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A survey of contagious ecthyma and molecular characterization of Orf virus in sheep and goats in Nigeria (2014–2016)

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Abstract

Background: Outbreaks of contagious ecthyma (CE) are frequently reported in sheep and goat flocks in Nigeria with severe clinical outcomes. CE is a debilitating and economically important disease primarily affecting sheep and goats caused by the Orf virus (ORFV). Despite field reports of CE in the country, there is no concise country-wide epidemiological data on the disease and limited genetic data of circulating Nigerian ORFV are available in the public domain.

Aim: An epidemiological survey of CE and molecular characterization of ORFV circulating in Nigeria from 2014 to 2016.

Method: Data were collected using designed questionnaires, administered to veterinarians and farmers in selected States of Nigeria. Samples were collected during passive surveillance for CE from 2014 to 2016 which were analyzed by polymerase chain reaction (PCR). The *A32L* and *B2L* genes of circulating ORFV were also characterized.

Results: Analysis of the questionnaire showed that 69.54% (n = 82/118) of the farmers claimed to have experienced CE in their flocks with average morbidity and mortality rates of 25% and 15%, respectively. A total of 113 veterinarians participated in the study, with 69.9% (n = 79) familiar with CE and claimed CE causes morbidity rates of 25%–37% and mortality rates of 10%–15% in sheep and goats. Laboratory results revealed that ORFV was detected in 72% (18/25) of outbreak samples analyzed by real-time PCR. Phylogenetic analysis of *A32L* and *B2L* genes revealed that Nigerian ORFV sequences belong to clusters I and II and are similar to viruses from India, Ethiopia, and China.

Conclusions: This study is the first nationwide epidemiological data on the status of CE in sheep and goats in Nigeria. It is also the first report of molecular characterization of two genes of ORFV circulating and causing outbreaks in small ruminants in the country. This study showed that CE is under-reported, widespread and of economic importance to sheep and goat farmers in Nigeria.

Keywords: *A32L* gene, *B2L* gene, Contagious ecthyma, Orf virus, Small ruminants.

Introduction

Contagious ecthyma (CE) or Orf is a debilitating and economically important disease of sheep and goats caused by the epitheliotropic Orf virus (ORFV) a member of the genus *Parapoxvirus* and family *Poxviridae* (Spyrou and Valiakos, 2015). The ORFV primarily affects sheep and goats but has been reported in cattle, camels, and wild animals (Nandi *et al*., 2011; Adedeji *et al*., 2018a; Şevik, 2019). The genome of ORFV is linear double-stranded DNA with an estimated size of 134–139kb and it is the smallest member within the subfamily *Chordopoxvirinae* (Friederichs *et al*., 2014). Genetically, the *A32L* and *B2L* genes encode highly conserved segments of the ORFV, which have been used for detection and phylogenetic studies of the virus (Chan *et al*., 2009). The *A32L* gene of ORFV encodes an ATPase that mediates virion DNA packaging, while the envelope gene (*B2L*) is the highly immunogenic protein of the virus (Gelaye *et al*., 2016). CE is a zoonotic disease and occupational hazard to farmers, butchers, and veterinarians handling livestock (Essbauer *et al*., 2010; Veraldi *et al*., 2019; Andreani *et al*., 2019). Clinically, the disease is characterized by fever, papules, pustules, and thick tenacious scabs at oral commissures, lips, mouth, nose, ears, legs, genital region, and udders (Nandi *et al*., 2011). These skin lesions become ulcerative and painful, with the affected animals unable to either feed or walk, leading to weight loss and reduced productivity (Bala *et al*., 2018). However, CE is also characterized by high morbidity and low mortality, but, in young animals, mortality is high due to the inability to suckle (Windsor *et al*., 2017). ORFV is transmitted by contact through damaged skin during grazing and abrasions developed

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on the lips, nostrils, and mouth caused by dried feeds or plants (Spyrou and Valiakos, 2015). The ORFV is distributed worldwide, particularly in sheep and goat-producing countries (Nandi *et al*., 2011; Bala *et al*., 2018).

Diagnosis of CE is based on clinical signs and laboratory techniques such as electron microscopy, viral isolation, and molecular assays (Torfason and Guðnadóttir, 2002; Kottaridi *et al*., 2006; Chan *et al*., 2009).

Since the first report of CE in Nigeria in 1978, reported outbreaks of the disease have resulted in economic losses to sheep and goat farmers in the country (Odo, 2003; Adedeji *et al*., 2018b). Despite, reports of CE outbreaks in some parts of Nigeria with varying economic losses (Adedeji *et al*., 2017; Ifende *et al*., 2019). There is a paucity of epidemiological data on the disease nationally and limited genetic sequences of the circulating ORFV in Nigeria are available in the public domain. Albeit, only one sequence from one gene of ORFV circulating in Nigerian small ruminants flock has been deposited in the public domain or reported (Lawal *et al*., 2021).

Therefore, an epidemiological survey was carried out to determine the status of CE in Nigeria and characterized the circulating ORFV causing outbreaks.

