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Evaluation of Virulence of Tanzanian Strains of Fowlpox and Pigeonpox Viruses in Chickens

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Authors' contributions

This work was carried out in collaboration between all authors. Author SNM designed the study, did both In ovo and In vivo experiments (data collection), literature search and wrote the first draft of the manuscript. Author AM was involved during laboratory work (polymerase chain reaction). Authors CJK and PNW supervised the entire work and corrected the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To evaluate the virulence characteristics of recently isolated Tanzanian strains of fowlpox virus (FWPV) and pigeonpox virus (PGPV) in chickens.
Study Design: Experimental.
Place and Duration of Study: Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania; between January 2015 and April 2015.
Methodology: Ten-day embryonated chicken eggs were used for *In ovo* evaluation. The eggs were randomly grouped into four groups (I, II, III, and IV) of 5 eggs each. Each egg in group I, II, and III was inoculated with 0.1 ml of 10^6 EID_{50}/0.1 ml of reticuloendotheliosis virus (REV)-free FWPV inoculum; REV-integrated FWPV inoculum; and PGPV inoculum; respectively, through chorioallantoic membranes (CAMs). Group IV eggs served as control. All eggs were incubated at

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37ºC for 7 days, thereafter CAMs and chicken embryos were examined for gross pathological changes. One hundred and forty chicks were used for *In vivo* evaluation. At 26 days of age the chicks were randomly grouped into four groups (I, II, III, and IV) of 35 chicks each. Each chicken in group I, II, and III was inoculated with 0.1 ml of 10^6 EID_{50}/0.1 ml of REV-free FWPV inoculum; REV-integrated FWPV inoculum; and PGPV inoculum; respectively, subcutaneously. Chickens in group IV served as control. Thereafter from day zero to day 28 post-inoculation, the chickens were examined for development of clinical signs and deaths; followed by necropsy of dead chickens and examination of samples of cutaneous nodular lesions from chickens inoculated with REV-free FWPV or REV-integrated FWPV for the presence of FWPV by using standard procedures.

Results: Extensive pock lesions and severe haemorrhages were evident on CAMs and embryos, respectively, of eggs inoculated with REV-integrated FWPV. Chickens inoculated with REV-integrated FWPV developed a severe disease, characterized by mortality rate of 57%.

Conclusion: REV-integrated FWPV strains are more virulent in susceptible chickens than REV-free FWPV strains.

Keywords: Tanzanian avipoxvirus strains; REV-free FWPV strains; REV-integrated FWPV strains; PGPV; virulence; chickens.

ACRONYMS

APV = avipoxvirus; APVs = avipoxviruses; CAM = chorioallantoic membrane; CAMs = chorioallantoic membranes; COSTECH = commission for science and technology; DNA = deoxyribonucleic acid; ECE = embryonated chicken egg, ECEs = embryonated chicken eggs, EID_{50} = mean (50%) embryo-infectious dose; FVM = faculty of veterinary medicine; FWPV = fowlpox virus; i.e = that is; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; PGPV = pigeonpox virus; pi = post-inoculation; REV = reticuloendotheliosis virus; SPF = specific-pathogen free; SUA = sokoine university of agriculture.

1. INTRODUCTION

The extent of virulence is usually correlated with the ability of the pathogen to multiply within the host. This ability, which is mediated by virulence factors, represents a genetic component of the pathogen; and the overt damage done to the host is an outcome of the host-pathogen interactions [1,2]. Currently virulence is used to characterize the relative capacity of an infectious agent to cause disease in susceptible hosts and has traditionally been used to describe biological characteristics of infectious agents [2].

Fowlpox and pigeonpox viruses are DNA viruses belonging to the family *Poxviridae*, subfamily *Chordopoxvirinae* and genus *Avipoxvirus* [3–6]. Avipoxviruses (APVs) cause pox in birds, in chickens the disease is known as fowl pox [7]. Clinically, there are three forms of avian pox; the cutaneous, diphtheritic and systemic forms [8,9]. The cutaneous form is characterized by formation of proliferative lesions ranging from papules to nodules in the featherless or poorly feathered 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 oral cavity and upper respiratory tracts [7]. In the systemic form various body systems and tissues of an infected bird are involved [9]. The cutaneous form is common and causes no or low mortality. Mortality rates are high in the diphtheritic and systemic forms. However, these forms of avian pox occur rarely [10,11].

