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Molecular Based Detection, Validation of a LAMP Assay and Phylogenetic Analysis of Capripoxvirus in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TH and J. Kasiiti designed and managed the analysis of the study. Author MM wrote the protocol. Author DCAO carried out the study and wrote the first draft of the manuscript. Authors EM and J. Kinyua managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To optimize the different available molecular based techniques, validate the Loop-mediated isothermal amplification (LAMP) assay for detection of Capripoxviruses (CaPVs) DNA and deep sequencing to confirm the results of the LAMP assay and ensure that an effective and reliable detection method is recommended and adopted for use in Capripox diagnosis. The phylogenetic analysis was also to determine CaPVs genome variability, evolution, and diversity.
Study Design: It included sample collection, DNA extraction, analysis and sequencing.
Place and Duration of Study: Biosciences East and Central Africa-International Livestock Research Institute **(**BecA-ILRI) Hub, between May 2014 and March 2015.
Methodology: We analyzed 130 samples including blood, skin nodules and cell cultures from

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symptomatic and suspected sheep, goats and cattle. The samples were tested by, conventional PCR, LAMP assay and real time PCR as the gold standard method, the results obtained were compared and used to determine sensitivity and specificity. The Limit of detection (LOD) of real time PCR and LAMP assay was determined using a serially diluted positive sample. Deep sequencing of 18 LAMP positive samples from different parts of the country was done and the obtained sequenced data analyzed using bioinformatics platform.

Results: The disease prevalence in the population was 61%, The validated LAMP assay had a 97% sensitivity and a specificity of 100%. Using the Fisher's Exact Tests, a P-value of (P = .8) at 95% interval was obtained. This shows that there is no significant difference between the validated LAMP Assay and the gold standard method. The Phylogenetic analysis of the 3 important CaPVs genes revealed the distribution of CaPVs into the 3 major specific lineages.

Conclusion: This study supports the adoption of LAMP assays for CaPVs diagnosis as a rapid and robust method of detection, disease monitoring and controlling outbreaks. The sequence data confirms the possibility of cross infection, nonhost specificity of CaPVs and also provides a reference in genomic research and a basis for molecular epidemiological studies in Kenya.

Keywords: Capripoxviruses; phylogenetics; diagnosis; LAMP; capri pox.

1. INTRODUCTION

The genus Capripoxvirus, subfamily Chordopoxvirinae and the family Poxviridae comprise sheeppox virus (SPV), goatpox virus (GPV), and Lumpy skin disease virus (LSDV) which are important pathogens of ruminants. They cause sheep pox, goat pox and lumpy skin disease in sheep, goats and cattle respectively, which are economically important livestock diseases and are classified as notifiable animal diseases by the World Organization for Animal Health (OIE). These diseases are associated with significant production losses due to high morbidity, decreased milk production and increased susceptibility to other diseases mainly bacterial infections like Pneumonia as a result of compromised immunity, which are the direct cause of mortality [1]. Capripoxviruses (CaPVs) are classified based on the host from which the virus was originally isolated e.g. SPV from sheep, GPV from goats and LSDV from cattle [2].

The disease is transmitted by direct contact of susceptible and sick or recovered animals through skin abrasions and indirectly through contaminated fomites and transport vehicles. Inhalation of aerosols generated from lung lesions or contaminated dust and blood sucking insects like *Stomoxys calcitrans* may play a minor role in transmission [3]. CaPVs are highly resistant and may remain viable in infected tissue for at least four months, and probably longer. The virus is also present in blood, nasal and lachrymal secretions, semen and saliva, which may be sources for transmission [4]. Symptoms include pyrexia of 40-42℃, anorexia, depression, lethargy and excess lacrimation. Dermatological signs begin to develop soon after the onset and appear as round circumscribed areas of erect hair measuring between 5 to 50 mm in diameter [5]. These lesions are raised and firm and they may be surrounded by a ring of hemorrhage. The regional superficial lymph nodes are enlarged and edematous [6].

Other signs include nasal discharge and ptyalism, which is thought to be due to lesions in the nose and mouth. Lesions can be found in the respiratory tract and alimentary tract and so can cause coughing, increased respiratory rates and diarrhea [7]. The lesions are often secondarily infected by bacteria causing any discharge to be purulent and pneumonia is common sequelae of the disease. Lesions may eventually slough away to leave a hole of full skin thickness, known as "sit fast" [2].

CaPVs are enveloped brick-shaped virions with a linear dsDNA genome of about 151 kb; they are up to 96% genetically related between species and up to 99% within a given species [8]. This, therefore, makes it hard to distinguish them clinically or serologically.

LSD was first reported in Kenya in 1957 in areas of Nakuru and Baringo from a mixed farm of cattle and sheep [9] and almost at the same time SPV was isolated from sheep samples in Isiolo and Kedong Valley [10].

Lumpy skin disease being endemic in Kenya, its diagnosis has always remained a major challenge as well as the other Capri pox diseases. This is due to problems of cross-reactivity with other closely related viruses

always encountered with the readily available serological diagnostic methods like ELISA [11]. Other methods like cell cultures are more time consuming and labor intensive and can't be used for quick diagnosis [12].

