissa FV-Ludhia (ara/12/2 RFV/2015/ Erzinsk	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA	cccecceee	ACGACGCCGC T. T. T.	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACCG
ORFV/01 PIRGG24 V-Mysore V-Jaland V-Orissa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2	GTGGACATGT	ссатассаа	GTTCGTGGTÅ	ccceeccee	ACGACGCCGC T. T.	GAACAACACT	AAGCTGCTCA	тсетерасса	CACCTTCGCG	CACCTCACGG
ORFV/01 JERGG24 V-Jaland V-Orissa V-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 /India/ICA	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA GTTCGTGGTA	CCCGGCCGGG	ACGACGCCGC T. T. 	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
ORFV/01 IRGG24 EV-Mysore EV-Jaland EV-Orissa EV-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1	GTGGACATGT	. T G	GTTCGTGGTA	ccceccece	ACGACGCCGC T. T. T. T. T. T. T. 	GAACAACACT	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
_ORFV/01 _ IRGG24 FV-Jaland FV-Orissa FV-Ludhia cara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA 3Y-AHF1 _Chalin	GTGGACATGT	CCGTGCGCAA	GTTCGTGGGA	CCCGGCCGGG	ACGACGCCGC T. T. T. T. T. T. 	GAACAACACT GAACAACACT C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTOGOG	CACCTCACCG
ORFV/01 IRGG24 V-Mysore V-Jaland V-Joissa V-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA 3Y-AHF1 _Chalin _zuria/O	GTGGACATGT		GTTCGTGGTA GTTCCTGGGTA G		ACGACGCCGC 	GAACAACACT GAACAACACT C C C C C C C C C	AAGCTGCTCA	тсетерасса	CACCTTCGCG	CACCTCACGG
ORFV/01 IRGG24 V-Mysore V-Jaland V-Orissa V-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 Chalin _zuria/O zeti/O02	GTGGACATGT	CCGTGCGCAA	GTTCGTGGTA GTTCGTGGTA G	CCCGGCCGGG	ACGACGCCGC 	GAACAACACT GAACAACACT CCCCCCCCCCCCCCCCCC	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	CACCTCACGG
_ORFV/01 _ IRGG24 V-V-Jaland V-Orissa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 _Chalin _zuria/O zeit/O02 /002/2010	GTGGACATGT		GTTCGTGGTA		ACGACGCCGC TT	GAACAACACT 	AAGCTGCTCA	TCGTGGACGA	CACCTTOGOG	CACCTCACCG
ORFV/01 IRGG24 V-Mysore V-Jaland V-Orissa V-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 Chalin _zuria/O zeti/O02	GTGGACATGT	. T G	GTTCGTGGTA GTTCGTGGTA G T. C. CG G		ACGACGCCGC 	GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	тсетерасса	CACCTTCGCG	CACCTCACGG
_ORFV/01 _ IRGG24 V-V-Jaland V-Orissa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 _Chalin _zuria/O zeit/O02 /002/2010	GTGGACATGT		GTTCGTGGTA GTTCGTGGTA G T. C. CG G		ACGACGCCGC 	GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	тсетерасса	CACCTTCGCG	CACCTCACGG
