British Microbiology Research Journal
4(7): 759-771, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Isolation and Molecular Biological Characterization of Fowlpox Virus from Specimen of Cutaneous Nodular Lesions from Chickens in Several Regions of Tanzania

S. N. Masola[1,2*], A. Mzula[2], H. N. Tuntufye[2], C. J. Kasanga[2] and P. N. Wambura[2]

[1]Tanzania Livestock Research Institute, P.O.Box 202, Mpwapwa, Tanzania.
[2]Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture, P.O.Box 3019, Morogoro, Tanzania.

Authors' contributions

This work was carried out in collaboration between all authors. Author SNM designed the study, did the field work (samples collection), laboratory work (data collection), data editing, analysis of the sequence data, literature search and wrote the first draft of the manuscript. Author AM was involved in purchasing of consumables such as DNA extraction kits, primers, TBE buffer, nuclease-free water, loading dye and DNA molecular weight marker. Author AM was also involved in some laboratory work. Author HNT participated in editing and analysis of the sequence data. Authors CJK and PNW read and corrected the entire manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 13th December 2013
Accepted 16th February 2014
Published 25th March 2014

ABSTRACT

Aim: To investigate molecular and evolutionary characteristics of genes of fowlpox virus (FWPV) isolates from chickens in Tanzania.
Study Design: Experimental.
Place and Duration of Study: Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania; between November 2011 and October 2013.
Methodology: Samples of cutaneous nodular lesions were collected from featherless parts of chickens (n = 154) suspected to have fowl pox in 14 regions of Tanzania followed by virus isolation, DNA extraction, polymerase chain reaction (PCR) amplification of the P4b gene, gel electrophoresis of PCR products, purification of PCR products, sequencing

**Corresponding author: Email: snmasola@yahoo.co.uk;*

of purified PCR products and finally analysis of sequence data using standard procedures.
Results: The disease was confirmed in 12 regions, out of 154 investigated samples 66 (42.86%) were found to contain FWPV, indicating that the 66 chickens from which the samples were collected had fowl pox as a result FWPV infection. Sequence analysis revealed that the Tanzanian FWPV isolates were 99.65 – 100% identical to each other and 99 – 100% identical to several published sequences of FWPV isolates from various countries in different continents of the world, including Europe and Asia. Phylogenetic analysis revealed that all Tanzanian isolates belong to clade A, subclade A1.
Conclusion: Based on the findings of this study it is concluded that currently fowl pox is prevalent in several regions of Tanzania, caused by FWPVs which are genetically and phylogenetically closely related. However, these findings do not rule out the possibility of existence of genetic divergence among FWPVs currently prevalent in Tanzania. In order to rule out or detect genetic divergence (if any) among FWPVs currently prevalent in the country, other studies aimed at investigating molecular and evolutionary characteristics of genes in other genomic regions are highly recommended.

Keywords: Fowlpox virus; fowl pox; cutaneous nodular lesions; virus isolation; PCR; sequencing; Tanzania.

1. INTRODUCTION

Fowlpox virus (FWPV) is a DNA virus that belongs to the family *Poxviridae*, subfamily *Chordopoxvirinae* and genus *Avipoxvirus* [1]. The virus causes fowl pox in chickens. Clinically, the disease presents itself in three forms; the cutaneous, diphtheritic and systemic form [2,3]. The cutaneous form is characterized by formation of proliferative lesions ranging from papules to nodules in the unfeathered parts of the body, which eventually hardens to form scabs. The diphtheritic form is characterized by formation of fibrous necrotic proliferative lesions in the mucous membrane of the digestive and upper respiratory tracts [4, 5]. In the systemic form various body systems and tissues of an infected bird are involved [2].

Fowl pox is an important disease because it causes great economic losses to farmers due to mortality and decreased productivity of birds. The mortality rate, which is higher in birds with the diphtheritic form, may approach 50% in severe outbreaks, especially when accompanied by secondary bacterial and/or fungal infection(s). Economic losses are largely due to transient decrease in egg production in laying birds and decreased growth in young birds [6].

Provisionally, fowl pox is suspected if papules or nodular lesions are evident on chickens during clinical or postmortem examination [5]. The definitive diagnosis is attained if the presence of FWPV in infected tissue samples is confirmed by using one or a combination of two or more of the following techniques: isolation of FWPV in cell culture of avian origin, or the chorioallantoic membrane (CAM) of embryonated chicken eggs (ECEs) [7,8,9]; demonstration of the characteristic viral inclusion bodies by examination of affected tissue specimen stained with Haematoxylin and Eosin under a light microscope, or by fluorescent antibody and immunohistochemical techniques [9]; demonstration of viral particles with typical morphology of FWPV by negative staining electron microscopy [3,5,9,10] or by other techniques of transmission electron microscopy [3,10,11]; demonstration of the presence of DNA of FWPV in the infected tissue specimen by using polymerase chain reaction (PCR) and identification of FWPV based on the sequence blast results [12]. Fowl pox can also be confirmed by serological assays [5].