Materials and Methods

Study area

Nigeria is located in West Africa and divided into 36 states. There are five major agro-ecological zones in the country consisting of humid (rainforest), sub-humid (southern Guinea savannah, Northern Guinea Savannah), semi-arid (Sudan Savannah), and arid (Sahel Savannah) zones (Fadiga *et al*., 2013). There are two main climatic seasons in Nigeria, namely; Rainy season (May to September) and dry season (October to April). The rainy season extends to October-December in the southern coastal states of the country (Fadiga *et al*., 2013).

Nigeria has a population of 42 million sheep and 73 million goats (Bolajoko *et al*., 2019). Keeping sheep and goats is an integral part of the lives of rural households in Nigeria. These animals are kept for food, source of income, and religious purposes and serve as "bank" for saving money (Bolajoko *et al*., 2019). Most of the sheep and goats in Nigeria are reared under an extensive or semi-intensive system of management. The common breeds of sheep are Yankasa, Udah, and Balami reared mostly in sub-humid, semi-arid, and arid agro-ecological zones. The West African dwarf (WAD) sheep are reared mostly in humid zones (Ngere *et al*., 1984; Fadiga *et al*., 2013). In contrast, the common breeds of goats in Nigeria are Sahel, Sokoto Red and Kano Brown found in the sub-humid and semi-arid and arid agro-ecological zones. While WAD goats are mostly kept in the sub-humid and humid zones (Fadiga *et al*., 2013; Yusuf *et al*., 2018).

Field data collection

In this study, epidemiological data on CE was collected by administering standardized and pretested questionnaires to veterinarians, and farmers using interview, conducted in the selected study areas (Fig. 1). For veterinarians, the questionnaires were administered at their Annual National Conference in November 2016. Out of 130 questionnaires administered, 113 veterinarians from 25 of the 36 states of Nigeria returned their filled questionnaires (Fig. 1). While 118 farmers from 7 states in Nigeria participated in the study (Fig. 1), the interviews were conducted between February and May 2017. Purposive sampling methodology was used, and the availability of the veterinarians and farmers to be interviewed was utilized. The questionnaire for veterinarians focused on diagnosis/identification of CE, age, sex, breeds of sheep and goats affected by CE, and season of the year with the highest incidence of CE. Other information obtained were the cost of management, morbidity rates, mortality rates, and economic importance of CE. Information from livestock farmers was collected using structured interviews with the aid of pictures showing lesions of CE for easier understanding. The questions were translated into local languages as appropriate. The interview focused on the livestock management system, familiarity with CE, age, and occurrence of CE outbreak in their flock in the last year before the interview. Other questions include factors associated with CE outbreaks, morbidity/mortality rates and breeds of the sheep and goats mostly affected by CE. Questions were also asked on the economic importance of disease such as the effect of CE on the trade value of affected animals.

Sample collection

Scab samples were collected from suspected CE outbreaks in different parts of Nigeria (Fig. 1). Twenty-five samples were submitted from 2014 to 2016, and samples from the same flocks were pooled together. The samples were collected from a livestock market, abattoirs, and backyard smallholder flocks from five states in Nigeria (Fig. 1). The samples were submitted to the Virology Division, National Veterinary Research Institute, Vom, Nigeria for laboratory analysis.

Laboratory analysis

The scab samples were homogenized, and genomic DNA extracted using QIAamp® DNA Mini kit (QIAGEN, Hilden Germany) following the manufacturer's instructions.

ORFV freeze-dried vaccine (Onderstepoort, South Africa) was used as positive control and subjected to DNA extraction. All the samples were analyzed using a polymerase chain reaction (PCR) assay for the detection of eight poxviruses of livestock based on high-resolution melting curve analysis (HRMCA) (Gelaye *et al*., 2017). The panel of controls were ORFV, Pseudocowpox virus (PCPV), GTPV, SPPV, Lumpy

