Genetic characterization and Temporal dynamics of Orf virus in Small Ruminants from Republic of Niger and parts of Northern Nigeria sold in Livestock Market in Abuja

Babatunde Motayo[1], Anyebe Bernard ONOJA[2], Oluwaseyi AJAGBE[3], Andrew Musa ADAMU[4], Cornelius Arome OMATOLA[5], Ismaila Shittu[6], and Adenike AJAGBE[7]

[1]Federal Medical Centre Abeokuta
[2]University of Ibadan College of Medicine
[3]University of Abuja
[4]James Cook University Australian Institute of Tropical Health and Medicine
[5]Kogi State University Faculty of Natural Sciences
[6]National Veterinary Research Institute
[7]University of Maiduguri

January 19, 2023

Abstract

Background: Orf virus (ORFV) is an important zoonotic parapox virus, with 100% morbidity. It affects mainly domesticated ruminants such as sheep and goats. This study determined the molecular epidemiology of ORFV local as well as imported sheep and goats in Nigeria and evolution of ORFV in Africa. **Methods**: A total of 30 small ruminants with orf were sampled in a livestock market Abuja Nigeria.Skin scabs from pathological tissues were collected and processed for viral genomic DNA. PCR and Sanger sequencing of B2L gene of ORFV. Phylogenetic analysis, phylogeography, and Bayesian skygird reconstruction (BSK), including mutational changes were performed on B2L gene sequences. **Results**: An ORFV positivity rate of 67% was determined from samples. Animals <2 years had the highest prevalence of 76.5%.Hundred percent attack rate was observed among the Uda and WAD breeds, followed by Niger 71.4%, Kano Brown 66.7%, Yankasa 25% and Balami 0% breeds. ORFV from Africa breeds clustered into 2 major lineages, Asian and African, with an evolutionary rate of 7.45×10^{-4}, 95% HPD (3.46×10^{-4} to 1.17×10^{-3}) substitutions/ site/year. Viral population demography showed a constant population growth with a slight rise in viral population growth towards year 2020. **Conclusion**: We report molecular evolution of ORFV in Africa and identified gap in molecular data; we recommend regional molecular surveillance of ORFV and other zoonotic trans-boundary diseases in global health prevention and control effort.

Introduction

Orf virus (ORFV) is a member of parapoxvirus genus which belongs to the family *Poxviridae* . Ithas a linear double-stranded DNA which is about 135 kbp in size. Orfis (also known as contagious ecthyma) is an infectious pustular dermatitis which primarily affects sheep, goats and wild ruminants[1] . It is characterized by proliferative skin lesions on the lips, muzzle, ears, eyelids, and found around the mouth and nostrils of lambs[2] . Transmission within the herd is by direct contact between animals. The morbidity may reach 100%, but mortality is usually less than 1%. It is zoonotic and humans can become infected through direct contact with sick animals. In humans, it has predilection for the hands where it appears as rash-shaped papule and can progressively extend to form pustules[3] . Sheeppox and goatpox are important trans-boundary diseases that are notifiable to the Office Internationale des Epizooties[4] . Mortality and morbidity in young animals can

be high[5,6] . Exotic breeds of sheep and goats are more susceptible than indigenous breeds that exhibit some level of natural immunity[7] while in naïve animals, mortality can reach100%[8] .The disease is endemic in North African countries of Morocco, Tunisia, Algeria and Libya because of porous land borders coupled with legal and illegal trade of animals. It is widespread in Central Africa[9] . It has been reported in Southern Europe, the Middle East, Central Asia, India and China[10] . In West Africa, pastoral farming is widely practiced because of unrestricted trans-border movement leading to importation of ORFV which infects sheep and goats. Nigeria has the largest small ruminant population in Africa with about 73.8 million goats and 42.1 million sheep located mainly in the Northern part of the country[11] . Clinical and serological evidence of Orf has been reported in Nigeria[12] , with few reports of molecular identification. Since there is increased livestock trade and cross border activities between Nigeria and neighboring countries, it is important to determine the evolutionary diversity of ORFV in Nigeria. Also, the zoonotic nature of ORFV underpins the public health importance of this study. We determined the prevalence, and molecular epidemiology of ORFV in sheep and goats imported into Nigeria's capital Abuja from neighboring countries, the temporal dynamics and evolution of ORFV across Africa.

Materials and method

Study design

This is a prospective observational study which carried out in August 2019. A total of 30 small ruminants with contagious ecthyma were sampled in a livestock market at the Federal Capital Territory (FCT) Abuja. These ruminants were raised under extensive farming system in Kano (5), Yobe (4), Katsina (5), Jigawa (1), Niger (1) and Niger Republic (14) from where they were shipped to the FCT (Figure 1). Common clinical signs include weakness, inappetence, lymphadenopathy, rough hair coat, cough, scabby lesions on the commissures of the lips, mouth, muzzle, skin, eyes, nose ears and head region.