Prior this work no study on fowl pox and FWPV had been conducted in Tanzania, consequently no data on fowl pox and strain(s) of FWPV circulating in the country were available. The aim of this study was to investigate molecular and evolutionary characteristics of genes of FWPV isolates from chickens in Tanzania.

2. MATERIALS AND METHODS

2.1 Study Location

Field work (collection of samples of cutaneous nodular lesions from chickens suspected to have fowl pox) was conducted in various geographical locations in 14 regions of Tanzania (Fig. 1, Table 1). Laboratory work was conducted at the Faculty of Veterinary Medicine (FVM), Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The country is located in Eastern Africa between latitudes 1º-12º South and longitudes 29º-41º East [13, 14].

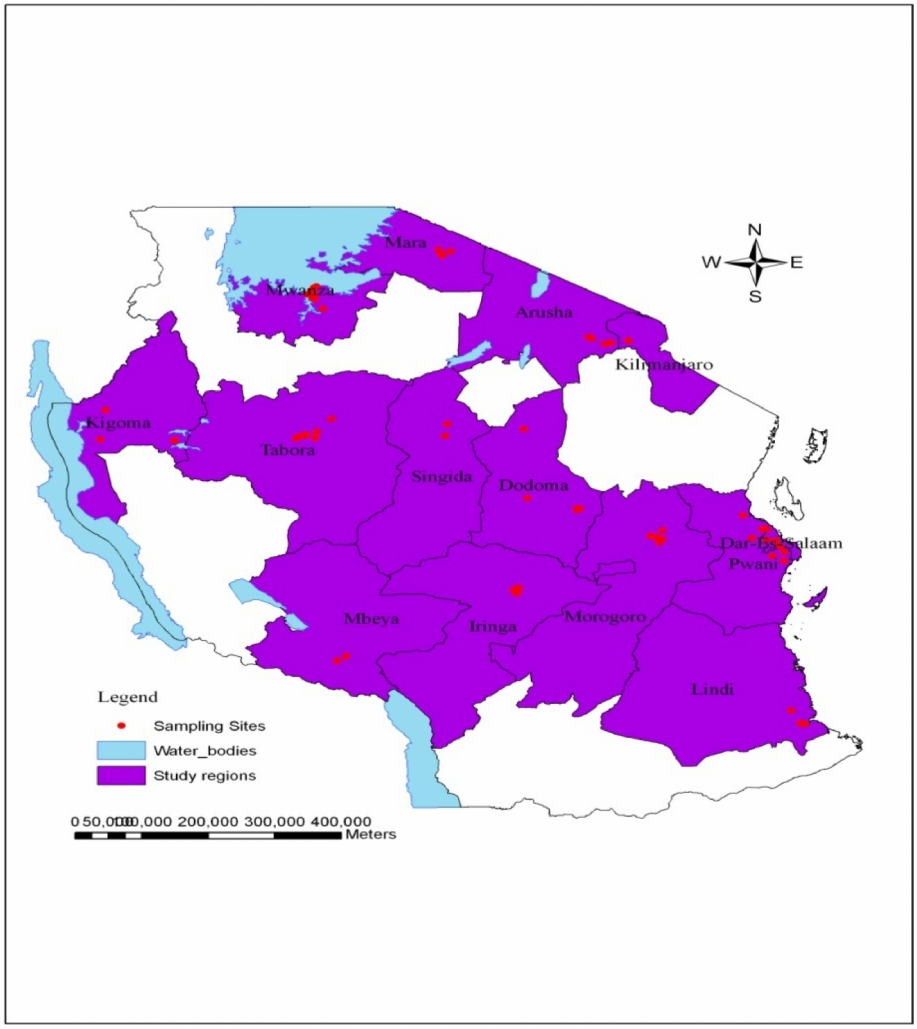

Fig. 1. Map of Tanzania showing study regions and sampling sites

Table 1. Details of samples of cutaneous nodular lesions used in this study

Source of samples		Number of samples collected
Geographical location	**Region**	
Eastern Tanzania	Dar es Salaam	11
Eastern Tanzania	Morogoro	20
Eastern Tanzania	Pwani	2
Central Tanzania	Singida	13
Central Tanzania	Dodoma	21
Western Tanzania	Kigoma	7
Western Tanzania	Tabora	13
North-western Tanzania	Mwanza	20
Northern Tanzania	Mara	5
Northern Tanzania	Arusha	9
North-eastern Tanzania	Kilimanjaro	1
Southern Tanzania	Mbeya	15
Southern Tanzania	Iringa	12
South-eastern Tanzania	Lindi	5
Total		**154**

2.2 Samples Collection and Storage

Between November 2011 and May 2013 samples of proliferative cutaneous nodular lesions were collected from chickens (n = 154) suspected to have fowl pox. Pieces of cutaneous nodular lesions collected from the same cadaver or live bird were put in one plastic vial and were considered as one sample. Each sample was labeled and stored in a deep freezer at -20ºC at a nearby Veterinary Investigation Centre (VIC), Local Government Authority or Regional Secretariat office. Thereafter all samples were transported (in cool boxes) to the FVM, SUA, Morogoro; where they were also stored at -20ºC until required.