ORFV/01 IRGG24 V-Mysore V-Jaland V-Jaland V-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 _Chalin _zuria/O2 zeit/002 /002/2010 Giorgis/O	GTGGACATGT	. T G	GTTCGTGGTA GTTCGTGGTA G	T. CC.	ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	
ORFV/01 IRGG24 V-Mysore V-Jaland V-Jorissa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 Chalin _zuria/O zeit/O02 //002/2010 Giorgis/O 05/2012	GTGGACATGT	. T G	GTTCGTGGTA		ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCETEGACEA	CACCTTCGCG	T.T.
_ORFV/01 _IRGG24 V-Mysore V-Jaland V-Orissa V-Ludhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 _Chalin _zuria/O zeti/O02 /002/2010 Giorgis/O D5/2012 PA11/B2	GTGGACATGT	. T G	GTTCGTGGTA		ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCETEGACEA	CACCTTCGCG	T.T.
_ORFV/01 _IRGG24 =V-Mysore =V-Jaland =V-Jaland =V-Ludhia acra/12/2 RFV/2015/ =rzinsk //AH-FD/2 India/ICA GY-AHF1 _Chalin _zuria/O02 zoti/002 /002/2010 Giorgis/O 55/2012 =A11/B2 V_Assam/	GTGGACATGT	. T G	GTTCGTGGTA GTTCCTGGGTA G	T. CC.	ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	T. T.
_ORFV/01 _IRGG24 V-Mysore V-Jaland V-Joirsa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA 3Y-AHF1 _Chalin _zuria/O zeit/O02 /O02/2010 Giorgis/O 05/2012 PA11/B2 V_Assam/ //ShanXi/	GTGGACATGT		GTTCGTGGTA G T. C. CG G	T, CC,	ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	
_ORFV/01 _ IRGG24 FV-Mysore EV-Jaland FV-Orissa EV-Ludhia ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 _Chalin zuria/O zeit/O02 //002/2010 Giorgis/O D5/2012 PA11/B2 V_Assam/ //ShanXi/ //XinJian	GTGGACATGT		GTTCGTGGTA		ACGACGCCGC TT	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	
ORFV/01 IRGG24 V-Mysore V-Jaland V-Jaland V-Judhia kara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA GY-AHF1 Chalin _zuria/O2 zeit/002 /002/2010 Giorgis/O D5/2012 V_Assam/ //ShanXi/	GTGGACATGT	. T G	GTTCGTGGTA GTTCCTGGGTA G		ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	
_ORFV/01 _IRGG24 V-Mysore V-Jaland V-Joirsa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 India/ICA 3Y-AHF1 _Zuria/O zeit/002 /002/2010 Giorgis/O 05/2012 PA11/B2 V_Assam/ //ShanXi/ //XinJian A/goat V/HuB/200	GTGGACATGT		GTTCGTGGTA GTTCCTGTGGTA G	CCCGGCCGGG	ACGACGCCGC 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	
_ORFV/01 _IRGG24 V-Mysore V-Jaland V-Jorissa V-Ludhia (ara/12/2 RFV/2015/ Erzinsk //AH-FD/2 /India/ICA GY-AHF1 _Chalin _zuria/O zeit/O02 //002/2010 Giorgis/O 05/2012 PA11/B2 V_Assam/ //ShanXi/ //XinJian A/goat //HuB/200 tdeswar/09_c	GTGGACATGT		GTTCGTGGTA G	T. CC.	ACGACGCCGC T. 	GAACAACACT GAACAACACT GAACAACACT C C C C C C C C C C C C C C C	AAGCTGCTCA	TCGTGGACGA	CACCTTCGCG	