DNA Extraction

Skin scabs from pathological tissues were centrifuged at 10,000 g for 10 min at +4 °C. The centrifuged scabs was used for viral genomic DNA extraction using Blood-Animal-Plant DNA Preparation kit (Jena Biosciences®, Germany). A total of 300 µl of lysis buffer was added to 200 µl of tissue homogenate and 2 µl RNAse A inhibitor was added and vortexed for 30 seconds, then 8µl of proteinase K was added to digest the tissue and placed on heating block at 60°C for 30 minutes. This was cooled for 5mins and 300 µl binding buffer was added and centrifuged at 10,000g for 5mins. The supernatant was decanted into a spin column and washed twice with wash buffer. Fifty microliter of viral DNA was eluted into a new eppendorf tube, labeled and kept at -4°C briefly before PCR.

Molecular detection using conventional Polymerase Chain Reaction

Fragments containing the B2L genes were amplified by PCR in a 25 µl reaction volume containing 20UM forward primer, 20 UM reverse primer, 2 mM dNTPs, 5x PCR Buffer (Applied Biosystem), 5X Taq Polymerase (Jena Biosciences) and 5 µl template DNA. Cycling conditions were: initial denaturation at 94°C for 3 mins, followed by 35 cycles at 94°C for 50s, 52°C for 60s and 72°C for 90s, and final extension at 72°C for 7 min. Primers ORFV-B2Lf-For 5'-GACCTTCCGCGCTTTAATTT-3 and ORFV-B2Lf-Rev 5'-CCCGCCTGCTAAAAGACT-3'. Each PCR run included a negative control consisting of 5µl of PCR grade water instead of the DNA template. Amplified PCR products were subjected to electrophoresis on 1.5% agarose gel stained with ethidium bromide and visualized using Biorad® transilluminator.

Sequencing and phylogenetic analysis

Amplified DNA fragments of 1,210bp were used forthe dideoxynucleotide sequencing reactions. PCR-amplified products were analyzed on an ABI Prism 3700 automated DNA sequencer (Applied Biosystems, Foster City, California). Low quality sequences were trimmed with Chromas version 2.6.2 for windows licensed by Technelysium Pty Ltd 1998-2016 (www.technelysium.com.au). The sequences were combined with those currently available from GenBank (as shown in Table 2). Pairwise and multiple genomic alignments

were done with Clustal W[13] alignment programs. The evolutionary history was inferred by using the Maximum Likelihood (ML) method and Kimura 2-parameter model[14] . The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying ML algorithm with a GTR+G model, and 1000 bootstrap resampling. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 55 nucleotide sequences with 556 positions in the final dataset. Evolutionary analyses were conducted in iqtree[15] . Sequences were submitted to the GenBank and accession numbers MW916908-MW916918 were assigned for 11 isolates in this present study. Information regarding the study sequences can be accessed in supplementary table 1.

Phylogeography and Bayesian Skyride analysis

Phylogenetic trees were generated by Bayasian inference through Markov chain Monte Carlo (MCMC) implemented in BEAST version 2.5. To reconstruct the ancestral-state phylogeographic transmission across countries, the discrete-trait model implemented in BEAST version 2.5[16] . The coding genes were partitioned into first+second and third codon positions and applied a separate Hasegawa-Kishino-Yano (HKY+G) substitution model with gamma-distributed rate heterogeneity among sites to each partition[17] .The relaxed clock with Gausian Markov Random Field Skyride plot (GMRF) coalescent prior was selected for the final analysis. The MCMC was set at 100, 000, 000 states with10% as burn in. Results were visualized using Tracer version 1.8. (http://tree.bio.ed.ac.uk/software/tracer/) all effective sampling size ESS values were >200 indicating sufficient sampling. Bayesian Skyride analysis was carried out to visualize the epidemic evolutionary history using Tracer v 1.8. (http://tree.bio.ed.ac.uk/software/tracer/).

Statistical analysis

Statistical Packages for Social Sciences (SPSS) version 16.0 was used to analyze the data. Prevalence was computed by dividing the number of positive animals by the total animals tested and multiplied by 100. Univariate logistic regression at 95% confidence level was performed to assess potential influence of demographic parameters on the occurrence of Orf. Level of statistical significance was p value [?]0.05.