2.3 Commercial Fowl Pox Vaccines

Two samples of imported lyophilized fowl pox vaccines commercially available in Tanzania were purchased from some commercial sources in Morogoro Municipality, and stored at SUA in a refrigerator at 4ºC until required.

2.4 Virus Isolation

Inoculums for CAM were prepared from 154 samples of cutaneous nodular lesions, followed by inoculation of each inoculum in 10 day-old ECEs through CAMs as described previously [15] with some modification the eggs were incubated at room temperature (25 - 28ºC) instead of 37ºC. Briefly, each sample from a suspected chicken was finely ground in a pestle and mortar with sterile sand and suspended in phosphate buffered saline (PBS). Thereafter the suspension was centrifuged (500g for 10 minutes at room temperature) and the supernatant was collected. The supernatant was filtered using a 0.45 µm membrane filter in order to prepare inoculum for CAM. Then 0.1 ml of inoculums was inoculated in 10 day-old ECEs through CAMs. Similarly, 0.1 ml of each sample of the FWPV vaccine was inoculated in 10 day-old ECEs through CAMs (these served as positive control). Nothing was inoculated in the negative control ECEs. The eggs were incubated at room temperature for 5-7 days, thereafter examined for the presence of nodular lesions on CAMs or generalized

thickening and haemorrhage of the CAMs. The inoculums were passaged in the CAMs four times.

2.5 DNA Extraction

DNA samples were extracted from samples of CAM containing virus cultures after the 4th passage by using ZR Tissue and Insect DNA MiniPrep™ Kit Catalog Number D6016 (Zymo Research Corp., USA) according to the manufacturer's instructions.

2.6 PCR for Amplification of the P4b Gene

Conventional PCR was conducted in Takara PCR Thermal Cycler (Takara Bio Inc., Japan) using a set of primers indicated below (Table 2). Each amplification reaction consisted of 12.5 µl of master mix, 1 µl of each primer, 5.5 µl of nuclease-free water, and 5 µl of template DNA; making a total volume of 25 µl . After an initial heat denaturation at 94ºC for 2 minutes; each mixture was subjected to 40 cycles of heat denaturation at 94ºC for 1 minute, primer annealing at 60ºC for 1 minute, and DNA extension at 72ºC for 1 minute. After the 40th cycle a final extension step was performed at 72ºC for 2 minutes.

Table 2. Primers used in this study

Primer	Sequence	Expected fragment size	References
Forward primer (P1)	5'-CAGCAGGTGCTAAACAACAA-3'	578 bp	[1,12,16,17, 18,19,20,21]
Reverse primer (P2)	5'-CGGTAGCTTAACGCCGAATA-3'		

2.7 Gel Electrophoresis

Five microlitre (5 µl) of each PCR product was loaded in a 1.5% agarose gel with ethidium bromide (10 mg/ml). Electrophoresis was conducted in 1X Tris-Borate-EDTA (TBE) buffer for 45 minutes at 100V. A DNA ladder with 100-bp increments was used as a molecular weight marker. Thereafter, gels were visualized under UV light using UVI tec transilluminator and photographed using a digital camera.

2.8 Purification of PCR Products, Sequencing and Analysis of Sequence Data

Samples of PCR products were purified using EXOSAP Amplicon Purification Kit (Affymetrix, USA) according to the manufacturer's instructions. The sequencing reaction was cleaned using ZR-96 DNA Sequencing Clean-up Kit™ Catalog Numbers D4052 and D4053 (Zymo Research Corp., USA) according to the manufacturer's instructions. Thereafter purified samples were injected in the ABI 3500XL with P0P7 and a 50 cm array (Applied Biosystems, USA). The primers used for PCR were used for sequencing. The samples were sequenced using ABI V3.1 Big Dye Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's instructions. Thereafter the sequences of each PCR product were assembled using CLC Main Workbench version 6.7.1 software to get a consensus sequence.

After manual editing the sequence homology was investigated using BLAST algorithm [12, 16]. Similarities among the Tanzanian FWPV isolates were investigated using BLAST two

sequences programme which gives alignment of two sequences of interest [16]. Prior phylogenetic analysis the nucleotide sequences reported in this study (Table 3) were aligned with reference avipoxvirus (APV) sequences from the GenBank (Table 4) using Clustal Omega programme [22]. Thereafter possible phylogenetic relationships and grouping of the APVs were investigated using procedures described previously [19] except that in the present study MEGA version 5.2.2 [23] was used instead of MEGA version 3.1, also no pairwise genetic and amino acid distances were calculated because none of the analyzed isolates displayed greater variability in the phylogenetic tree.