Molecular diagnostic tests including Polymerase Chain Reaction (PCR) and Loop-Mediated Isothermal Assay (LAMP) offer a more reliable, sensitive and rapid way of detecting the CaPVs compared to serological methods [3,13]. Real time PCR assays are highly sensitive but require very expensive and fragile equipment that cannot be used in the field and poorly equipped laboratories [14]. LAMP is a DNA – dependent method that uses four to six primers targeting six to eight genomic regions. It utilizes the activity of a strand displacing DNA polymerase [15,16]. It is a simple, inexpensive test that can be used in the field and also in resource-limited laboratories, and this is mainly due to its isothermal nature and easy detection methods like turbidity and color change [12].

This will, therefore, provide a rapid, sensitive and reliable diagnosis of the Capri pox that can lead to effective implementation of control measures in cases of a disease outbreak.

2. MATERIALS AND METHODS

A total of 130 samples were analyzed in this study. These samples were from symptomatic animals received on a routine basis at the Central Veterinary Laboratories (CVL)-Kabete diagnostic laboratory from farmers or from reported cases in the course of the study. Blood samples were collected in EDTA tubes and also on FTA cards; organs were from postmortem sections from the pathology department of the Labs in sample bags and skin nodules in screw - cap tubes. To obtain cell cultures, CaPVs were grown on primary Lamb testis (LT) cells on Glasgow minimum essential medium (GMEM) supplemented with 10% fetal blood serum (FBE) and antibiotics at 10^3 cells /ml in 25-cm^2 tissue culture flasks. The monolayer was passaged three times before inoculation with the virus and incubation at 37℃. The infected cells were monitored for virus-induced cytopathic effects (CPE) like cell lysis which were observed at around day 13 and 14 after infection. The Lumpivax vaccine, an attenuated LSD vaccine from KEVEVAPI was also used in this study.

DNA was extracted from all the 130 samples including whole blood, serum, organs, skin nodules, LT cell cultures and the vaccine using PureLink® Genomic DNA kits (Invitrogen) according to the manufacturer's instruction and eluted in the 30 µl elution buffer. The extracted DNA was used to perform conventional PCR, LAMP assay, and qPCR. All were done in duplicates with both appropriate positive control (cloned positive CaPVs) and negative control (no- template control-water).

2.1 LAMP Assay

The LAMP assay was performed using the VP 39 primers by [17] derived from Poly (A) polymerase small subunit (VP39) which consists of four primers.

Forward primer F3:
TGGTAAGTATATTAAAACCAGCAG,

Backward primer B3:
GAATCATCCTTTGTGATGCA

Forward inner primer FIP:
TTCATTTCCGTGAGGAATATAGAAAATCTAGT TTAAAATGGCGATG

Backward inner primer BIP:
TTCAACCATTTGCGCCTAAAGCTTTATAGGAT TACCGCTA

The final working primer mix for each reaction consisted of 0.2 µM (each) F3 and B3, 2 µM (each) FIP and BIP and 7.5 µl of Optigene master mix prepared according to Manufacture's instruction. The final reaction volume used for the assay was 12.5 µl including 10 µl master mix and 2.5 µl of the template DNA in LAMP assay Optigene tubes and incubated at 65ºc for 1 hour in the genie II LAMP reader machine. The assay was performed in duplicates except the sensitivity test done in triplicate and monitored in real time by the amplification curves.

Optigene master mix which contains fast novel DNA polymerase, proprietary thermostable inorganic pyrophosphate, optimized reaction buffer, Magnesium sulphate, dNTPs and a ds-DNA binding dye (FAM detection channel).

2.2 Conventional PCR

This was done to validate and compare its sensitivity with that of the LAMP assay results. The LAMP assay, VP39 primers F3 and B3 designed to target the conserved poly (A) polymerase small subunit gene (ORF068) of the CaPVs genome were used. The PCR was carried out in 20 µl Bioneer tubes with a dye using ABI 9700 Thermocycler. The reaction master mix consisted of 10 µM (each) forward primer (F3) and reverse primer (B3), 25 mM

Magnesium chloride, 2 µl template DNA plus the required amount of nuclease-free water to adjust the volume to 20 µl. Amplification reactions were performed on the ABI 9700 Thermocycler under these cycling conditions; initial denaturation at 95ºC for 3 minutes, 35 cycles of denaturation at 95ºC for 30 seconds, annealing at 65ºC for 30 seconds, elongation at 72ºc for 30 seconds and the final extension at 72ºC for 7 minutes. All the conventional PCR assays were performed in duplicates and 2% agarose gel electrophoresis used to analyze the PCR products.