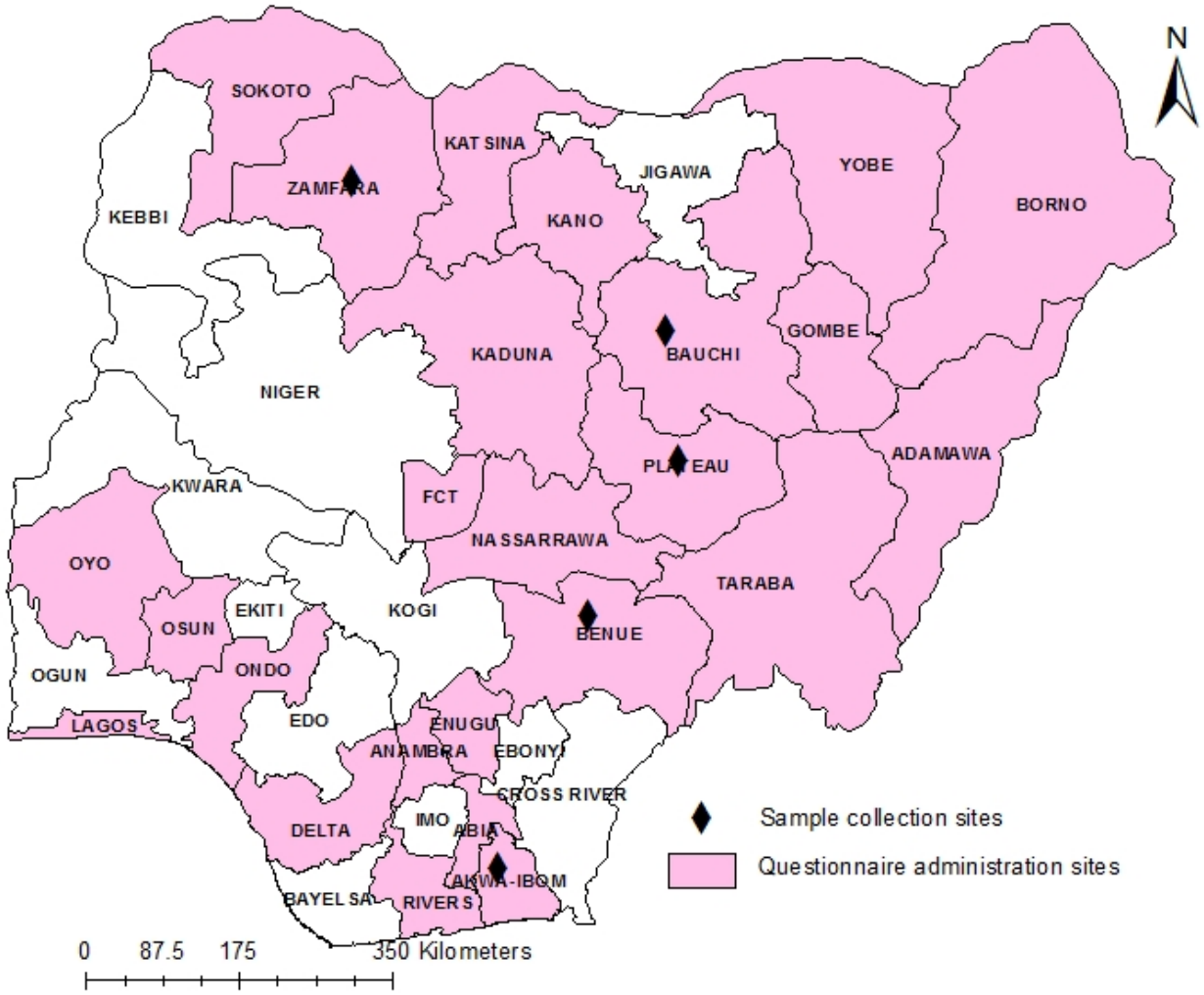

Fig. 1. Map of Nigeria showing states where the questionnaire survey was carried out and samples collected.

skin disease virus (LSDV), Bovine papular stomatitis virus, Cowpox virus (CPXV), and Camel pox virus. The positive control panels for the PCR assay were provided by the Animal Production and Health Section, Joint Food and Agriculture Organization-International Atomic Energy Agency, Seibersdorf, Vienna, Austria. Positive samples were characterized by amplification and sequencing of *A32L* and *B2L* genes fragments of the ORFV using primers as previously described (Gelaye *et al*., 2016).

Data analysis

Data obtained from the questionnaires were entered into a spreadsheet and analyzed using descriptive statistics and tabulated.

Phylogenetic analysis of A32L and B2L genes of ORFV

Eight ORFV positive samples were selected showing spatial temporal spread for molecular characterization targeting two genes of the virus (Table 1). The *A32L* gene of ORFV encoding an ATPase that mediates virion DNA packaging and the *B2L* envelope gene which are the highly immunogenic proteins were charactered using the Sanger sequencing method (Chan *et al*., 2009; Gelaye *et al*., 2016). ORFV sequences were generated commercially by Macrogen Inc. (South Korea). The raw sequence data were assembled using Staden Package (http://staden.sourceforge.net/) and multiple alignments were performed using the MUSCLE condon algorithm implemented in MEGA X (Kumar *et al*., 2018). Sequences of the two gene segments of ORFV were compared to available sequences in the GenBank using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast) at default settings. MEGA X was used for phylogenic analysis by Neighbor-joining tree inferred using *p-distance method* and data resampled 1,000 times in bootstrap method (Kumar *et al*., 2018).To construct the phylogenic trees, 13 *A32L* and 15 *B2L*gene sequences representing the two known ORFV clusters as well as

Table 1. List of Nigerian ORFV from field outbreaks and their GenBank accession numbers.

Lab No	Location	Year of sample collection	Breed/species	History/clinical signs observed	Type of sample collected	GenBank accession number	
						B2L	*A32L*
KR45	Kuru, Plateau	2016	WAD goat	Proliferative skin lesion around the mouth	Skin scab	MG757228	MG757220
UY54E	Uyo, Akwa Ibom	2014	WAD goat	Proliferative skin lesion around the mouth, nose and ear. Enlarged superficial lymph nodes	Skin scab	MG757229	MG757221
BA74C	Bauchi, Bauchi	2016	Kano brown goat	Female, 3 years, Proliferative skin lesion around the mouth, nose and ear.	Skin scab	MG757230	MG757222
KU77A	Kuru, Plateau	2016	Kano brown goat	Male, 3 years, Proliferative skin lesion around the mouth, nose, scrotum and hoof	Skin scab	MG757231	MG757223
GMB80B	Gamawa, Bauchi	2016	Kano brown goat	Male, 3 years, Pox like lesions around the mouth and nose	Skin scab	MG757232	MG757224
MK83A	Makurdi, Benue	2015	WAD goat	Proliferative skin lesion around the mouth and nose	Skin scab	MG757233	MG757225
JS103A	Jos, Plateau	2016	WAD goat	Male, adult, scab skin lesion around the mouth and nose	Skin scab	MG757234	MG757226
JO118	Jos, Plateau	2016	Yakansa, sheep	Female, adult proliferative skin lesion on the back	Skin scab	MG757235	MG757227

the sequences of BSPV and PCPV were retrieved from GenBank, and listed in Table 2 (Gelaye *et al*., 2016). The nucleotide sequences generated in this study were deposited in the NCBI GenBank sequence database with accession numbers MG757220–MG757227 for the *A32L* gene and MG757228–MG757235 for the *B2L* gene (Table 1).