Isolates of APVs from several avian species have been characterized in several countries based on their antigenic, genetic or biological properties. Some of the countries include Norway [12], Hawaii [13,14], Tanzania [15-17], Galápagos islands [18], Czech Republic [19], Croatia [20], the United States [21,22], Italy [23], Hungary [24], Japan [25], Great Britain [26], Egypt [27], Australia [28], Israel [29] and India [30]. As far as biological characterization is concerned, reports [12,14,31-34] indicate that several studies have been conducted to determine the pathogenicity or virulence of avipoxvirus (APV) isolates from birds belonging to different avian species.

In recent years fowl pox has been reported to cause high mortalities of chickens (particularly chicks and growers) in Tanzania [35,36]. It has been speculated that the increased mortality rate

of chickens (chicks and growers in particular) due to fowl pox could be attributed to emergence of variant strains of APVs which are more virulent than FWPV strains which were circulating in the country in the past decades. This necessitated biological characterization of Tanzanian APV strains currently circulating in the country. The objective of this study was to investigate the virulence characteristics of recently isolated Tanzanian strains of FWPV and PGPV in chickens.

2. MATERIALS AND METHODS

2.1 Study Location

The study involved both *In ovo* and *In vivo* evaluation. *IN ovo* evaluation was carried out in the virology laboratory, at the Faculty of Veterinary Medicine (FVM), Sokoine University of Agriculture (SUA), Tanzania. *In vivo* evaluation was conducted at the Animal Research Unit of the FVM, SUA, Tanzania.

2.2 Source of Chickens and Chicken Eggs

One hundred and forty (140) day-old layer chicks and 130 specific-pathogen free (SPF) eggs were purchased from Interchick Company Limited, Dar es Salaam, Tanzania; and a commercial farm in Morogoro, Tanzania, respectively.

2.3 Incubation of Eggs

The eggs were incubated at 37ºC for 10 days so as to obtain embryonated chicken eggs (ECEs) for *In ovo* evaluation and determination of mean (50%) embryo-infectious dose (EID_{50}).

2.4 Preparation and Storage of Virus Inocula

Samples of chorioallantoic membranes (CAMs) with pock lesions due to Tanzanian strains of fowlpox virus (FWPV) and pigeonpox virus (PGPV), previously isolated and genetically characterized [15-17], were homogenized. Thereafter, each homogenate was suspended in phosphate-buffered saline (PBS) along with antibiotics [i.e Gentamycin (10% w/v) and Penistrept®, consisting of Procaine Penicillin (200 mg/ml) and Dihydrostreptomycin Sulphate (200 mg/ml)]. The homogenates were centrifuged at 500g for 10 minutes at room temperature (25-28ºC); thereafter the supernatants were collected and filtered using 0.22 µm membrane filters to get the inocula. Each inoculum was stored at -20ºC in plastic vials containing 10^6 EID_{50}/0.1 ml of either reticuloendotheliosis virus (REV)-free FWPV inoculum, REV-integrated FWPV inoculum, or PGPV inoculum.

2.5 Management of Chickens

The chicks were reared at the Animal Research Unit of the FVM, SUA; in a well-ventilated concrete floor house, littered by rice husks. The chicks were given standard feed (chick starter) and water *ad libitum* and held in isolation until they were 21 days of age for maternally derived antibodies to wane. At this age all birds were screened for fowl pox and reticuloendotheliosis antibodies and were found to be seronegative.

2.6 Study Design

This was an experimental study design and it involved both *In ovo* and *In vivo* evaluation as described below:

2.6.1 *In ovo* evaluation

Twenty 10-day-old SPF ECEs were used for *in ovo* evaluation. The eggs were randomly grouped into four groups (I, II, III, and IV) of 5 eggs each. Each egg in group I, II, and III was inoculated 0.1 ml containing 10^6 EID_{50}/0.1 ml of REV-free FWPV inoculum; REV-integrated inoculum; and PGPV inoculum; respectively, through CAMs. Group IV eggs served as control, they were injected 0.1 ml of PBS through CAMs. All eggs were incubated at 37ºC for 7 days, thereafter CAMs were examined for the presence of pock lesions or generalized thickening and haemorrhages. Chicken embryos were also examined for gross pathological changes. The experiment was replicated one time.