Table 3. Details of the Tanzanian FWPV isolates analyzed in this study

Source of isolates		Virus name	Acronym	Host	Nature	GenBank accession numbers
Geographical location	Region					
Central Tanzania	Dodoma	Fowlpox TZ 3/DOM	FWPV3DOM	Chicken	Clinical isolate	KF722863
Eastern Tanzania	Morogoro	Fowlpox TZ 6/MOR	FWPV6MOR	Chicken	Clinical isolate	KF722864
Western Tanzania	Tabora	Fowlpox TZ 19/TBR	FWPV19TBR	Chicken	Clinical isolate	KF722865
Southern Tanzania	Mbeya	Fowlpox TZ 28/MBY	FWPV28MBY	Chicken	Clinical isolate	KF722866
Southern Tanzania	Iringa	Fowlpox TZ 41IRG (TPV1)	FWPV41IRG (TPV1)	Chicken	Clinical isolate	KF032407
North-western Tanzania	Mwanza	Fowlpox TZ 47/MWZ	FWPV47MWZ	Chicken	Clinical isolate	KF722860
Northern Tanzania	Arusha	Fowlpox TZ 60/ARS	FWPV60ARS	Chicken	Clinical isolate	KF722861
Eastern Tanzania	Pwani	Fowlpox TZ 63/CST	FWPV63CST	Chicken	Clinical isolate	KF722859
Western Tanzania	Tabora	Fowlpox TZ 65/TBR	FWPV65TBR	Chicken	Clinical isolate	KF722858

Table 4. Details of reference APV sequences obtained from the GenBank

Virus name	Acronym	Host	P4b locus GenBank accession numbers
Fowlpox 174/4/04	FWPV174	Chicken	AM050377
Fowlpox Mild (Websters; Fort Dodge)	FWPVM	Chicken	AM050378
Turkeypox 2/11/66	TKPV66	Turkey	AM050387
Turkeypox 10/12/98	TKPV98	Turkey	AM050388
Albatrosspox 353/87	ABPV	Black-browed albatross	AM050392
Falconpox 1381/96	FLPV1381	Falcon	AM050376
Falconpox GB362-02	FLPV36202	Falcon	AY530306
Canarypox 1445/97/33	CNPV1445	Canary	AM050375
Canarypox (Duphar; Fort Dodge)	CNPVV	Canary	AM050384
Great titpox GTPV-A310	GTPVA310	Great tit	AY453173
Great titpox-A311	GTPVA311	Great tit	AY453174
Pigeonpox 950 24/3/77	PGPV950	Pigeon	AM050386
Pigeonpox B7	PGPVB7	Pigeon	AY453177
Starlingpox /27	SLPV	Starling	AM050391
Macawpox 1305/86	MCPV	Macaw	AM050382
Parrotpox 364/89	PRPV	Parrot	AM050383
Agapornispox APIII	AGPV	Agapornis	AY530311

Source: [19]

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Virus isolation

Gross pathological changes on CAMs were first observed at the third passage, when two to three nodular lesions about 1 mm in diameter were observed on some CAMs without thickening of the CAMs. At the fourth passage marked proliferative nodular lesions were observed, the nodules had increased in number and size ranging from 1 to 2 mm in diameter, most of the nodules had coalesced to form large mass (Fig. 2). The lesions were demonstrated in CAMs inoculated with inoculums prepared from 66 samples of cutaneous nodular lesions from chickens in 12 regions (Table 5) and in all CAMs inoculated with inoculums prepared from samples of imported commercial fowl pox vaccines. None of these lesions were demonstrated in the CAMs of negative control ECEs and CAMs of ECEs inoculated with inoculums prepared from the other 88 samples of cutaneous nodular lesions.