Ethical approval

This study was approved by National Veterinary Research Institute Animal Ethics Committee (AEC/03/58/18).

Results

Questionnaire data analysis

Sheep and goats farmers interviewed were from seven states of Nigeria (Akwa Ibom, Bauchi, Kano, Kaduna, Plateau, Yobe, Zamfara) (Fig. 1). The farmers interviewed were of ages 30–80 years old and 92.4% (*n* = 109/118) of them practice extensive husbandry system of livestock of farming (Table 3). Data from the survey also revealed that 69.54% (*n* = 82/118) of the farmers were familiar with CE and had observed CE outbreaks in their flocks at least once. Additionally, 31.4% (n = 37/118) of the farmers said they had observed cases of CE in their flocks in the last year before the interview was conducted. Also, 69.54% (*n* = 82/118) of the farmers indicated that CE is a disease of economic importance in Nigeria. Similarly, the analyzed data showed that 60.1% (*n* = 79/118) of the farmers indicated that CE affects both young and adult animals, while 69.54% (*n* = 82/118) of farmers claimed CE affects sheep and goats of both sexes. Furthermore, 60.1% of the farmers claimed that CE occurs during both dry and rainy seasons. Regarding breed of goats mostly affected, 58.5% (n = 69/118, Table 3) stated that WAD as the breed of goats mostly affected by CE. At the same time, all the respondents did not identify any breed of sheep as most susceptible to CE. Furthermore, 24.6% (*n* = 29) of farmers claimed CE affects the

Table 2. List of *B2L* and *A32L* gene sequences of ORFV and other parapoxviruses used for phylogenetic analysis.

GenBank accession number	Country	Year of collection	Specie	Gene	References
AY386263	USA	1982	Sheep	*A32L*	Yogisharadhya *et al*., 2012
JN183066	India	2004	Sheep	*A32L*	Yogisharadhya *et al*., 2012
JN183069	India	2010	Goat	*A32L*	Yogisharadhya *et al*., 2012
JN183074	India	2005	Goat	*A32L*	Yogisharadhya *et al*., 2012
JN183075	India	2010	Sheep	*A32L*	Yogisharadhya *et al*., 2012
JN183076	India	2008	Sheep	*A32L*	Yogisharadhya *et al*., 2012
EU327509	Taiwan	2006	Goat	*A32L*	Yogisharadhya *et al*., 2012
KT438533	Ethiopia	2012	Sheep	*A32L*	Gelaye *et al*., 2016
KT438538	Ethiopia	2012	Sheep	*A32L*	Gelaye *et al*., 2016
KT438539	Ethiopia	2008	Sheep	*A32L*	Gelaye *et al*., 2016
KT438543	Ethiopia	2012	Sheep	*A32L*	Gelaye *et al*., 2016
AY386264	USA	NA	Goat	*A32L*	Delhon *et al.* 2004
DQ184476	New Zealand	NA	Sheep	*A32L*	Mercer *et al*., 2006
GQ329670	Finland	NA	Cattle	PCPV(*A32L*)	Hautaniemi *et al*., 2010
AY386265	USA	NA	Cattle	BSPV(*A32L*)	Delhon *et al*. (2004)
DQ263303	India	2004	Sheep	*B2L*	Hosamani *et al*., 2006
MH790951	India	2005	Goat	*B2L*	Yogisharadhya *et al*., 2012
JN088052	Brazil	1992	Sheep	*B2L*	NA
KF703747	China	2013	Goat	*B2L*	Yang *et al*., 2014
DQ263305	India	2004	Sheep	*B2L*	Hosamani *et al*., 2006
KT191487	India	2013	Black buck	*B2L*	Sharma *et al*., 2016
JN565694	China	2011	Goat	*B2L*	Li *et al*., 2013
JN846834	India	2009	Goat	*B2L*	Bora *et al*., 2012
HQ694772	China	2009	Sheep	*B2L*	NA
GQ328006	South Korea	2009	Goat	*B2L*	Oem *et al*., 2009
KC568396	China	2012	Goat	*B2L*	Chi *et al*., 2013
KT438513	Ethiopia	2012	Goat	*B2L*	Gelaye *et al*., 2016
KT438521	Ethiopia	2008	Sheep	*B2L*	Gelaye *et al*., 2016
MF462346	India	2017	Sheep	*B2L*	Nazir *et al* (Unpublished)
MW748471	Botswana	2017	Sheep	*B2L*	Modise *et al* 2021
AY424972	USA	2003	Bovine	PCPV (*B2L*)	Guo *et al*., 2004
AY424973	USA	2003	Bovine	BSPV (*B2L*)	Guo *et al*., 2004