Figure 3. Nucleotide sequence comparison between More strain and reference sequences downloaded from the GeneBank: NCBI (cont.). The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the nucleotide.

reported a mortality rate of 100% [4] in two out of the three flocks investigated, which was higher than usually observed in CE outbreaks. This necessitated the need to molecularly characterize the orf virus circulating within the northern region of Nigeria in order to appreciate the relationship between Nigerian isolates and other ones prevalent in other parts of the world. Moreover, to the best of our knowledge, detailed molecular epidemiological studies of CE has not been carried out in northern Nigeria. Consequently, the present study was designed to isolate and genetically characterize Orf virus obtained from an outbreak involving a goat farm in Sokoto metropolis, northwestern Nigeria.

Between May and September 2019, a number of suspected CE outbreaks have occurred in Sokoto State, Nigeria. One of those outbreaks involved a goat farm located in More village within Sokoto metropolis. Scabs were obtained from the two affected animals and prepared for inoculation into CEE via the CAM route for virus isolation. Expectedly, the CAM developed characteristic pock lesions typical of orf viruses, signifying virus replication. Although, this method of virus isolation is simple and relatively rapid for orf diagnosis, to the best of our knowledge, this is the first report of orf virus isolation using specific antibody free CEE in Nigeria.

Molecular characterization & phylogenetic analysis of orf virus isolated from goats in Sokoto Metropolis, Nigeria Research Article

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100 TCGCCAACCT CGACGGCACG CACTACCGCT ACCACGCCTT CGTGAGCGTG AACGCCGAGA AGGGCGACAT CGTCAAGGAC CTGTCCGCGG TCTTCGAGCG G

U06671.1/orfv/NZ-2/B2L MT272780.1/More_strain MN422332.1/Orfv isolate MH790955.1/isolate_ORI MH790954.1/isolate_ORF MH790953.1/isolate ORI MH790947.1/isolate_ORF MF997468.1 Orfv/TR-Ank LC208799.1 B2L gene OF KY652170 1/Orfv/strain KX951407.1/strain_ORF\ KX129982.1/ORFV/Goat/ KX029228.1/Orfv/strain (KU851936.1/Orfv/isolate KT438530.1/Orfv/Gondar KT438526.1/Orfv/Debre_ KT438524.1 Orfv/ATARC KT438520.1 Orfv/Amba_0 KT438517.1 Orfv/Adet/O0 JQ349520.1 Orfv/strain JN846834.1/isolate ORF JN565696.1/strain_ORFV JN565694.1/strain ORFV JN088053 1 Orfv/isolate A GU320351.1/strain_ORF GU139356.1 ORFV Muk GQ328006 1/OBEV/2009 AY278209.1/Orfv/vaccine

olate_Chalin	······
ondar_zuria/O	······ T······ T······
bre_zeit/002	G
ARC/002/2010	
nba_Giorgis/O	······ T····· T·····
et/O05/2012	
ain_PA11/B2	
ORFV_Assam/	
)RFV/ShanXi/	·····
DRFV/XinJian	
late A/goat	······································
ORFV/HuB/200	······
_Mukteswar/09_c	
2009/Korea/B2L	······································
ccine_strain	
	1110 1120 1130

gene/Ne		GCAAGCCAAT AAATTAA
_ORFV/01	•••••••••	· · · · A · · · · · · · · · · · · · · ·
e_IRGG24		· · · · · · · · · · · · · · · · · · ·
FV-Mysore		
FV-Jaland		
FV-Orissa		
FV-Ludhia		
kara/12/2		
RFV/2015/		· · · · · · · · A · · · · · · · ·
Erzinsk		
V/AH-FD/2		· · · A · · · · · · · · · · · · · · · ·
/India/ICA		
GY-AHF1	••••••••	· · · A · · · · · · · · · · · · · · · ·
_Chalin	•••••••••	•••••
_zuria/O	••••••••	•••••
zeit/O02	••••••••	•••••
/002/2010	••••••••	••••••
Giorgis/O	••••••••	· · · · · · · · · · · · · · · · · · ·
05/2012	•••••••••	· · · · · · · · · · · · · · · · · · ·
PA11/B2	••••••••	••••••
V Assam/	••••••••	· · · · · · · · · · · · · · · · · · ·
//ShanXi/		•••••
//XinJian	••••••••	•••••
A/goat	·····	· · · · · · · · · · · · · · · · · · ·
V/HuB/200	•••••••••	· · · A · · · · · · · · · · · · · · · ·
kteswar/09 c	·····	· · · · · · · · · · · · · · · · · · ·
/Korea/B2L	••••••••	•••••
e_strain	•••••••••	•••••

Figure 3. Nucleotide sequence comparison between More strain and reference sequences downloaded from the GeneBank: NCBI (cont.). The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the nucleotide.