2.6.2 *In vivo* evaluation

This involved inoculation of chickens with Tanzanian strains of REV-free FWPV, REV-integrated FWPV (previously demonstrated to be integrated with a near-full length REV provirus i.e 807 bp of REV envelope gene and 370 bp of 5' REV long terminal repeat [16]) or PGPV [17], clinical examination of both inoculated and control chickens, necropsy of dead chickens, examination of cutaneous nodular lesions from chickens inoculated with Tanzanian strains of FWPV for the presence of FWPV, antigen preparation and measurement of humoral immune responses as described below:

2.6.2.1 Inoculation of chickens with FWPV or PGPV

At 26 days of age the chicks were randomly grouped into four groups (I, II, III, and IV) of 35 chicks each. Each group was kept in a separate room. Each chicken in group I, II, and III was inoculated 0.1 ml containing 10^6 EID_{50}/0.1 ml of REV-free FWPV inoculum; REV-integrated FWPV inoculum; and PGPV inoculum, respectively, through the subcutaneous route at the ventral side of the neck. Chickens in group IV served as control, they were injected 0.1 ml of PBS subcutaneously. Blood samples were collected from wing vein of each chicken before inoculation and at 4-day intervals post-inoculation (pi) up to day 28 pi.

2.6.2.2 Clinical examination

From day zero to day 28 pi all chickens were clinically examined for development of clinical signs and deaths.

2.6.2.3 Necropsy

Post-mortem examination was carried out to all dead chickens using routine procedures described previously [37], so as to establish gross pathological changes in internal organs.

2.6.2.4 Examination of cutaneous nodular lesions for the presence of FWPV

Samples of lesions were examined by using both virus isolation and polymerase chain reaction (PCR) techniques. The samples were collected from chickens inoculated with REV-free FWPV or REV-integrated FWPV strains and stored at -20ºC. Thereafter virus inocula were prepared as described earlier [15]. The inocula were inoculated on SPF CAMs of 10-day-old chicken embryos. The infected embryos were incubated at 37ºC for 7 days. Thereafter they were cooled for 2 hours and the CAMs were removed. The CAMs thickness and the presence of characteristic pock lesions were evaluated grossly. Thereafter samples of genomic DNA were extracted from samples of CAMs containing virus cultures as described earlier [15], followed by examination for the presence of FWPV-specific DNA by using PCR as described previously [15] using gene specific oligonucleotide primers indicated in Table 1. After PCR the amplicons were run in a 1.5% agarose gel as described earlier [15] to determine the positive samples.

2.6.2.5 Antigens preparation and measurement of humoral immune responses

The antigens were prepared by using procedures described previously [12]. Humoral immune responses of chickens after inoculation with REV-free FWPV, REV-integrated FWPV or PGPV were measured by using enzyme-linked immunosorbent assay as described earlier [12].

2.7 Biosecurity Measures

In order to ensure that no environmental contamination occurred during *In vivo* experiment, the following biosecurity measures were taken:

i) A disinfectant footbath was available at the entry point of the experiment facility, each personnel involved during the experiment had to disinfect his/her feet before getting in or out of the experiment facility.
ii) Each personnel involved during the experiment had to put on protective clothing (i.e coveralls), gum boots and gloves that were devoted solely to the experiment facility.
iii) All other equipments and supplies such as feeders and drinkers used during the experiment were solely devoted to the experiment facility.
iv) Each personnel involved during the experiment observed personal hygiene which included frequent hand washing with warm water and soap, cleaning and disinfection of gum boots, coveralls and equipments used for post-mortem examination.
v) All dead chickens were burnt and ashes were buried. All chickens that were alive at the end of the experiment were euthanized. The euthanized birds were burnt and ashes were buried.
vi) After completion of data collection the experiment facility was disinfected.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 *In ovo* evaluation

3.1.1.1 Pathological findings

Gross examination of CAMs and chicken embryos revealed development of pock lesions on CAMs of ECEs inoculated with PGPV,