trade price of animals with the disease. Farmers also indicated that the average morbidity and mortality rates of CE were 25% and 15% respectively during outbreaks in sheep and goats. Additionally, 37.2% (n = 44) of farmers claimed that CE causes skin infections in humans. A total of 113 veterinarians participated in the questionnaire survey from 25 states of Nigeria (Table 3). Amongst the veterinarians that participated in the study, 69.9% (n = 79) were familiar with CE. In comparison, 45.1% (n = 51) said CE is of economic importance with average morbidity of 25% in sheep and 37.5% in goats, respectively, as well as average mortality of 10% in sheep and 15% in goats. Concerning breed of goats, 29.2% (n = 33) of veterinarians claimed the WAD as the most affected goats while others indicated Red Sokoto (25.6%, n = 29) and Sahel (17.6%, n = 20) as other breeds affected by CE in Nigeria. The data collected from the questionnaire survey revealed that 47.7% (n = 54) of veterinarians indicated that CE affects sheep and goats of all ages, while 61.9% (n = 70) said CE affects both male and female animals. Regarding the season, 30.9 % (n = 35) of veterinarians stated that CE occurs

Table 3. Summary of epidemiological data collected from questionnaire survey on CE of sheep and goats in Nigeria.

Variable	Farmers *n*=118 (%)	Veterinarians *n*=113 (%)
Age of farmers	30–80 years	NA*
Husbandry system		
Extensive	109 (92.4%)	NA
Intensive	9 (7. 6%)	NA
Familiar with CE	82 (69.54%)	79 (69.9%)
Observed cases of CE in farmers' flocks at least once	82 (69.54%)	NA
Observed cases of CE in farmers' flocks in the last 1 year	37 (31.4%)	NA
CE is disease of economic importance	82 (69.54%)	51 (45.1%)
Affects both young and adult animals	79 (60.1%)	54 (47.7%)
CE affects both sexes	82 (69.54%)	70 (61.9%)
Cases of CE observed mostly in both season of the year	79 (60.1%)	35(30.9%)
Breeds observed with cases of CE:		
Goats		
All breeds	-	18 (15.9%)
WAD	69 (58.5%)	33 (29.2%)
Sahel	-	29 (25.6%)
Sokoto Red	-	20 (17.6%)
No response	49 (41.5%)	
Sheep		
All breeds	79 (60.1%)	31(27.4%)
Yankasa	-	18 (15.9%)
Udah	-	14 (12.4%)
Balami	-	11 (9.7%)
No response		39 (34.5%)
Affects trade value of sheep and goat	29 (24.6)	-
Cause skin infections in humans	44 (37.2%)	-
Average morbidity rate	25%	25%–37.5%
Average mortality rate	15%	10%–15%

NA: Not applicable; CE: Contagious ecthyma.

mainly during the rainy season. The veterinarians' questionnaire survey also revealed that the average cost of managing a CE outbreak is estimated to be $2 per animal (sheep or goat).

Field observations and laboratory results

During field investigation of CE outbreaks, clinical signs observed were proliferative scab lesions around the mouth, noses, mammary glands, and hoofs (Table 1, Fig. 2A–D). Twenty-five samples were collected from sheep and goats in the following states of Nigeria, namely, Akwa Ibom, Benue, Bauchi, Plateau and Zamfara (Fig. 1, Table 4). The samples were collected from Kano Brown (Fig. 1 A, B, and D) and WAD breeds of goats and Yankasa sheep, (Fig. 2C). ORFV was detected by multiplex Real-time PCR in 72% (18/25) of the samples analyzed. SPPV, GTPV, LSDV, PCPV, and CPXV were not detected by the multiplex Real-time PCR in the samples analyzed.

Phylogenetic analysis of A32L and B2L genes of ORFV

Phylogenetic analysis of *A32L* gene segment of Nigerian ORFV showed 7 Nigerian ORFV *A32L* gene sequences (KR45, UY54E, BA74A, KU77A, MK83 and JS103) with 96%–98% similarity and clustering in Cluster I, sub-cluster I and II (Fig. 3). Meanwhile, ORFV sample JO118 grouped Cluster I together with ORFVs from Ethiopia, India and Taiwan.

A32L gene Sub-cluster II of Cluster I was the dormant and widespread ORFV in Nigeria between 2014 and 2016, detected in Akwa Ibom, Bauchi, Benue, and Plateau states (Fig. 3). The *B2L* gene segment of seven of Nigerian ORFV isolates (KR45, UY54E, BA74A, KU77A, MK83, and JS103) had 97%–99% similarity. The Nigerian ORFV isolates grouped with Cluster II when inferred on the phylogenic tree with ORFVs from Brazil, Ethiopia, and South Korea (Fig. 4). While, ORFV isolate JO118 grouped with Cluster I together with ORFVs from Botswana, China Ethiopia, and India.