The full length *B2L* gene sequence obtained when blasted confirmed the identity of the virus isolate as orf virus using the NCBI BLASTN tool. The virus when aligned with deposited orf virus sequences downloaded from NCBI database showed similarities with the reference sequences at both nucleotide and amino acid levels. A unique amino acid change observed only in the strain under study was the W2G seen at amino acid position 2. Another mutation observed was the P3A change at position 3 that was seen only in the Ankara strain (MF997468) from Turkey. However, the significance of these two substitution mutations as they affect the virulence and pathogenicity of the orf virus isolate under study need to be investigated. This is necessary in order to determine the extent of genetic diversity of the orf virus isolates circulating in Sokoto state and its environs since no vaccination against CE are currently practiced in the country. Moreover, the number of samples analyzed in the current study is not enough to determine the significance of the mutations observed, this can be achieved by a larger study covering the whole state.

Phylogenetic tree analysis based on the *B2L* gene showed four major branches on the tree, with the isolate under study (More_strain, MT272780.1) forming a distinct cluster with Korean (GQ328006.1), Zambian (LC208799.1)

	10	20	30	40	50	60	70	80	00	100
		ī								
U06671.1/orfv/NZ-2/B2L gene/Ne		ADCRVVETLP								
MT272780.1/More_strain_ORFV/01		•••••								
MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore										
MH790954.1/isolate ORFV-Jaland										
MH790953.1/isolate ORFV-Orissa										
MH790947.1/isolate ORFV-Ludhia										
MF997468.1 Orfv/TR-Ankara/12/2			.к					N.		
LC208799.1 B2L gene ORFV/2015/		PQAP.LG								
KY652170.1/Orfv/strain_Erzinsk										
KX951407.1/strain_ORFV/AH-FD/2		•••••								
KX129982.1/ORFV/Goat/India/ICA		. . R								
KX029228.1/Orfv/strain_GY-AHF1		•••••								
KU851936.1/Orfv/isolate_Chalin										
KT438530.1/Orfv/Gondar_zuria/O KT438526.1/Orfv/Debre zeit/O02										
KT438526.1/Offv/Deble_2eft/002 KT438524.1 Orfv/ATARC/002/2010										
KT438520.1 Orfv/Amba Giorgis/O										
KT438517.1 Orfv/Adet/O05/2012										
JQ349520.1 Orfv/strain PA11/B2										
JN846834.1/isolate ORFV Assam/										
JN565696.1/strain ORFV/ShanXi/										
JN565694.1/strain_ORFV/XinJian	V.									
JN088053.1 Orfv/isolate A/goat										
GU320351.1/strain_ORFV/HuB/200										
GU139356.1_ORFV_Mukteswar/09_										
GQ328006.1/ORFV/2009/Korea/B2L										
AY278209.1/Orfv/vaccine_strain	YF	•••••	•••••	•••••	•••••	•••••	•••••	•••••N•	•••••	•••••
	11	0 120) 130) 140) 150 ••••	D 160) 170) 180) 190	
U06671.1/orfv/NZ-2/B2L gene/Ne	VNYYKVKVST	KEGVGNLLGS	FWLSDAGHWY	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IV <mark>E</mark> PKVPFTR		TNFHLDHSGG
MT272780.1/More_strain_ORFV/01	VNYYKVKVST	KEGVGNLLGS	FWLSDAGHWY	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	LCCAI VTPTA	TNFHLDHSGG
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24	VNYYKVKVST	KEGVGNLLGS	FWLSDAGHWY	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	LCCAIVTPTA	TNFHLDHSGG
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore	VNYYKVKVST	KEGVGNLLGS	FWLSDAGHWY	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR		TNFHLDHSGG N N N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland	VNYYKVKVST	R	FWLSDAGHWY	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	LCCAIVTPTA	TNFHLDHSGG
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa	VNYYKVKVST	RR	FWLSDAGHWY	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	LCCAIVTPTA	TNFHLDHSGG
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia	VNYYKVKVST	RRR	FWLSDAGHWY	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR		N N N N N N N N N N N N N N N N N N N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Crissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2	VNYYKVKVST	RR RR RR RR	FWLSDAGHWY	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	UCCAIVTPTA	N N N N N N N N N N N N N N N N N N N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790954.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/	VNYYKVKVST	RR. R. R. R. R. R. R. R. R.	FWLSDAGHWY	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	UCCAIVTPTA	II TNFHLDHSGG N N N N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Crissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2	VNYYKVKVST	RR RR RR RR	FWLSDACHWY	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	LCCAIVTPTA	II TNFHLDHSGG N N N N N N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk	VNYYKVKVST	RR. RR. RR. RR. RR. RR.	FWLSDAGHWY T.T.T.N.	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	UCCAIVTPTA	II TNFHLDHSGG N N N N N N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Ludhia MH7909471.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2	VNYYKVKVST	R. R. R. R. R. R. R. R. R. R. R. R. R. R	FWLSDAGHWY 	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA	VNYYKVKVST	RRRRR	FWLSDAGHWY T.T.T.N. T.N. T.N.	VGSASLTGGS	VSTIKNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	UCCAIVTPTA	N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790954.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/Strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Gat/India/ICA KX029228.1/Orfv/strain_GY-AHF1	VNYYKVKVST	RR. RR. RR. RR. R	FWLSDAGHWY T T T.N. T.N. 	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	E	UCCAIVTPTA	II TNFHLDHSGG N
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790971.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/Strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/strain_GY-AHF1 KU851936.1/Orfv/solate_Chalin KT438530.1/Orfv/Debre_zeit/O02	VNYYKVKVST	RR. RR. RR. RR. RR. RR. RR. RR. RR. RR. RR.	FWLSDAGHWY 	VGSASLTGGS	VST I KNLGLY	STNKHLAWDL	MNRYNTFYSM	IVEPKVPFTR	UCCAIVTPTA	I I TNFHLDHSGG N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N
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Figure 4. Amino acid sequence comparison between More_strain and reference sequences downloaded from the GeneBank: NCBI. The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (...), while areas of differences were represented by a letter denoting the amino acid.