2.3 Real Time PCR

A highly sensitive quantitative Capripox TaqMan Probe Hybridization assay used for the detection of CaPVs was employed as the gold standard method for validating the LAMP assay in this study. The sequences of the primers and probes used for the real time PCR were as follows:

Forward Primer: 5'-GGCGATGTCCATCCCTG-3'

Reverse primer:
5'-AGCATTTCATTTCCGTGAGGA-3'

Probe: 5'-6FAM –CAATGGGTAAAAGATTTCTA –MGBNFQ-3'

The reaction master mix consisted of 25 µM FAM /MGB probe; 90 µM (each) forward and reverse primers, 5 µl of the template DNA, Fast Start Universal Probe master (ROX) Ref.04913957001 and 6.5 µl of nuclease-free water to adjust the volume to 25 µl. Amplification reactions were performed on an Applied Biosystems 7900HT real time PCR system using a 2 step PCR cycling profile of 95ºC for 5 min and 40 cycles of 95ºC for 15 seconds and 60ºC for 1 minute. All qPCR assays were performed in duplicates except for the sensitivity test that was performed in triplicates.

2.4 Determination of the Diagnostic Sensitivity and Specificity

The diagnostic sensitivity of the LAMP assay and qPCR used as the gold standard method was determined by preparing a serial dilution of 50 ng/µl of a positive CaPVs DNA. The dilutions were then tested in triplicates both by qPCR and LAMP assay to determine the limit of detection (LOD). The diagnostic specificity was determined by testing the CaPVs DNA alongside other closely related viruses.

The results of all the 130 samples were also analyzed to determine the sensitivity.

2.5 Next Generation Sequencing

After analyzing all the samples, 18 LAMP positive samples were selected for deep sequencing. These included 14 samples representing different regions of the country, the 3 CaPVs pure cell cultures, and the Lumpivax vaccine. Nextera XT libraries were prepared as per the Illumina Nextera XT Library preparation protocol using extracted DNA. It prepares pooled indexed end libraries genomic DNA for DNA sequencing.

The DNA concentration was determined using Qubit. Qubit solution was prepared by adding 1 µl of dsDNA Qubit broad range reagent to 199 µl of the dsDNA Qubit broad range buffer, then dispensing 200 µl of the Qubit solution to the Qubit tubes and then adding 2 µl of the DNA sample. The dsDNA BR standards were used to calibrate the Qubit machine. After determining the concentration DNA was diluted to 0.2 ng/µl. and used as the input DNA for the Nextera XT libraries. The libraries were prepared as per the Illumina Nextera DNA sample preparation Guide.

2.5.1 Quality control of the generated Nextera XT libraries

The quality of the generated Nextera XT libraries was confirmed using real time PCR. Illumina Nextera PCR cocktail primer containing both the reverse and forward primer (1 µl) and Free start universal SYBR green master mix (12.5 µl) was used with 1 µl of the prepared library. Amplification was done in 2 steps, step 1(95ºC for 600 secs.) and 20 cycles of step 2 at 95ºC for 10 mins and 60ºC for 45 seconds using the LightCycler® Thermocycler.

2.5.2 Library pooling and miseq sample loading

This was done as per the Illumina Nextera DNA sample preparation Guide and the prepared Libraries sequenced as per the Miseq System User Guide.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Molecular methods and validation of the LAMP assay

All the 130 samples were used for diagnostic analysis and different results were obtained

depending on the method. This can be attributed to the sensitivity of the assays. Real time PCR showed the highest level of detection at 60%, closely followed by LAMP assay at 59% and conventional PCR at 28% as shown in Table 1.

In conventional PCR, expected amplicons of about 199 bps were obtained for positive samples, though only 28% of the samples were positive. Fig. 1 shows a gel photo of some of the positive samples. The 2% agarose gel was stained with gel red.

The LAMP assay was run on the Optigene II LAMP machine and Fig. 2 shows the amplification curves of some of the samples plotted by fluorescence against time.

3.1.2 Sequenced data

All the 18 samples sequenced were positive for the LAMP assay and this was confirmed by the deep sequencing where the sequence analysis showed the presence of Capripoxvirus in all the samples. The samples revealed varied viral titers with the Denovo assembly of the trimmed sequenced data, and blast against a viral database. The samples with the highest viral titers were bovine samples from Kajiado (S1) and Homabay (S8) that had up to 98% of the virus sequences being specifically LSDV and the lowest being 7% with only one contig; (S4) from Kitui, the only isolate from whole blood sequenced. This, therefore, confirms the sensitivity of the LAMP assay, being able to detect very low viral load.

Table 1. Results of the 130 analyzed samples tested using different assays

Type of samples analyzed	No. of samples analyzed	Conventional PCR VP39 primers (Das et al. 2012) [17]		LAMP assay VP39 primers (Das et al. 2012) [17]		Real time PCR	
		Positive	Negative	Positive	Negative	Positive	Negative
Tissues	32	20	12	25	7	25	7
Whole blood	94	12	82	48	46	50	44
Cell cultures	4	4	0	4	0	4	0
Total	130	36	94	77	53	79	51