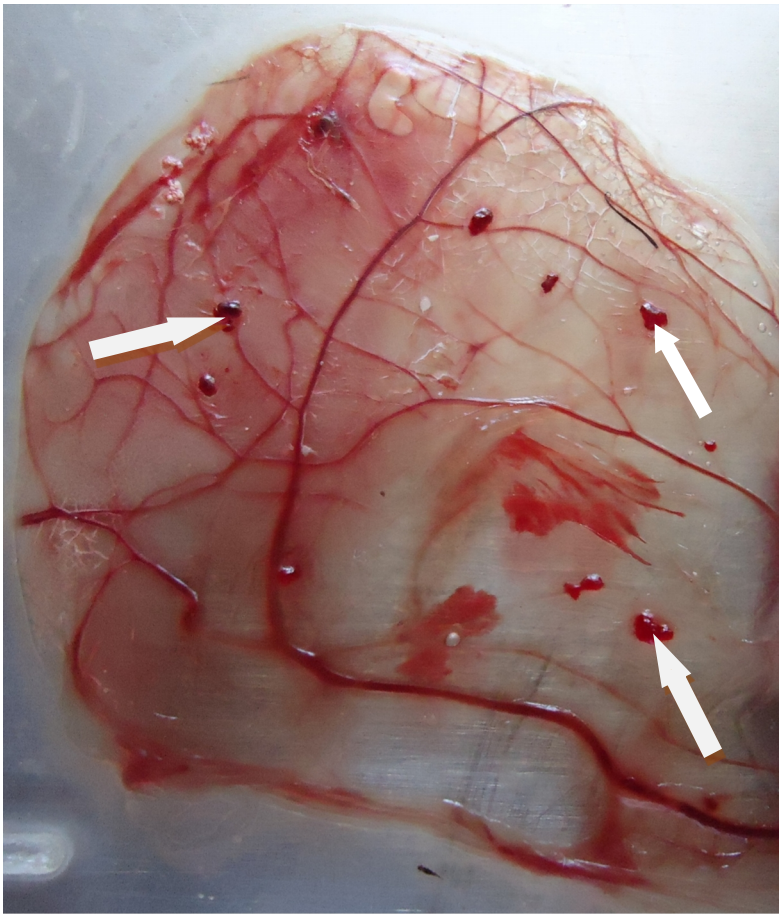

Fig. 2. Chicken CAM during the fourth passage showing nodular lesions (arrows) characteristic of poxvirus infection

3.1.2 PCR and gel electrophoresis

DNA samples were isolated from samples of cutaneous nodular lesions collected from chickens suspected to have fowl pox, and from a sample of CAM with nodular lesions at the fourth passage after inoculation with inoculum prepared from imported commercial fowl pox vaccine currently used in Tanzania; followed by PCR for amplification of the P4b gene, and agarose gel electrophoresis. Positive results were indicated by migration of PCR products to approximately 578 bp, an expected size for the P4b gene amplicon for FWPV and other avipoxviruses (APVs) (Fig. 3). Out of 154 total samples tested 66 (42.86%) were positive (Table 5).

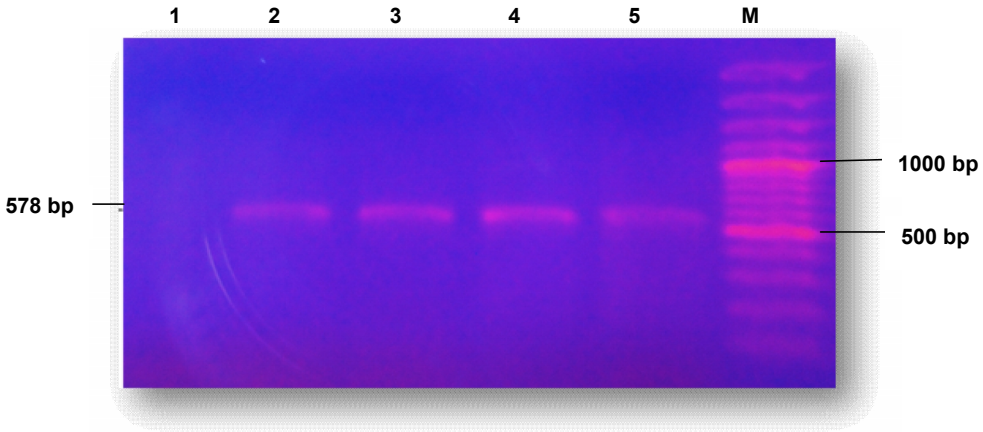

Fig. 3. Agarose gel electrophoresis of PCR products of DNA extracted from samples of cutaneous nodular lesions collected from chickens suspected to have fowl pox (lanes 2, 3, and 4), and from a sample of CAM infected with inoculum prepared from imported commercial fowl pox vaccine currently used in Tanzania which served as a positive control (lane 5). The amplicons migrated to approximately 578 bp, which is an expected fragment size for the P4b gene of FWPV and other APVs. Lanes 1 and M are negative control and 100-bp molecular weight marker, respectively

3.1.3 Sequencing and analysis of sequence data

In order to identify the virus the obtained consensus nucleotide sequences were blasted in the GenBank. Each blasted sequence showed 99 – 100% identity to several published sequences of FWPV isolates (GenBank accession numbers AM050378, AM050379, AY453171, AY453172, AY530302, FR852586, GQ180201, GQ180212, GQ221269, GU108500, GU108501, GU108502, GU108503, GU108504, GU108505, GU108506, GU108507, GU108508, GU108509, JQ665838, JX464819 and JX464820) from various countries in different continents of the world, including Europe and Asia.