and Ethiopian (KT438524.1) isolates, suggesting a common ancestor. Indeed, the Nigerian isolate and Ethiopian (KT438524.1) isolate are so phylogenetically related that they are believed to share a common most recent ancestor during evolution. Expectedly, a high degree of nucleotide and amino acid similarities of 99.0 and 98.9% respectively was demonstrated between the two isolates. Another branch on the tree had four major sub-branches where Chinese isolates (red line) diverged from the others, while the New Zealand (U06671), USA vaccine (AY278209) and two Brazilian strains (JQ349520 and JN088053) clustered together (green line) in a separate branch. Isolates from India (blue line) clustered together with one Ethiopian strain in a separate sub-branch. Finally, three Ethiopian isolates clustered with Erzinsky strain (KY652170) from Russia and XinJiang strain (JN565694) from China in a separate sub-branch. This analysis indicated that the Nigerian isolate investigated is genetically different with other ORFV isolated from different geographic regions of the world but closely related with other orf viruses circulating in Ethiopia, Zambia and Korea. The nucleotide and amino acid sequence analyses conducted in this study further confirmed usefulness of the *B2L* gene for genetic and phylogenetic analysis due to its conserved nature and the percentage similarity at both nucleotide and amino acid being in agreement with previous studies [3,37–39].