Prevalence of the disease: Total disease/ Total×100
79/130 × 100 = 61%

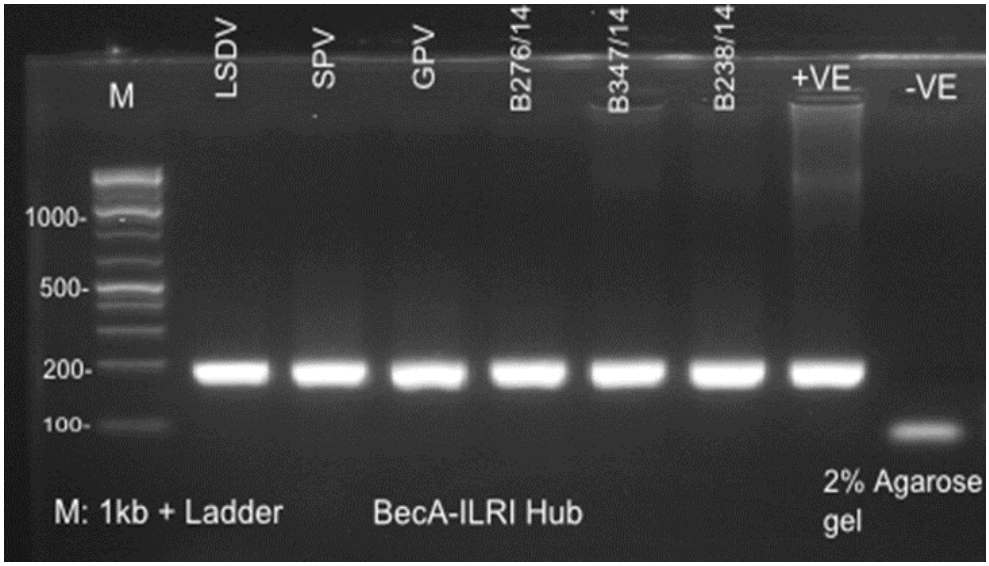

Fig. 1. Agarose gel photo showing conventional PCR amplicons

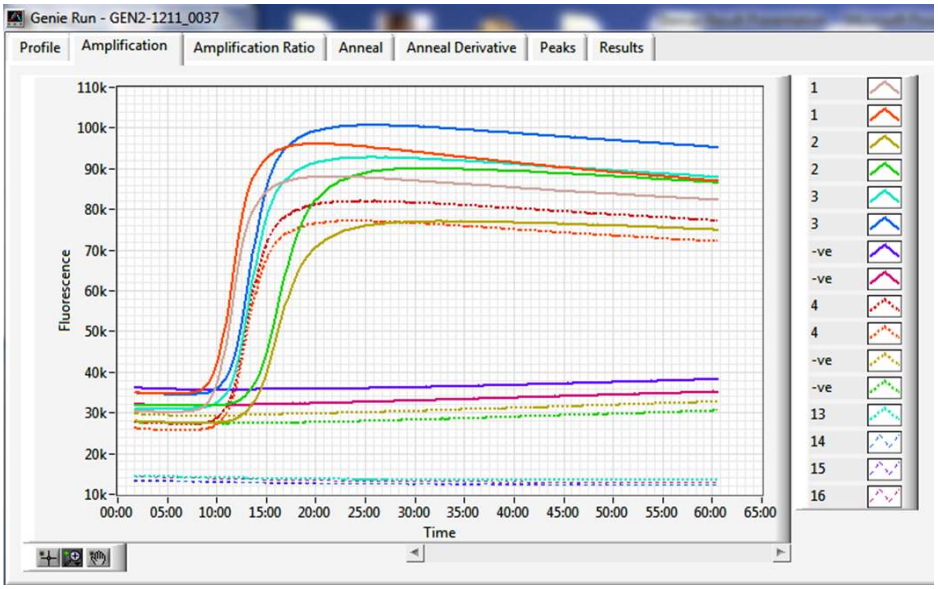

Fig. 2. LAMP results of some samples tested using the Optigene II machine

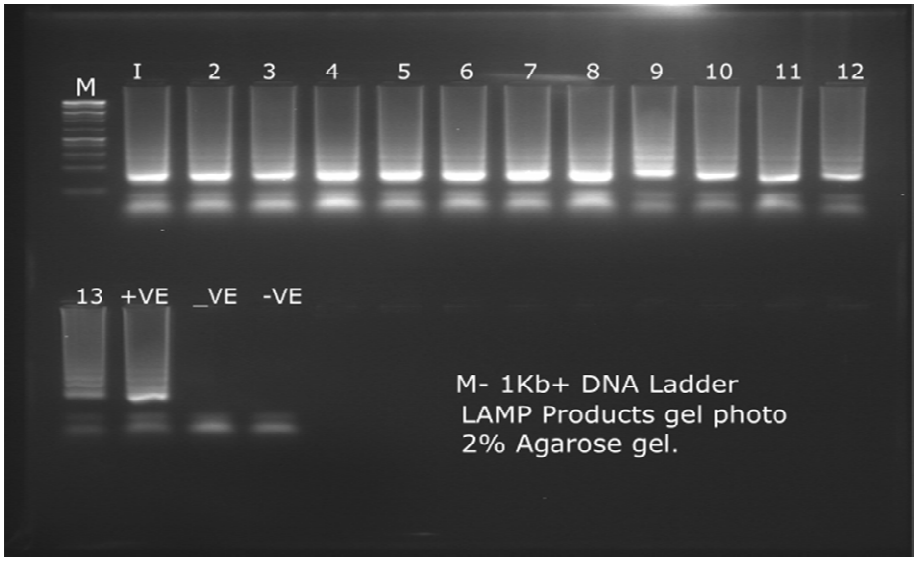

Fig. 3. A LAMP product gel photo

Table 2. Sensitivity and specificity of the LAMP assay to the gold standard method (real time PCR)