Moreover, sequence analysis revealed that the Tanzanian FWPV isolates are 99.65 – 100% identical to each other. Phylogenetic analysis revealed that all Tanzanian isolates belong to clade A subclade A1 (Fig. 4). After analysis sequences of the Tanzanian FWPV isolates were deposited in the GenBank data base under accession numbers KF722858, KF722859, KF722860, KF722861, KF722862, KF722863, KF722864, KF722865 and KF722866.

Table 5. Results based on virus isolation and PCR

Source of samples		Number of samples of cutaneous nodular lesions analyzed	Number of positive samples (percentage positive)
Geographical location	**Region**		
Eastern Tanzania	Dar es Salaam	11	9 (81.82)
Eastern Tanzania	Morogoro	20	4 (20.00)
Eastern Tanzania	Pwani	2	1 (50.00)
Central Tanzania	Singida	13	4 (30.77)
Central Tanzania	Dodoma	21	10 (47.62)
Western Tanzania	Kigoma	7	3 (42.86)
Western Tanzania	Tabora	13	4 (30.77)
North-western Tanzania	Mwanza	20	8 (40.00)
Northern Tanzania	Mara	5	4 (80.00)
Northern Tanzania	Arusha	9	2 (22.22)
North-eastern Tanzania	Kilimanjaro	1	0 (0.00)
Southern Tanzania	Mbeya	15	14 (93.33)
Southern Tanzania	Iringa	12	3 (25.00)
South-eastern Tanzania	Lindi	5	0 (0.00)
Total		**154**	**66 (42.86)**

3.2 Discussion

In this study 154 samples of cutaneous nodular lesions were analyzed to demonstrate the presence of FWPV in the samples. Out of 154 analyzed samples 66 (42.86%) were found to contain FWPV. This implies that the 66 chickens from which the samples were collected had fowl pox due to FWPV infection as revealed by virus isolation, PCR and sequence blast results. The findings of this study (Table 5) indicate that currently fowl pox is prevalent in various geographical locations and regions of Tanzania.

However, most of the analyzed samples [88 (53.14%)] were found to be FWPV-negative. This implies that the 88 chickens from which the samples were collected were not infected with FWPV. The proliferative cutaneous nodular lesions found on featherless parts of the chickens during samples collection could be attributed to other diseases such as papillomatosis [24] and/or mange [25,26] which have clinical signs similar to those of the cutaneous form of fowl pox.

Although results in Table 5 show that none of the analyzed samples from Kilimanjaro region in North-eastern Tanzania, and Lindi region in South-eastern Tanzania was confirmed to be FWPV-positive; this does not rule out fowl pox in these regions due to the fact that the analysis involved few samples of cutaneous nodular lesions from chicken(s) in Kilimanjaro (n = 1) and Lindi (n = 5) regions.

A close genetic relationship of the Tanzanian FWPVs to each other (99.65 – 100% identity) and to FWPV isolates of several other countries in the world (99 – 100% identity) demonstrated in this study shows how highly conserved the P4b gene is [17,19]. Phylogenetically all Tanzanian isolates belong to clade A subclade A1 (Fig. 4), this implies that based on sequences of P4b gene the FWPVs currently prevalent in Tanzania are phylogenetically closely related.