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U06671.1/orfv/NZ-2/B2L gene/Ne					MVPVIKHASA					
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24					G.					
MH790955.1/isolate ORFV-Mysore					G.					
MH790954.1/isolate_ORFV-Jaland					G.					
MH790953.1/isolate ORFV-Orissa					V					
MH790947.1/isolate_ORFV-Ludhia					V					
MF997468.1 Orfv/TR-Ankara/12/2						· · · · · R · · · ·	D			
LC208799.1 B2L gene ORFV/2015/					G.					
KY652170.1/Orfv/strain_Erzinsk										
KX951407.1/strain_ORFV/AH-FD/2					G.					
KX129982.1/ORFV/Goat/India/ICA KX029228.1/Orfv/strain GY-AHF1					G.					
KU851936.1/Orfv/isolate Chalin					G.					
KT438530.1/Orfv/Gondar zuria/O										
KT438526.1/Orfv/Debre zeit/O02										
KT438524.1 Orfv/ATARC/O02/2010					G.	· · · · · B · · · ·		· · · · · · · · · · · · · · · · · · ·		
KT438520.1 Orfv/Amba_Giorgis/O	•••••									
KT438517.1 Orfv/Adet/005/2012										
JQ349520.1 Orfv/strain_PA11/B2					• • • • • • • • • • •					
JN846834.1/isolate_ORFV_Assam/										
JN565696.1/strain_ORFV/ShanXi/ JN565694.1/strain_ORFV/XinJian					G.					
JN088053.1 Orfv/isolate A/goat										
GU320351.1/strain_ORFV/HuB/200					G.					
GU139356.1_ORFV_Mukteswar/09_c					V					
GQ328006.1/ORFV/2009/Korea/B2L					GD	· · · · · R · · · ·	D	M		
AY278209.1/Orfv/vaccine_strain							D	· · · · · · · · · · · · · · · · · · ·		
	310	320	330	340) 35	360	370			
U06671.1/orfv/NZ-2/B2L gene/Ne	310 VDMSVRKFVV) 320 PGRDDAANNT	330 KLLIVDDTFA	340 HLTVANLDGT) 350 HYRYHAFVSV) 360 NAEKGDIVKD	370 LSAVFERDWR) SEFCKPIN*		
MT272780.1/More_strain_ORFV/01	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT) 350 HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790955.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790955.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Orissa MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2	VDMSVRKEVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790954.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2	VDMSVRKEVV	PGRDDAANNT	KLLIVDDTFA	HLTVANLDGT	HYRYHAFVSV	NAEKGDIVKD	LSAVFERDWR	SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790954.1/isolate_ORFV-Jaland MH790947.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA	VDMSVRKEVV	PGRDDAANNT	KLLIVDDTFA	.F.	HYRYHAFVSV	NAEKODIVKD		SEFCKPIN*		
MT272780.1/More_strain_ORFV/01 MN422332.1/Orfv_isolate_IRGG24 MH790955.1/isolate_ORFV-Mysore MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Jaland MH790953.1/isolate_ORFV-Ludhia MF997468.1 Orfv/TR-Ankara/12/2 LC208799.1 B2L gene ORFV/2015/ KY652170.1/Orfv/Strain_Erzinsk KX951407.1/strain_ORFV/AH-FD/2 KX129982.1/ORFV/Goat/India/ICA KX029228.1/Offv/strain_GY-AHF1	VDMSVRKFVV	PGRDDAANNT	KLLIVDDTFA		HYRYHAFVSV	NAEKGD I VKD	LSAVFERDWR	SEFCKPIN*		
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Figure 4. Amino acid sequence comparison between More_strain and reference sequences downloaded from the GeneBank: NCBI (cont.). The strain ORFV/NZ-2 from New Zealand (accession number U06671.1) was used as the guide sequence. Areas of similarity with guide sequence were represented as dots (....), while areas of differences were represented by a letter denoting the amino acid.

However, there is sufficient mutation to allow for genetic differentiation between the Nigerian isolate and the reference sequences. The presence of some certain mutations at position 196 (D-N), 256 (Q-R) and 267 (N-D) observed in this study was previously reported by Olivero *et al.* in Uruguayan orf viruses [3] indicating their genetic relatedness of the Nigerian isolate. The absence of the sheep specific conserved residue S at position 249 [37] in the isolate under study may indicate that the G residue found in that same amino acid position may be regarded as goat specific residue to be found in isolates that have adapted to goats. However, this assertion needs to be confirmed by further studies using several orf virus *B2L* gene sequences isolated from goats. The presence and significance of the unique mutation at position W2G as well as P3A present in only the Turkish Ankara strain (MF997468) among the reference sequences examined needs to be further investigated by both *in vivo* and *in vitro* studies in relation to virulence and pathogenicity This study provided the first insight on the genetic diversity of the orf viruses circulating within the Nigerian goat population using complete *B2L* gene nucleotide and amino acid sequence analyses as well as phylogenetic approach.