		Truth		
		Disease	**Non-disease**	**Total**
Test results	Positive	(True positive)	(False positive)	(Total test positive)
		77	0	77
	Negative	(False negative)	(True negative)	(Total test negative)
		2	51	53
		(Total disease)	(Total non-disease)	(Total)
		79	51	130

Sensitivity: True Positive/Total disease ×100 *Specificity: True Negative/Total Non-disease × 100*
77/79 × 100 = 97% *51/51 × 100 = 100%*

Table 3. Sensitivity of conventional PCR to the gold standard method (real time PCR)

		Truth		
		Disease	**Non-disease**	**Total**
Test results	Positive	(True positive)	(False positive)	(Total test positive)
		36	0	36
	Negative	(False negative)	(True negative)	(Total test negative)
		43	51	94
		(Total disease)	(Total non-disease)	(Total)
		79	51	130

Sensitivity: True Positive/Total disease ×100
36/79 × 100 = 45.6%

Table 4. Limit of detection (LOD) of the LAMP assay

Sample dilution	Peaks	Annealing point
Neat	15.2	82.17
$10^{-}2$	17.17	82.58
$10^{-}4$	17.32	82.64
$10^{-}6$	19.17	82.54
$10^{-}8$	21.17	82.59
$10^{-}10$	23.05	82.54
$10^{-}12$	25.02	82.32
$10^{-}14$	28.36	82.58
$10^{-}16$	29.42	82.56
$10^{-}18$	_	_
$10^{-}20$	_	_

The molecular genetics and phylogenetic analysis were done from the sequenced data based on different genes. The RP030 and GPCR genes have been reported by [18] to contain lineage-specific signatures for sheeppox and goatpox and could be used for differentiation as well as molecular epidemiological studies. The P32 gene that is located on the membrane surface of mature intracellular viral particles and is highly conserved among the Capripoxviruses was also analyzed. The complete ORF of these genes were analyzed in this study and compared with the sequences of Capripoxviruses already available in the database. The findings of the phylogenetic analysis were in agreement with previously reported results where three main clusters representing different lineages of Capripoxviruses were obtained based on different genes. The phylogenetic trees showed the similar topological structure in all the three genes.

The samples clustered differently and only those with a complete coverage of the particular gene were used for the analysis. The live cultures were obtained from a stock at KALRO. The supposed SPV virus was confirmed to be LSDV and the analysis based on the 3 genes showed that it clusters with the LSDV. The live cultures were given the names according to the animal which the virus had been isolated from, they have not been previously sequenced.

The isolated Kenyan LSDV viruses clustered differently, the LUMPIVAX (S18) vaccine currently being used in Kenya and the bovine sample (S17) from Kakamega clustered together in the goatpox virus lineage with other isolates from India China and other earlier GPV isolates from Kenya; Kedong and Isiolo. The goat sample from Isiolo (S16) and bovine sample from Garisa (S9) clustered with the sheeppox virus in the SPV lineage with other sheeppox viruses from Nigeria, Tunisia, Turkey, and China. Other Bovine samples from the Western part of Kenya like Siaya (S13), Homabay (S8), Kisumu (S7) and Kakamega (S6) clustered together with other LSDV isolates previously from Kenya and others from Sudan, South Africa and Egypt in the LSDV lineage.

3.2 Discussion

Capripoxviruses remain a major challenge to farmers in endemic areas like Asia and Africa and presents a real threat of spreading to new geographical regions. Lumpy skin disease is endemic in Africa and has continued to be a big problem to farmers in Kenya as it results in loss of livestock and reduced production of skin, hides, and milk. This has been proved by the fact that out of the 130 samples more than half were positive confirming the presence of the disease in the population. That is a prevalence rate of 61% was revealed from the population. Outbreaks occur in different parts of the country the latest during this study being in the year 2014, where outbreaks were reported in western Kenya (Kakamega - Malava) and North and West Pokot. These are areas on the Kenyan border with Uganda, and the transboundary movements of cattle were suspected to have resulted in these infections with approximately 1000 deaths reported before vaccination.