Table 1. Primers used in this study

Primer specific for amplification of	Primer sequence (5' → 3')	Expected fragment size	Reference
P4b gene of APVs	CAGCAGGTGCTAAACAACAA[a] CGGTAGCTTAACGCCGAATA[b]	578 bp	[38]

[a]Forward primer. [b]reverse primer

REV-free FWPV and REV-integrated FWPV strains. Apart from development of pock lesions the CAMs were thickened and haemorrhagic (Fig. 1). Embryos of ECEs inoculated with PGPV, REV-free FWPV and REV-integrated FWPV strains were haemorrhagic (Fig. 2). Table 2 shows virulence scores of PGPV and FWPV strains based on damage induced by the viruses on the embryos as indicated by the extent of haemorrhages on the embryos.

3.1.2 *In vivo* evaluation

3.1.2.1 Clinical findings

Cutaneous nodular lesions started to be evident on featherless parts of chickens such as eyelids and nostrils on day 10 pi, the lesions were evident in chickens inoculated with REV-free FWPV or REV-integrated FWPV. In addition to cutaneous nodules, on day 26 pi abnormal feathering started to be evident in five of 35 (14%) chickens inoculated with REV-integrated FWPV. Chicken deaths occurred on days 23, 25, 26, and 28 pi; where 3, 7, 6, and 4 chickens died, respectively. Chicken mortalities related to inoculation of each virus inoculum by day 28 pi were as shown in Table 3. No clinical signs were observed in chickens which served as control and those which were inoculated with PGPV. Virulence scores based on severity of disease as indicated by the number of cutaneous nodular lesions on chickens that developed fowl pox were as indicated in Table 4.

3.1.2.2 Necropsy findings

Gross examination of dead chickens revealed atrophy of bursa of Fabricius and thymus, spleen and liver necrosis, enlarged peripheral nerves, proventriculitis and enteritis.

3.1.2.3 Examination of cutaneous nodular lesions for the presence of FWPV

Successful isolation of FWPV from samples of cutaneous nodular lesions from chickens which developed fowl pox was indicated by the presence of characteristic pock lesions on CAMs. This was confirmed by detection of FWPV-specific DNA in samples of CAMs containing virus cultures, as indicated by migration of PCR products to approximately 578 bp which is an expected fragment size for the P4b gene amplicons for FWPV (Fig. 3).

3.1.2.4 Humoral responses

All chickens inoculated with the APVs showed antibody responses against the APVs antigens. The highest antibody titre was reached on day 20 pi. Chickens inoculated with PGPV had the highest antibody response, followed by chickens inoculated with REV-free FWPV. Chickens inoculated with REV-integrated FWPV had relatively low antibody titres compared to chickens inoculated with PGPV or REV-free FWPV. Chickens in the control group did not show any humoral response (Fig. 4). No antibodies against the APVs antigens were detected in chickens before inoculation.

3.2 Discussion

In the present study, virulence characteristics of Tanzanian strains of FWPV and PGPV in chickens were evaluated both *In ovo* and *In vivo*. *In ovo* evaluation revealed that chicken embryos inoculated with REV-integrated FWPV were more affected, as indicated by the extent of haemorrhages, as compared to chicken embryos inoculated with REV-free FWPV or PGPV. This suggests that REV-integrated FWPV are more virulent in susceptible chickens than REV-free FWPV.

In *In vivo* evaluation; clinical examination revealed that chickens inoculated with REV-integrated FWPV developed severe fowl pox, as indicated by high number of cutaneous nodular lesions, as compared to chickens inoculated with REV-free FWPV. In addition to that, 20 of 35 (57%) chickens inoculated with REV-integrated FWPV died. Apart from chicken deaths; abnormal feathering, which is considered a pathognomonic clinical sign of reticuloendotheliosis caused by REV [40], was also observed in five of 35 (14%) chickens

inoculated with REV-integrated FWPV. The relatively low antibody titres demonstrated in chickens inoculated with REV-integrated FWPV, as compared to antibody titres in chickens inoculated with PGPV or REV-free FWPV, could be attributed to the immunosuppressive effects of REV provirus integrated in the genome of FWPV to the chickens [41,42]. It is likely that the integration of a near-full length REV provirus in the genome of a FWPV strain that was inoculated in the chickens gave rise to infectious REV which, in turn, caused immunosuppression and reticuloendotheliosis that led to deaths of 20 of 35 (57%) chickens inoculated with REV-integrated FWPV. Necropsy findings ruled out diphtheritic and systemic forms of fowl pox, which are usually characterized by high mortality rates [10,11]. This suggests that gross lesions observed in internal organs during necropsy could be attributed to a near-full length REV provirus integrated in the genome of the FWPV strain inoculated to the chickens.