Results

Out of 30 samples screened for ORFV, 20 were positive giving a rate of 67%. Animals <2 years had higher prevalence (76.5%) of ORF compared to older ages 2-3 years and >3 years with 50% and 66.7%, respectively. More females than males (80% versus 64%) were infected with ORFV despite having five-fold more of the later in our study. Logistic analysis showed rate of occurrence of Orf did not differ significantly with animals' ages and sexes (P>0.05). Goat with 71.4% prevalence demonstrated higher susceptibility to ORFV compared to sheep (55.6%). Hundred percent attack rate was observed among the Uda and WAD breeds followed by a decreasing susceptibility in Niger (71.4%), Kano Brown (66.7%), Yankasa (25%) and Balami (0%) breeds(Table 1). A differential distribution of Orf was observed in relation to sources of livestock sampled from the market. Animals sourced from Niger State (100%) were more infected followed by Kano State (80%), Yobe State (75%), Niger republic (71%) and Katsina State (40%). Though, current study did not observe any significant influence of source on the occurrence of ORFV infection (p>0.05).

The ML phylogenetic treeshowed a distinct diversification of the isolates analyzed into 2 lineages, African and Asian lineage, with majority of the Nigerian strains clusteringwithin the African lineage, a few Nigerian stains also clustered within the Asian lineage along with some South African strains and reference strains from China (Fig. 2).

Time scaled MCC phylogeny of the ORFV sequences indicates that the MRCA of the African strains was around 2002. The African Viruses clustered into 2 major clades with both clades containing West African (Nigerian) and South African viruses (Fig. 3a). There was also a strain from Botswana which clustered very closely with Nigerian isolates and shared same TMRCA of around 2014 highest posterior density interval (HPD 2012-2017), while the TMRCA for the entire African sequences was around 2004 (HPD 2000-2006).. The evolutionary rate of the African ORFV B2L genes was 7.45 x 10^{-4}, 95% HPD (3.46 x 10^{-4} to 1.17 x

10^{-3}) substitutions/ site/year. The demographic history of the African ORFV B2L genes showed a constant viral population expansion from 2005 to 2016, with a slight rise in population expansion between 2018 to 2020 (Fig. 3b). Amino acid alignment of the complete B2L protein coding sequence also showed some non-synonymous mutations (Suppl. Figure 1) which are summarized in Table 2. The sampling dates and Genbank accession numbers of the study sequences can be assessed in Suppl. Table 1.

Discussion

In this study, high prevalence of Orf occurred among animals of all ages which reinforces the endemicity which may jeopardize the animal productivity in the area. The greater incidence of ORF in ages <2years indicates increased susceptibility due to lower immunity in younger ages which has been corroborated in other studies[18,19] . The absence of statistical significance despite higher prevalence of ORF among female animals support reports from the eastern Sudan which posited that sex is not a significant predictor of Orf[19] . Higher rate of susceptibility in goats compared to ovine species has been reported[20] . The naturally aggressive behavior of the former compared to the latter predisposes them to injury, thereby increases the susceptibility to ORFV transmitted via direct contact. Most small livestock holders in Nigeria do not practice dehorning as observed in this study and this may increase the risk of deep wounds or injuries which serve as a predisposing factor for virus penetration via the skin wounds. Current study observed 100% morbidity among the Uda and WAD breeds, similar to previous observation in Ibadan (Onoja et al., unpublished data) and Jos[21] respectively. These findings suggest the influence of intrinsic genetic factors in certain breed susceptibility to Orf which needs to be fully investigated.

Over 70% morbidity rates were observed among animals imported from Niger republic and the ones sourced from Kano, Niger and Yobe States, raising the concern of trans-boundary and national sporadic spread to susceptible new herds which could be the starting point of contagious ecthyma outbreaks often associated with production loss in livestock and reduction in the market value[22] . The high morbidity observed in this study can be traceable to inadequate hygienic[20] and quarantine procedures as most of the sick animals are not isolated before mixing up with previously apparently healthy sheep and goats. Climatic change over the years has made more ruminants susceptible to this hitherto low pathogenic but highly contagious viral disease. In addition, Abuja been a commercial centre and the nation's capital receives a high influx of both human and livestock. With a burgeoning population, consumption of livestock is on the increase consequently increasing importation of sheep and goat from other African countries. It is noteworthy that all samples were collected from live markets where sheep and goats are ready to be sold. In the process of handling and bargain between the potential buyer and seller, transmission and infection with contagious ecthyma may occur. The highly contagious nature of contagious ecthyma affects production. Animals refuse or reduce intake of food which leads to poor market value consequently leading to low protein availability and loss to the farmers.