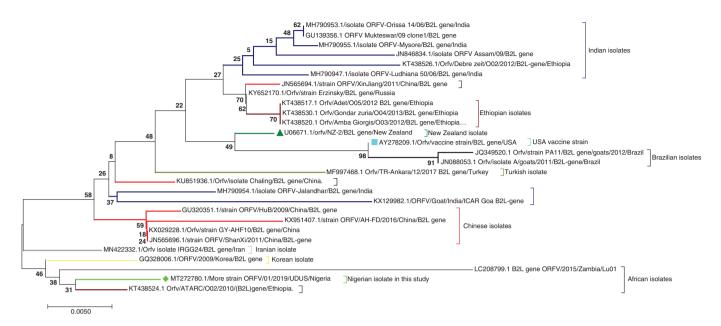


Figure 5. Evolutionary relationships of taxa. The figure showed the phylogenetic relationship between the Nigerian orf virus isolate 'More_strain' and other reference sequences from different parts of the world. The Nigerian More_strain clustered together more closely with Ethiopian and Zambian strains and the Iranian IRGG24 isolate is their common most recent ancestor. The evolutionary history was inferred using the neighbor-joining method with 2000 replicates bootstrap. The evolutionary distances were computed using the Poisson correction method involving 28 amino acid sequences. There were a total of 378 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Conclusion

In summary, the authors have examined the genetic characteristics of orf virus causing disease outbreaks in Sokoto metropolis, Nigeria and provided molecular signatures of the virus in the study area. The authors showed that the virus under study shared a close genetic relationship with an isolate from Ethiopia and harbors an amino acid change (W2G) that is unique to it and another mutation at P3A that is present in only in the Ankara strain (MF997468). We further highlighted the possibility of the G249 residue as being goat specific conserved residue in the same way as S249 is sheep specific conserved residue. This information is crucial to understand the molecular epidemiology of orf virus circulating in Nigeria, which is necessary for the design of effective CE vaccines which are currently lacking in the country.

Future perspective

There is the need to undertake a state-wide isolation and characterization study to fully understand the molecular epidemiology of the orf virus isolates circulating within the goat and sheep population in Nigeria. Whole genome sequencing can be done to add to the existing orf virus complete genome sequences since, currently, very few are in the public database. The significance of the W2G and P3A mutations with respect to orf virus virulence modulation and pathogenicity needs to be investigated.

Author contributions

N Lawal conceived the idea; N Lawal, M Ibrahim and DA Onawala designed and conducted the study; N Lawal, MB Bello, RM Aliyu, YS Baraya drafted the manuscript; N Lawal, MB Bello, A Aliyu and AM Ibrahim and A Sa'adu reviewed the manuscript.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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Summary points

- Scab lesions from goats suspected to be infected with orf virus based on clinical signs were obtained.
- Genomic DNA was extracted and amplified by PCR.
- The PCR products were analyzed by gel electrophoresis and sent for sequencing.
- The sequencing result was analyzed and aligned using ClustalW to determine its similarity with reference sequences downloaded from GeneBank.
- Deduced amino acid was determined and any change between the isolate and the reference sequences were noted.
- Phylogenetic tree was constructed to determine the taxonomic relationship between taxa.
- One unique amino acid change (W2G) and another (P3A) present only in a Turkish isolate were observed in the isolate under study.
- Phylogenetically, the isolate is more closely related to the Ethiopian isolate (KT438524) with which it clusters in the same clade different from other reference sequences.
- This is the first report on the molecular characterization of orf virus using full length B2L gene sequences.

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