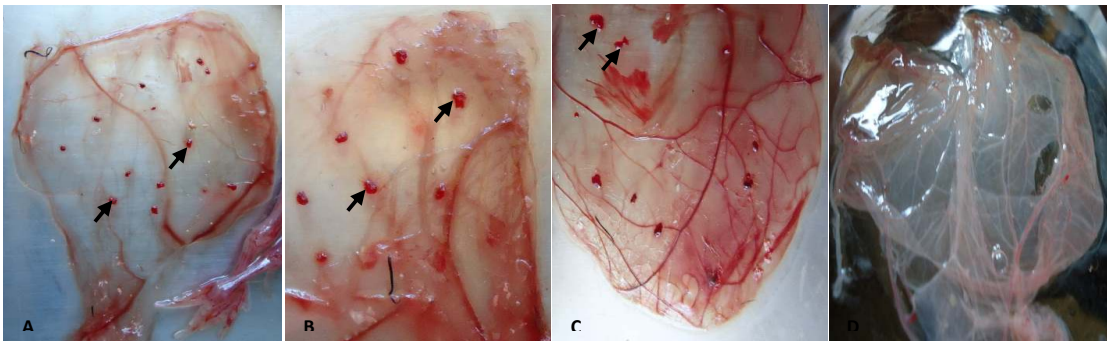

Fig. 1. A, B, C and D. Photographs showing gross pathological changes on CAMs of ECEs inoculated with Tanzanian strains of FWPV and PGPV. (A) pock lesions (arrows) on CAM of ECE inoculated with PGPV. (B) extensive pock lesions (arrows) and haemorrhages on CAM of ECE inoculated with REV-integrated FWPV. (C) pock lesions (arrows) and haemorrhages on CAM of ECE inoculated with REV-free FWPV. (D) CAM of ECE not inoculated with any APV strain (control)