Discussion

This study is the first nationwide epidemiological data collection on the status of CE in sheep and goats

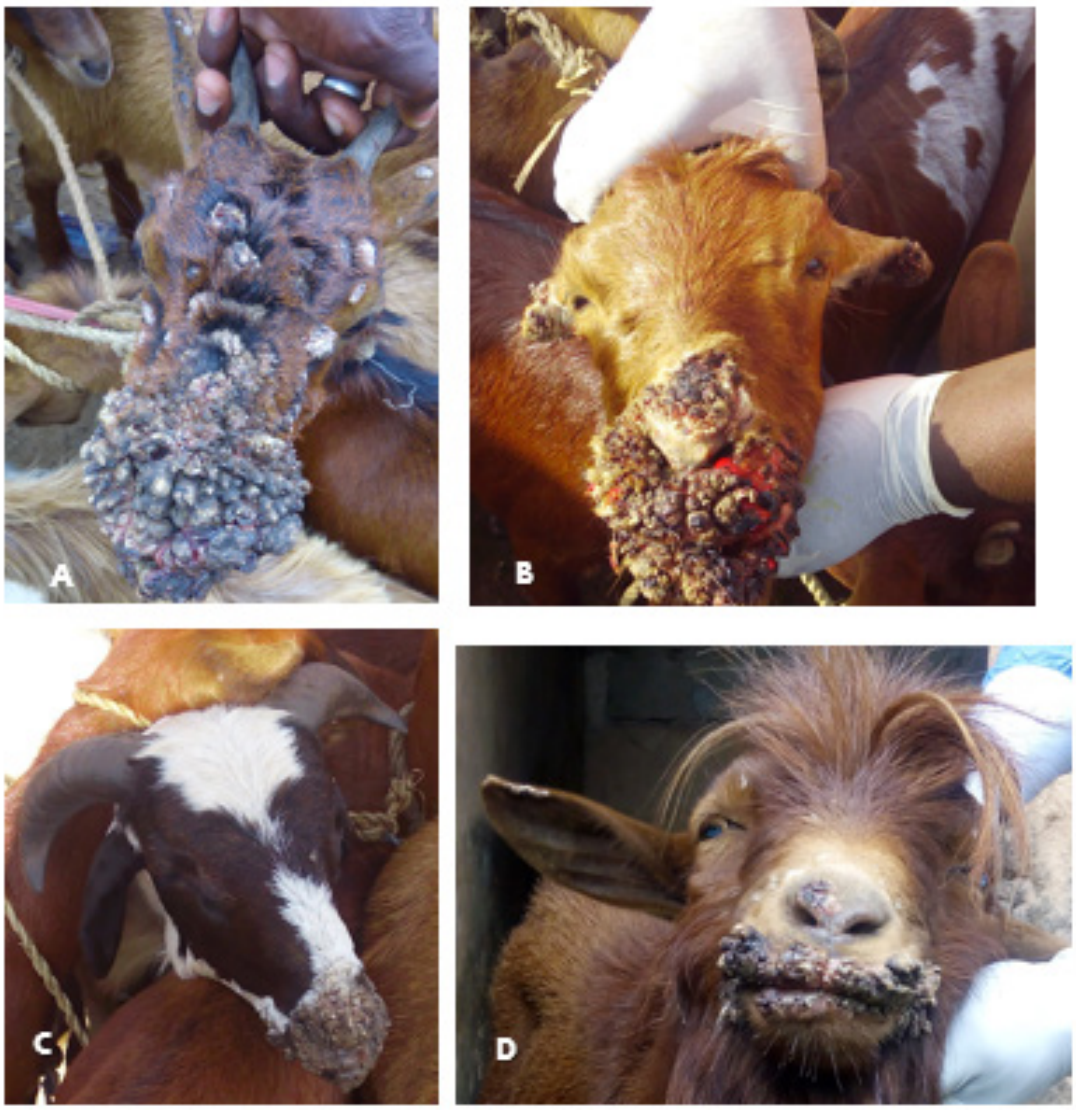

Fig. 2. (A): Proliferative lesions of CE around the mouth, nose and eyes of a Kano brown goat at a livestock market in Bauchi, Bauchi state. (B): Proliferative lesions of CE around the mouth, nose and eyes of a Kano brown goat in Bauchi, Bauchi state. (C): Scab lesions of CE on the mouth and nose of Yankasa ram in Gamawa, Bauchi state. (D): A Kano brown with proliferative lesions of CE in Kuru, Plateau.

Table 4. Distribution of samples collected during CE outbreaks and laboratory results in five states of Nigeria from 2014 to 2016.

Location	Number of outbreaks	Samples collected	Number positive samples
Akwa Ibom state	2	2	2
Bauchi state	10	10	7
Benue state	1	1	1
Plateau state	12	12	5
Zamfara state	1	1	0
Total	25	25	17