The phylogenetic analysis revealed that majority of the isolated Nigerian strains clustered within the African lineage, with a few stains isolated from goats sold within the FCT, clustering within the Asian lineage along with South African strains and reference strains isolated from China (Figure 2). This observation suggests that the goats sampled from Nigeria infected with the Asian Lineage, confirms Orf virus as a transboudary animal disease. This confirms that some of the Nigeria goats were breed outside the country in neighboring Countries such as Niger republic or Chad and might have had contact with other ruminants from North Africa or Arabia, where the Asian virus might have been introduced. Another theory for this observation is that the Asian virus might have been circulating within the African continent for decades undetected because of poor surveillance and weak diagnostic infrastructure. The MCC tree of African Orf B2L sequences was also clustered into a similar topology with majority of the African sequences falling in to the African Lineage and FCT, South African and Egyptian strains falling within the Asian lineage (Figure 3a). This clustering of sequences further buttresses our earlier observation that the sheep and goats may have been infected with the same strain during transit. Since majority of sheep and goats are from neighboring African countries especially Chad and Niger republic, sharing borders with Northern Nigeria, it is not unlikely that they came from a common source and were transported together across the border to Nigeria. Moreover,

the stress of trans-border transportation makes the animals more susceptible to contagious ecthyma virus. Unchecked and indiscriminate trans-border activity of both humans and animals is a very important factor in the dissemination of ORFV. The time to most recent common ancestor (TMRCA) for the Nigeria African lineage was around 2014, highest posterior density interval (HPD 2012-2017), while the TMRCA for the entire African sequences was around 2004 (HPD 2000-2006). This is quite recent and might not actually represent the true picture of when the virus was introduced into circulation because of the paucity of sequence information and regency of the data presented. However it shows the gap in knowledge posed by insufficient molecular data on this virus in Africa. In addition the calculated evolutionary rate of the African Orf virus B2L genes was 7.45 x 10^{-4}, 95% HPD (3.46 x 10^{-4} to 1.17 x 10^{-3}) substitutions/ site/year. This is higher than the calculated evolutionary mean rate of 1.32 x 10^{-6} derived for whole Orf gene sequences from a recent study[23] . This observation might be due to the small genome size analyzed, as well as the limited number of sequences, but reelects the relatively slower evolutionary rate for DNA viruses in comparism to RNA viruses. The viral demographic history portrays a steady population demography throughout the years past, except for a slight increase towards the year 2020, around 2016 (Fig. 3b). This observation should be taken with caution as it may not represent the true picture because of the lack of sequence data from Africa before the year 2000, however the rise in viral population, towards the more recent years shows an increase in detectable cases reflected by more sequence information being made available in various databases.

Table 2 shows a summary of major amino acid mutations observed among our B2L ORFV sequences in relation to the prototype vaccine strain (Accession number AY278208.1). The mutations observed are concomitant with that of a previously isolated strain reported in Sokoto State. The phenotypic properties of these mutations have not been well described, but some of them may affect the pathogenicity of the isolates[24] . Limitations of this study are paucity of African ORFV genomic data, and the small sample size.

In conclusion, we report the genetic diversity and molecular evolution of ORFV in Africa based on B2L sequences. Further, we highlighted the epidemiology of ORFV among goats and sheep sold in Northern Nigeria. The impact of trans-border movement of farm animals such as sheep and goats on the transmission pattern of trans-boundary disease such as ORFV, and have illuminated the information gap particularly genetic information of this virus in Africa. We hereby recommend a regional approach to molecular surveillance of ORFV and other zoonotic trans-boundary animal diseases to help in prevention and control effort.

Figure Legends

Fig. 1: Map showing locations were samples were collected in Nigeria and where animal were imported from Republic of Niger.

Fig. 2: Maximum likelihood phylogenetic tree of African ORFV B2L genes including global reference sequences. Blue lines represent study sequences, and other Nigerian isolates, red lines represent South African sequences, green lines represent Egyptian isolates. Light blue box represents the Asian lineage, light green box represents isolates within the African lineage.

Fig. 3a. Bayesian MCC tree showing African isolates, countries are represented by the color code the figure legend. Light blue box represents isolates within the African lineage, light green box represents isolates within the Asian lineage. **3b** . Population demography of African ORFV B2L genes represented by a Bayesian skyride plot showing effective viral population on the y axis and sampling time on the x-axis.

Author's Contributions

OAB designed the study and carried out molecular identification;AO, AAMcollected samples; OAB, AO, AAM, AA funded sequencing of the amplified products. OCAperformed data analysis,MBperformed bioinformatics analysis. IScleaned sequences, carried out the phylogenetic analysis OAB, AO, AAM wrote the initial draft. All authors reviewed and approved the final manuscript before submission.

Funding

This work was supported by grant award number D43TW010140 funded by Fogarty International Centre

of the National Institutes of Health to the University of Ibadan Medical Education Partnership Initiative Junior Faculty Project (UI-MEPI-J).

Data Availability Statement

The data that support the findings of this study are freely available in GenBank, reference numbers MW916908-MW916918.

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