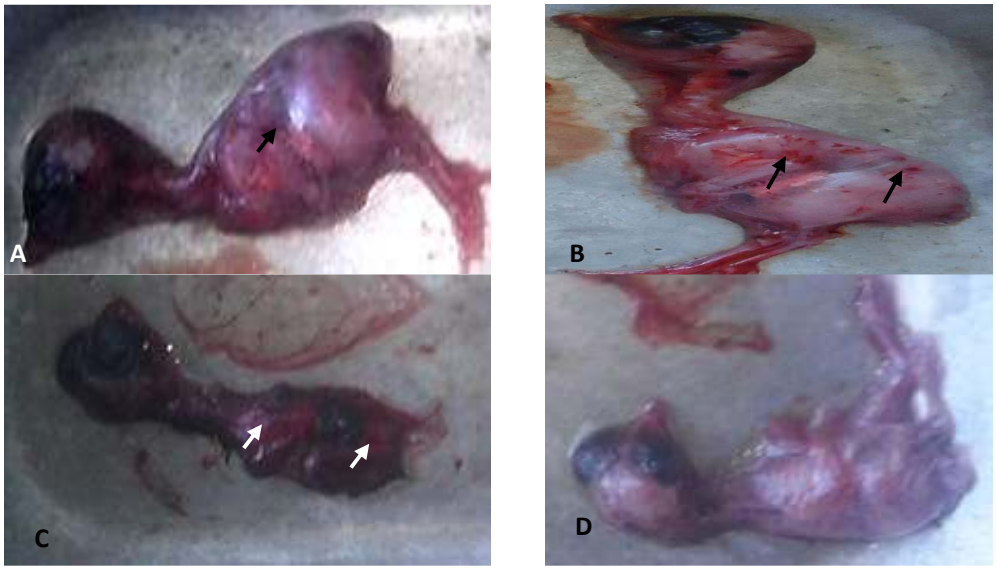

Fig. 2. A, B, C and D. Photographs showing haemorrhages on chicken embryos of ECEs inoculated with PGPV and FWPV strains. (A) few haemorrhagic foci (arrow) on embryo of ECE inoculated with PGPV. (B) embryos of ECE inoculated with REV-free FWPV demonstrating an increased number of haemorrhagic foci (arrows). (C) embryo of ECE inoculated with REV-integrated FWPV demonstrating generalized haemorrhage (arrows). (D) embryo of control ECE (not inoculated with any APV strain)

Table 2. Virulence scores based on the damage induced by PGPV and FWPV strains on chicken embryos as indicated by the extent of haemorrhage

Group	APV strains used for inoculation of ECEs	Virulence score	Reference
I	REV-free FWPV	2	[39]
II	REV-integrated FWPV	3	
III	PGPV	1	
IV	Control[a]	0	

[a]*ECEs were not inoculated with PGPV or FWPV strains. 0 = no haemorrhages, 1 = mild or slight haemorrhages, 2 = moderate haemorrhages, 3 = severe haemorrhages*

Table 3. Chicken mortalities related to inoculation of each virus inoculum by day 28 pi

Group	APV strains used for inoculation of chickens	Number of chickens			
		Inoculated	Infected	Contracted pox	Died
I	REV-free FWPV	35	35	35	0
II	REV-integrated FWPV	35	35	35	20
III	PGPV	35	35	0	0
IV	Control[a]	0	0	0	0

[a]*Chickens were not inoculated with any APV strain*

Table 4. Virulence scores based on infectivity or damage induced by PGPV and FWPV strains on chickens as indicated by the number of cutaneous nodular lesions by day 28 pi

Group	APV strains used for inoculation of chickens	Virulence score
I	REV-free FWPV	1
II	REV-integrated FWPV	2
III	PGPV	0
IV	Control[a]	0

[a]*Chickens were not inoculated with any APV strain. 0 = not affected, 1 = moderately affected (2 – 4 lesions), 2 = severely affected (> 4 lesions)*

Isolation of FWPV from samples of cutaneous nodular lesions collected from chickens inoculated with REV-free FWPV or REV-integrated FWPV, which was demonstrated by presence of characteristic pock lesions on CAMs, and thereafter confirmed by demonstration of FWPV-specific DNA in samples of CAMs containing virus cultures; indicates that the cutaneous nodular lesions on the chickens were attributed to inoculation of the chickens with the FWPV strains.

As opposed to a previous study by Weli et al. [12] which demonstrated a Norwegian strain of PGPV isolated from a Norwegian wood pigeon (*Palumbus palumbus*) to be pathogenic in chickens, and more virulent in chickens than a vaccine strain of FWPV; the present study has demonstrated that the Tanzanian strain of PGPV isolated from domestic pigeons (*Columba livia domestica*) can infect but does not cause disease (pox) in chickens, thus ruling out the possibility of a PGPV strain currently circulating in Tanzania being one of the attributing factors to the increased incidences and prevalence of fowl pox currently experienced in the country.

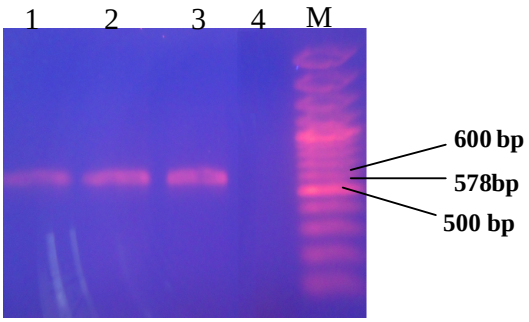

Fig. 3. Agarose gel electrophoresis of PCR products of DNA extracted from samples of cutaneous nodular lesions collected from chickens inoculated with REV-free FWPV (lane 1) and REV-integrated FWPV (lane 2). Lanes 3 and 4 are positive and negative control, respectively. Lane M is a molecular weight marker with 100-bp increments. The amplicons migrated to approximately 578 bp, which is an expected fragment size for the P4b gene of FWPV