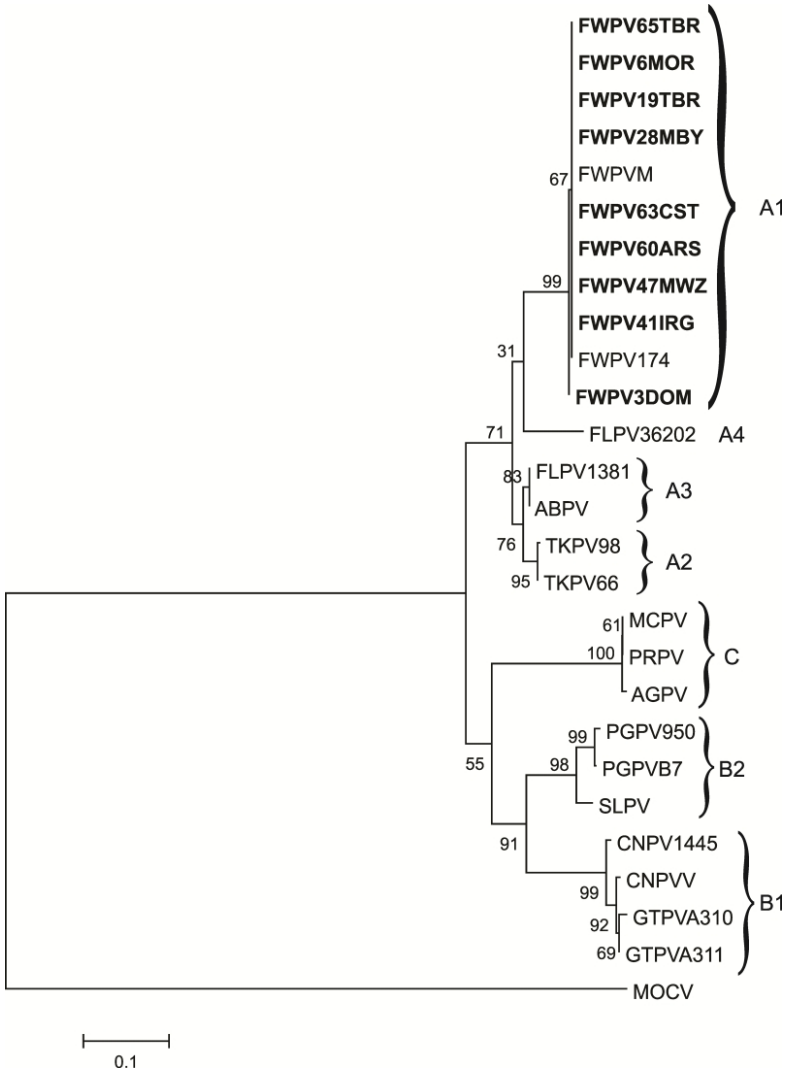

Fig. 4. Phylogenetic tree of nucleotide sequences of the P4b gene of APVs and the Molluscum contagiosum virus (MOCV) orthologue sequence, rooted on MOCV, showing the phylogenetic relationship of FWPVs currently prevalent in Tanzania (bolded). The tree was obtained by the neighbour-joining method calculated with the Jukes and Cantor model. Bootstrap testing of phylogeny was performed with 1000 replications and values equal to or greater than 30 are indicated in the branches (as a percentage). The length of each bar indicates the amount of evolution along the horizontal branches as measured by substitution per site. APV subclades A1 to A4, B1, B2 and clade C are labeled

4. CONCLUSION

Based on the findings of this study it is concluded that currently fowl pox is prevalent in several regions and geographical locations of Tanzania, caused by FWPVs which are genetically and phylogenetically closely related. However, these findings do not rule out the

possibility of existence of genetic divergence among FWPVs currently prevalent in Tanzania. In order to rule out or detect genetic divergence (if any) among FWPVs currently prevalent in the country, other studies aimed at investigating molecular and evolutionary characteristics of genes in other genomic regions are highly recommended.

ACKNOWLEDGEMENTS

Assistance from Mr. Charles S. Sanda, Miss Gudila Y. Mauki, Mr. Christopher M. Kaihura, Mr. Lazaro J. Malamla and Mr. Richard D. Mtilimbania of Kigoma, Misungwi, Serengeti, Lindi and Singida District Councils, respectively; Mr. Juma S. Marisa and Mr. Patrick S. Bushiri of Tanzania Veterinary Laboratory Agency (TVLA), Dar es Salaam; Mr. Emmanuel Mbise of VIC Arusha, Mr. Alex H. Mhenga of VIC Iringa, Mr. Godfrey N. Katambi of VIC Mwanza, Mr. Amos B. Kanire of VIC Mpwapwa (Dodoma brach), Mr. Bucheye R. Hamisi of VIC Tabora, Dr. Lucas B. Masawe of Makanyaga Veterinary Centre, Morogoro and Dr. Solomon W. Nong'ona of Mbeya Region Secretariat during field work is highly acknowledged. Mr. Jonas Fitwangile of FVM, SUA, Morogoro is thanked for his assistance during laboratory work, particularly virus isolation. Moreover, we thank Mr. Deogratias F. Masao of Tanzania Livestock Research Institute, Mpwapwa for his valuable assistance during editing of some figures. This study was funded by the Tanzania Commission for Science and Technology (COSTECH).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lüschow D, Hoffmann T, Hafez HM. Differentiation of avian poxvirus strains on the basis of nucleotide sequences of 4b gene fragment. Avian Dis. 2004;48:453-462.
2. Atkinson CT, Wiegand KC, Triglia D, Jarvi ST. Efficacy of commercial canarypox vaccine for protecting Hawai`i `Amakihi from field isolates of *Avipoxvirus*. Hawai`i Cooperative Studies Unit Technical Report HCSU – 01. Accessed 23 April 2012. Available:http://www.hilo.hawaii.edu/hcsu/documents/TRHCSU-019Atkinson_Avian_Pox.pdf
3. Kulich P, Roubalova E, Dubska L, Sychra O, Šmíd B, Literák I. Avipoxvirus in blackcaps (*Sylvia atricapilla*). Avian Pathol. 2008;37(1):101-107.
4. Thiel T, Whiteman NK, Tirapé A, Baquero MI, Cedeño V, Walsh T, et al. Characterization of canarypox-like viruses infecting endemic birds in the Galápagos Islands. J Wildl Dis. 2005;41(2):342-353.
5. Weli SC, Tryland M. Avipoxviruses: Infection biology and their use as vaccine vectors. Virol J. 2011;8:49. Accessed 6 November 2013. Available: http://www.virologyj.com/content/pdf/1743-422X-8-49.pdf
6. Quinn PJ, Marley BK, Carter ME, Donnelly WJ, Leonard FC. Veterinary microbiology and microbial disease. Oxford: Blackwell Science Ltd; 2002.
7. Carulei O, Douglass N, Williamson A-L. Phylogenetic analysis of three genes of Penguinpox virus corresponding to Vaccinia virus G8R (VLTF-1), A3L (P4b) and H3L reveals that it is most closely related to Turkeypox virus, Ostrichpox virus and Pigeonpox virus. Virol J. 2009;6:52. Accessed 6 November 2013. Available: http://www.virologyj.com/content/pdf/1743-422X-6-52.pdf