Table 5. Limit of detection (LOD) of real time PCR

Sample no.	Sample dilution	Detector name	Reporter	CT value (Mean)
1	Neat	17 beta	FAM	10.07231
2	$10^{-}2$	17 beta	FAM	13.08772
3	$10^{-}4$	17 beta	FAM	17.12098
4	$10^{-}6$	17 beta	FAM	19.72893
5	$10^{-}8$	17 beta	FAM	21.73447
6	$10^{-}10$	17 beta	FAM	24.89356
7	$10^{-}12$	17 beta	FAM	28.07662
8	$10^{-}14$	17 beta	FAM	30.94375
9	$10^{-}16$	17 beta	FAM	34.26325
10	$10^{-}18$	17 beta	FAM	37.05188
11	$10^{-}20$	17 beta	FAM	Undetermined (-)
12	$10^{-}22$	18 beta	FAM	Undetermined (-)

Confirmation of these outbreaks to ensure early control interventions has always been a challenge due to unreliable and nonspecific detection mechanisms and therefore this validated LAMP assay that was used to confirm the recent outbreaks provides a solution to these problems. The LAMP assay proved to be very sensitive and specific in the detection of Capripoxvirus, with a sensitivity of 97% and specificity of 100% in regard to the real time PCR (the gold standard method). The Limit of detection (LOD) of the LAMP assay was determined to be 10^{-16} and that of real time PCR 10^{-18} using the same serially diluted sample for both the test. The statistical analysis of the two methods using the Fisher's Exact Test indicated a p value of (P=.8), meaning that there is no significant difference between the two diagnostic tests. The simplicity of the LAMP assay and ability to be rolled out to our resource-poor Regional Veterinary Investigation laboratories (RVILs), therefore, offers a better and more reliable technique in CaPVs diagnosis. This, therefore, will ensure quick, reliable and specific detection which can result in early interventions in cases of disease presence with appropriate control measures.

The phylogenetic analysis showed that the Capripoxviruses present here in Kenya are varied and includes all the three viruses with different lineages. The three genes used in the phylogenetic analysis are highly conserved among the CaPVs. The P32 gene which codes for the envelope protein, the GPCR, and the RP030 genes. The phylogenetic analysis of the P32 gene showed the 3 main CaPVs lineages, with the SPV lineage having a 100% bootstrap value and LSDV and GPV lineages a bootstrap value of 96% and 99% respectively. However, the GPV lineage showed some 3 distinctive subgroups with between 93-99% bootstrap values. One of the subgroup with a 99% bootstrap value had two Kenyan LSDV samples clustered together, that is the Lumpivax vaccine (S18) and the sample (S17) that resulted in the most recent outbreak. The sample was obtained from a cattle and the vaccine is a Lumpy skin disease vaccine yet they all clustered together and in the same lineage with other GPVs from India, China, and Vietnam. This could be as a result of a possible mutation in the gene resulting in the differences and hence the formation of the sub-clusters. The LSDV lineage had no subgroups and all the samples clustered originated from cattle except one, (S12) from KALRO that was obtained from a sheep. The SPV lineage showed Kenyan samples (S16) and (S9) both from cattle clustering together with SPV from India China.

The phylogenetic analysis of the GPCR gene revealed the existence of the three distinct lineages [18,19] with a bootstrap value of 95% and 100%. The LSDV lineage had all LSDV strains but clustered into two subgroups, WITH one of the subgroups having only one LSDV strain from South AFRICA. The GPV lineage had three distinctive subgroups same as with the P32 gene analysis and the SPV lineage also had three subgroups.

The phylogenetic analysis of the RP030 gene that encodes for RNA polymerase subunit also revealed the formation of the three distinct lineages with the LSDV lineage having two subgroups containing all LSDV stains. In one subgroup, bovine samples (S13) from Siaya,

(S6) from Kakamega and (S1) from Kajiado clustered together with previously isolated LSDV strains from Kenyan and have been used as vaccines. The Lumpivax vaccine and the (S17) FROM Kakamega still clustered together under the GPV lineage while the SPV lineage had two samples, (S9), bovine sample from Garisa and (S11), bovine sample from KALRO, a likelihood of it being an SPV isolated from cattle.

All these, therefore, indicates the possibility of a mixed infection and also cross infection among the three CaPVs viruses and that they are no longer host specific as earlier reported by other scientists [7]. This was also observed from the 3 pure cultures that were used in this study. The pure cultures were obtained from sheep, goats, and cattle respectively and so named as has always been the case, according to the host of origin. The pure culture that was obtained from sheep on analysis was found to be Lumpy Skin Disease virus, (S12) and that obtained from cattle found to be sheep pox virus, (S11). Other field samples most of which were obtained from cattle clustered differently in the 3 CaPVs lineages. Like sample (S17) from Kakamega was obtained from cattle as what was generally known and reported as a Lumpy skin disease outbreak killing several cattle before a mass vaccination was carried out. Being a "Lumpy Skin