With regard to humoral responses, antibody titres in chickens inoculated with REV-integrated FWPV were low as compared to antibody

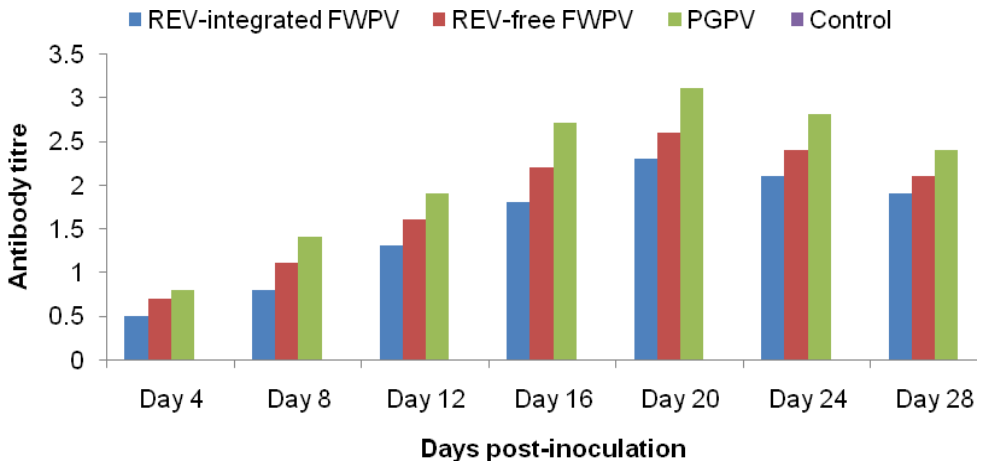

Fig. 4. Humoral responses of chickens following inoculation with REV-integrated FWPV, REV-free FWPV or PGPV

antibody titres in chickens inoculated with PGPV or REV-free FWPV. This could be attributed to immunosuppressive effect of REV provirus integrated in the genome of FWPV to chickens inoculated with REV-integrated FWPV.

4. CONCLUSION

Based on the findings of this study it is concluded that integration of REV provirus in the genome of FWPV renders REV-integrated FWPV strains more virulent in susceptible chickens than REV-free FWPV strains; and the Tanzanian strain of PGPV isolated from domestic pigeons is not pathogenic in chickens. This implies that the increased incidences and prevalence of fowl pox currently experienced in chickens in Tanzania are attributed to emergence of variant strains of FWPV which are REV-integrated. It also implies that, the Tanzanian strain of PGPV is not one of the attributing factors to the increased incidences and prevalence of fowl pox currently experienced in the country.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (National Institutes of Health [NIH] publication No. 85-23, revised 1985) were followed, and the Tanzania Animal Welfare Act of 2008 was complied. All experiments were approved by the Research, Publication and Ethics Committee of the FVM, SUA, Tanzania. The reference number for the ethical approval is SUA/VET/012/03.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Casadevall A, Pirofski L. Host-pathogen interactions: Redefining the basic concepts of virulence and pathogenicity. Infect Immun. 1999;67:3703-3713.
2. Casadevall A, Pirofski L. Host-pathogen interactions: The attributes of virulence. J Infect Dis. 2001;184:337-344.
3. Hendrickson RC, Wang C, Hatcher EL, Lefkowitz EJ. Orthopoxvirus genome evolution: The role of gene loss. Viruses. 2010;2:1933-1967.
4. 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.