8. Farias MEM, LaPointe DA, Atkinson CT, Czerwonka C, Shrestha R, Jarvi SI. Taqman Real-Time PCR Detects *Avipoxvirus* DNA in Blood of Hawai`I `Amakihi (*Hemignathus virens*). Plos One. 2010;5(5):10745. doi: 10.1371/journal.pone.0010745. Accessed 23 April 2012.
Available:
http://www.plosone.org/article/infro%Adoi%F10.1371%2Fjournal.pone.0010745
9. Tripathy DN. Fowlpox. In: Cynthia MK, Scott L, editors. The merck veterinary manual. 9th ed. Whitehouse station (NJ): Merck & Co., Inc; 2005.
10. Weli SC, Okeke MI, Tryland M, Nilssen Ø, Traavik T. Characterization of avipoxviruses from wild birds in Norway. Can J Vet Res. 2004;68:140-145.
11. Prukner-Radovčić E, Lüschow D, Ciglar Grozdanić I, Tišljar M, Mazija H, Vranešić D, et al. Isolation and Molecular Biological Investigations of Avian Poxviruses from Chickens, a Turkey and a Pigeon in Croatia. Avian Dis. 2006;50:440-444.
12. Palade EA, Biró N, Dobos-Kovács M, Demeter Z, Mándoki M, Rusvai M. Poxvirus infection in Hungarian Great Tits (*Parus major*): Case Report. Acta Vet Hung. 2008;56(4):539-546.
13. Adimola B, Asgele T, Gahima C, Mmbando JS, Owuor S. Pearson secondary atlas. 3rd ed. Edinburgh Gate: Pearson Education Limited; 2013.
14. Kireri KK. Steps in Primary Geography – Pupils Book 6. Arusha: Adamson Educational Publishers; 2012.
15. Wambura PN, Godfrey SK. Protective immune response of chickens to oral vaccination with thermostable live Fowlpox virus vaccine (strain TPV-1) coated on oiled rice. Trop Anim Health Prod. 2010;42:451-456.
16. Biswas SK, Jana C, Chand K, Rehman W, Mondal B. Detection of fowlpox virus integrated with reticuloendotheliosis virus sequences from an outbreak in backyard chickens in India. Vet Ital. 2011;47(2):147-153.
17. Jarmin S, Manvell R, Gough RE, Laidlaw SM, Skinner MA. Avipoxvirus phylogenetics: Identification of a PCR length polymorphism that discriminates between the two major clades. J Gen Virol. 2006;87:2191-2201.
18. Lierz M, Bergmann V, Isa G, Czerny CP, Lueschow D, Mwanzia J, et al. Avipoxvirus infection in a Collection of Captive Stone Curlews (*Burhinus oedicnemus*). J Avian Med Surg. 2007;21(1):50-55.
19. Manarolla G, Pisoni G, Sironi G, Rampin T. Molecular biological characterization of avian poxvirus strains isolated from different avian species. Vet Microbiol. 2010;140:1-8.
20. Rampin T, Pisoni G, Manarolla G, Gallanzi D, Sironi G. Epornitic of avian pox in common buzzards (*Buteo buteo*): Virus isolation and molecular biological characterization. Avian Pathol. 2007;36(2):161-165.
21. Terasaki T, Kaneko M, Mase M. Avian Poxvirus Infection in Flamingos (*Phoenicopterus roseus*) in a Zoo in Japan. Avian Dis. 2010;54:955-957.
22. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7:539. Accessed 4 December 2013.
Available: http://www.nature.com/msb/journal/v7/nl/pdf/msb201175.pdf
23. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-2739.
24. Literák I, Šmíd B, Valíček L. Papillomatosis in chaffinches (*Fringilla coelebs*) in the Czech Republic and Germany. Vet Med. 2003;48(6):169-173.
25. Center for food security and public health. Mange and Scabies; 2012. Accessed 29 July 2013. Available: http://www.cfsph.iastate.edu/FastFacts/pdfs/acariasis_F.pdf

26. Office International des Épizooties. Mange. In: OIE Terrestrial Manual; 2013. Accessed 29 July 2013.
Available:
http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.09.08_MANGE.pdf

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=469&id=8&aid=4122