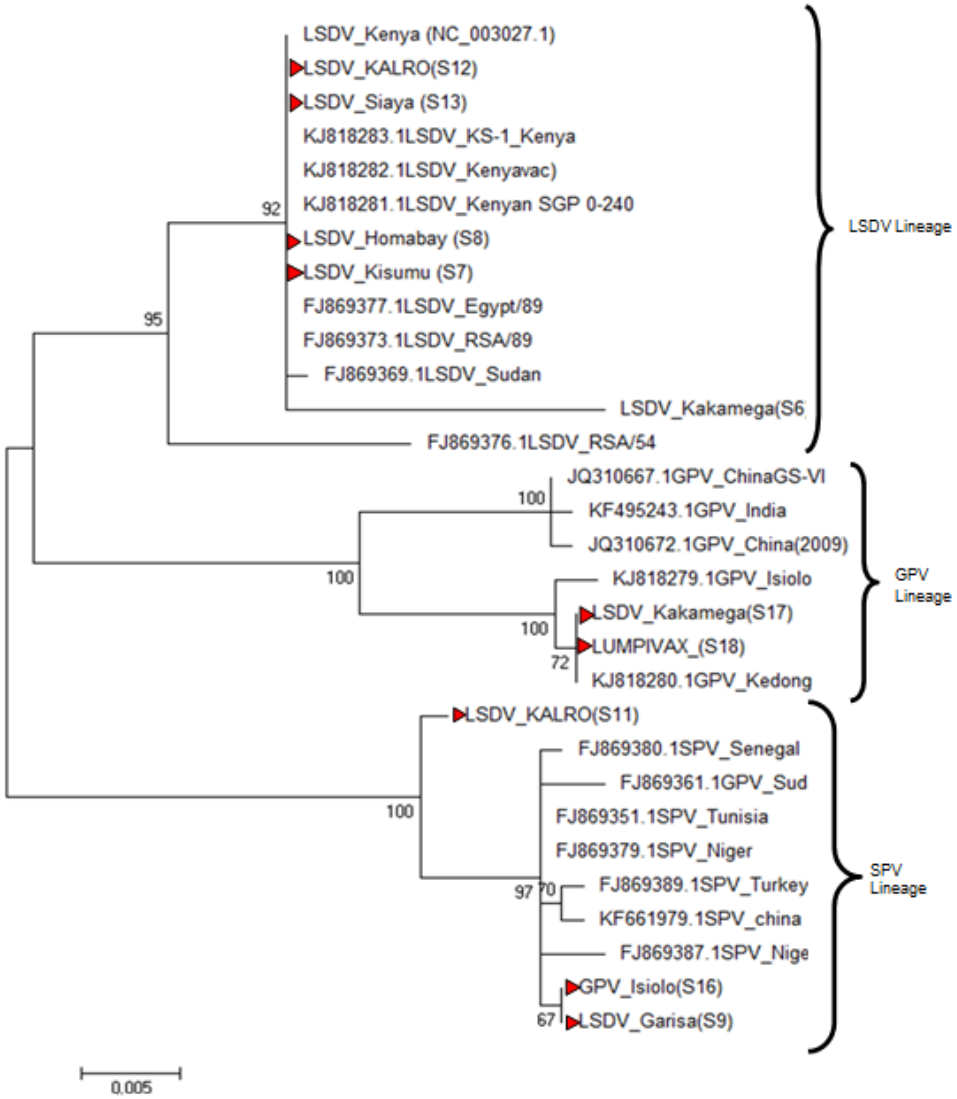

Fig. 4. Phylogenetic analysis based on GPCR gene
: Analyzed samples

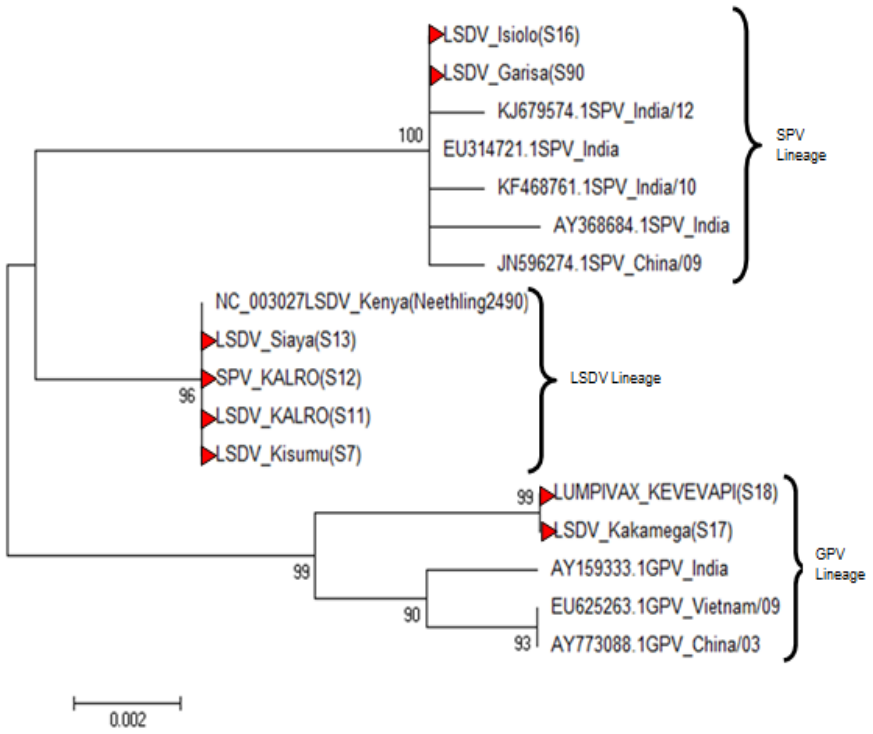

Fig. 5. Phylogenetic analysis based on the P32 gene

▶ *: Analyzed samples*

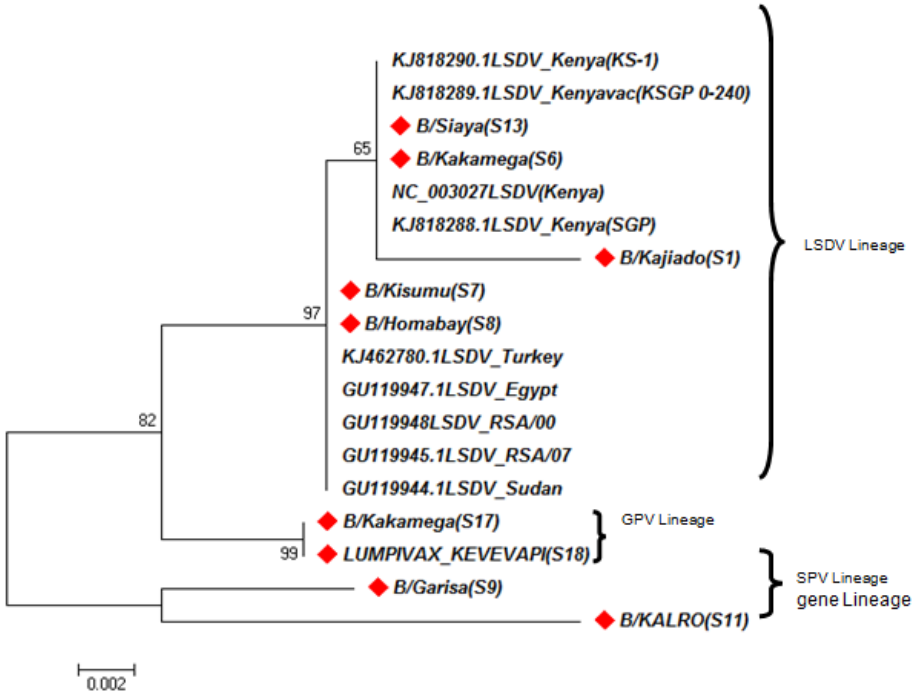

Fig. 6. Phylogenetic analysis based on the RP030 gene

◆ *: Analyzed samples*

Disease" the assumption was that it was caused by a Lumpy skin disease virus (LSDV), and therefore the mass vaccination carried out was done using the LUMPIVAX vaccine for Lumpy skin disease caused by LSDV virus. This phylogenetic analysis, based on all the 3 highly conserved genes shows the virus clustering in the GPV lineage, an indication that it was possibly a goat pox virus (GPV).

The Lumpivax vaccine is an attenuated live vaccine currently used in the control of LSD in Kenya. Its phylogenetic analysis in this study showed that it has a goatpox virus lineage and clustered closely together with the Kakamega isolate (S17) which was isolated from the recent outbreak in Kenya. This was depicted through the analysis of all the 3 genes. It, therefore, confirms that the recent outbreak of the disease in cattle that was thought to be Lumpy skin disease virus just because it was in cattle could then have been caused by goat pox virus.

With the fact that the sequences analysis results have proved that there is a possibility of cross infection and also mixed infection, more emphasis could be put on developing a combined vaccine that is able to offer cross-protection against all the 3 viruses in all the animals. This can be more economical than using the different vaccines in cases of disease outbreaks or genotyping to determine the virus responsible for