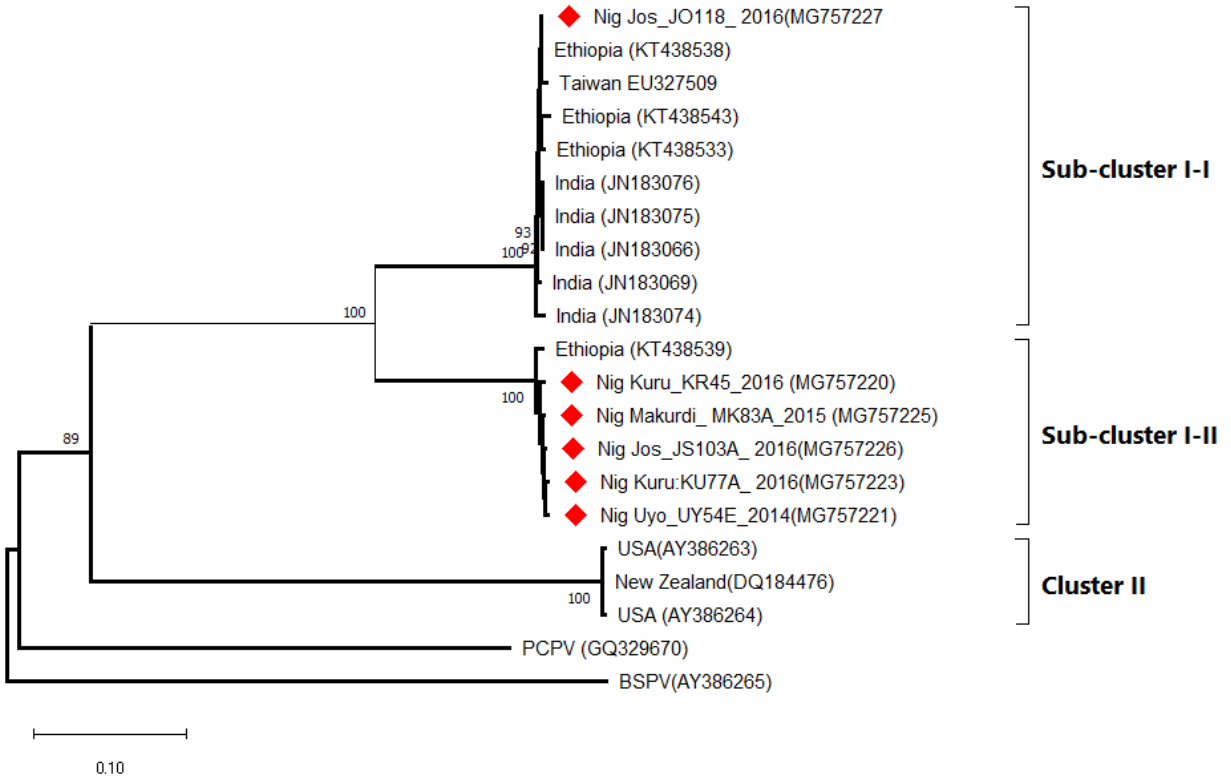

Fig. 3. The Phylogenic tree of *A32L* gene of the ORFV samples collected from outbreaks in Nigeria. The Nigerian ORFV samples are marked with red. The tree was constructed using the Neighbor-joining method at 500 bootstrap.

in Nigeria. It is also the first report of molecular characterization of two genes of ORFV circulating and causing outbreaks in small ruminants in the country. Previously, only case reports and studies at the regional level were documented for CE in Nigeria without a countrywide concise study of the disease (Adedeji *et al*., 2017; Ifende *et al*., 2019). In this study, most veterinarians (69.9%), farmers (69.4%), and butchers (64.03%) that participated in the questionnaire survey were familiar with CE, which suggests that cases of the disease frequently occur in various parts of the country. It also implies that CE outbreaks may be widespread in Nigeria. Interestingly, based on findings in this study, a higher percentage of farmers considered CE a disease of economic importance compared to veterinarians (Table 3). Also, farmers claimed average mortality rates of 15% as a result of CE outbreaks in flocks of sheep and goats which is high, particularly for poor rural communities in Nigeria that depend on livestock for their livelihood. Recent studies in parts of Nigeria and Laos reported mortality rates of 33%–100% in smallholder goats flocks (Windsor *et al*., 2017; Adedeji, *et al*., 2018b). This further suggests that CE may be causing greater economic losses, but is underreported by farmers and neglected by relevant authorities. Besides, CE incidence rates of 10%–90% have also been reported in some parts of the world (Bala *et al*., 2018). Concerning the economic importance of CE, majority of farmers and veterinarians interviewed stated that CE is of economic importance. A study conducted in Nigeria also reported that traders and farmers agreed that CE affects the trade prices of affected sheep and goats (Gambo *et al*., 2018). In rural communities in Nigeria, it is common practice for livestock farmers to quickly sell their sick animals directly to livestock traders or butchers to mitigate losses during disease outbreaks (Bolajoko *et al*., 2019). In another study in Nigeria, 7,258 cases of CE in sheep and goats were reported during ante mortem inspections over 9 years at an abattoir (Ifende *et al*., 2019). This finding suggests that farmers sell animals showing clinical signs of CE to butchers for slaughter without reporting to relevant veterinary authorities. Most veterinarians in this study claimed that CE affects goats more than sheep, which agrees with a report in South Africa with a similar finding (Scagliarini *et al*., 2012). In addition, veterinarians and farmers claimed a higher incidence of CE occurs in WAD goats than other breeds of goats in Nigeria. Likewise, our earlier study in Nigeria suggested a similar finding (Adedeji *et al.,* 2018b). The laboratory result confirms that CE outbreaks occur in Nigeria based on analysis with the multiplex real-time PCR. Due to the possibility of co-infections and the similarity of CE with other poxvirus infections of sheep and goats, a multiplex real-time PCR was used to analyse the samples. All the samples were negative for sheep pox and goat pox-viruses. The ORFV was detected in samples collected