disease outbreak before vaccination can be undertaken. The complaints by farmers on the ground of disease upsurge in population after every vaccination could, therefore, be because of the assumption that is made every time there is a disease outbreak in cattle that it is LSDV that is causing the infection, yet it could be any of the 3 CaPVs and with this assumption the wrong vaccine is administered. This, therefore, calls for the need of a commonly combined vaccine that can protect against all the 3 CaPVs.

4. CONCLUSION

Considering the analytical and diagnostic sensitivities of LAMP assay and real time PCR being in close agreement, LAMP assay should be adopted as a diagnostic tool for use in developing countries where resources are limited due to its simplicity and cost-effectiveness advantage.

The phylogenetic analysis has confirmed that there is Capri pox virus cross infection in livestock and the viruses are no longer host specific and are diversified. The virus found in Kenya are related to others found in other parts of the world and therefore not very specific to the country.

There is a need to develop a combined vaccine to protect livestock from the 3 CaPVs due to possibilities of cross infection and close relatedness of the virus just as the modern diagnostic methods also capitalize on a single primer for the detection of all the 3 CaPVs.

There is little information on the molecular characterization of the Capripoxvirus in Kenya and therefore, the deeply sequenced data obtained is being used to perform whole genome and multiple gene analysis to make available the molecular data.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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