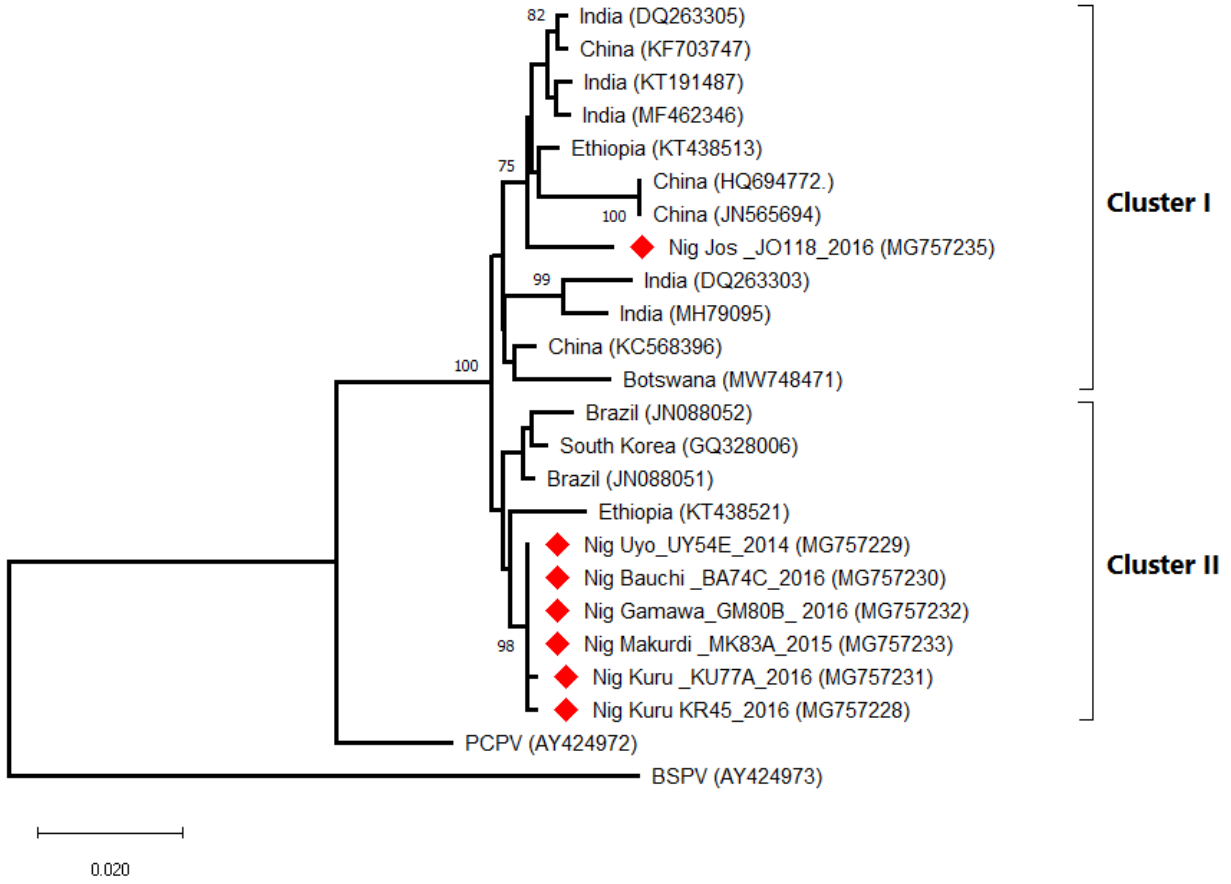

Fig. 4. The Phylogenic tree of the *B2L* gene of the ORFV collected from outbreaks in Nigeria. The Nigerian ORFV samples are marked with red. The tree was constructed using the neighbor-joining method at 1,000 bootstrap.

from four out of five states. Before this study, CE had not been reported in Bauchi and Benue states of Nigeria. The phylogenetic analysis of the *A32L* and *B2L* gene showed the diversity of the ORFV circulating in Nigeria, which clustered with sequences of ORFV from Brazil, Ethiopia, China, and India (Figs. 3 and 4). All the Nigerian ORFV isolates in this study cluster together apart from sample JO118_Nig, which was collected at a livestock market in Jos Plateau State. ORFV belonging to cluster I, sub-cluster II, and cluster II based on the two gene sequences analysed were the most dominant strain of the virus circulating in Nigeria. This is the first report of the phylogenetic analysis of two gene fragments of ORFV circulating in Nigeria. Although CE is not a notifiable disease, however, based on the data collected in this study, farmers have expressed concern about the impact of the disease on their flocks.

Conclusions

This study provides the first concise country-wide epidemiological data on CE in sheep and goats in Nigeria. It is also the first molecular characterization of two gene fragments of ORFV circulating and causing outbreaks in small ruminants. Findings from this study suggest that CE is an economically important disease of sheep and goats in Nigeria. It is, therefore, recommended that further studies be undertaken to investigate the impact of the disease on sheep and goat farming in the country.

Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

AJA, MBB TYW, and VII designed the study, AJA, JAA, AAG, and NAM collected the data and samples. AJA, MBB, AJ, JAA, NCC, YSW, and PDL analysis data and samples. AJA, OOA, PDL, TYW wrote the manuscript. All authors read and approved the manuscript.

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