5. Parker PG, Buckles EL, Farrington H, Petren K, Whiteman NK, Ricklefs RE, et al. 110 Years of Avipoxvirus in the Galapagos Islands. PLoS ONE. 2011;6(1):e15989. DOI: 10.137/journal.pone.0015989.
6. Gyuranecz M, Foster JT, Dán A, Ip HS, Egstad KF, Parker PG, et al. Worldwide Phylogenetic Relationship of Avian Poxviruses. J Virol. 2013;87(9):4938-4951.
7. Tripathy DN. Fowlpox. In: Cynthia MK, Scott L, editors. The Merck Veterinary Manual. 9th ed. Whitehouse station (NJ): Merck & Co. Inc; 2005.
8. Kulich P, Roubalova E, Dubska L, Sychra O, Šmíd B, Literák I. Avipoxvirus in blackcaps (*Sylvia atricapilla*). Avian Pathol. 2008;37(1):01-107.
9. 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 HCCU-01; 2010.
Available:http://www.hilo.hawaii.edu/hcsu/documents/TRHCSU-019Atkinson_Avian_Pox.pdf
(Accessed 23 April 2012)
10. van Riper C, Forrester D. Avian Pox. In: Thomas NJ, Hunter DB, Atkinson CT, editors. Infectious diseases of wild birds. Ames, Iowa, USA: Black well Publishing; 2007.
11. Alehegn E, Chanie M, Mengesha D. A systematic review of serological and clinicopathological features and associated risk factors of avian pox. Br J Poult Sc. 2014;3:78-87.
12. 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.
13. Tripathy DN, Schnitzlein WM, Morris PJ, Janssen DL, Zuba JK, Massey G, et al. Characterization of poxviruses from forest birds in Hawaii. J Wildl Dis. 2000;36:225-230.
14. Kim T, Tripathy DN. Evaluation of pathogenicity of avipoxvirus isolates from endangered Hawaiian wild birds in chickens. Avian Dis. 2006;50:288-291.
15. Masola SN, Mzula A, Tuntufye HN, Kasanga CJ, Wambura PN. Isolation and molecular biological characterization of fowlpox virus from specimen of cutaneous nodular lesions from chickens in several regions of Tanzania. Br Microbiol Res J. 2014;4(7):759-771.
16. Masola SN, Mzula A, Kasanga CJ, Wambura PN. Integration of reticuloendotheliosis virus in most of Tanzanian fowlpox virus isolates is not attributed to imported commercial fowl pox vaccines. Br Biotechnol J. 2014;4(6):659-669.
17. Masola SN, Mzula A, Mwega ED, Kasanga CJ, Wambura PN. Detection and genetic characterization of an avipox virus isolate from domestic pigeon (*Columba livia domestica*) in Morogoro region, Eastern Tanzania. Adv in Res. 2015;3(5):460-469.
18. 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.
19. Kulich P, Roubalova E, Dubska L, Sychra O, Šmid B, Literák I. Avipoxvirus in black caps (*Sylvia atricapilla*). Avian Pathol. 2008;37(1):101-107.
20. Prukner-Radovčic 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.
21. Shivaprasad HL, Kim T-J, Woolcock PR, Tripathy DN. Genetic and Antigenic Characterization of Poxvirus Isolate from Ostriches. Avian Dis. 2002;46:429-436.
22. García M, Narang N, Reed W, Fadly AM. Molecular characterization of reticuloendotheliosis virus insertions in the genome of field and vaccine strains of fowlpox virus. Avian Dis. 2003;47:343-354.
23. 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.
24. 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.
25. Terasaki T, Kaneko M, Mase M. Avian poxvirus infection in flamingos (*Phoenicopterus roseus*) in a zoo in Japan. Avian Dis. 2010;54:955-957.
26. Lawson B, Lachish S, Colvile KM, Durrant C, Peck KM, Toms MP, et al. Emergence of a novel avian pox disease in British tit species. PLoS ONE. 2012;7(11): e40176. DOI:10.1371/journal.pone.0040176.

27. Abdallah FM, Hassanin O. Detection and molecular characterization of avipoxviruses isolated from different avian species in Egypt. Virus Genes. 2013;46(1):63-70.
28. Hertig C, Coupar BET, Gould AR, Boyle DB. Field and vaccine strains of fowlpox virus carry integrated sequences from the avian retrovirus. Reticuloendotheliosis Virus. Virol. 1997;235:367-376.
29. Davidson I, Shkoda I, Perk S. Integration of reticuloendotheliosis virus envelope gene into the poutry fowlpox virus genome is not universal. J Gen Virol. 2008;89: 2456-2460.
30. 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.
31. Holt G, Krogsrud J. Pox in wild birds. Acta Vet Scand. 1973;14:201-203.
32. Cox WR. Avian pox infection in Canada goose (*Branta canadensis*). J Wildl Dis. 1980;16:623-626.
33. Winterfield RW, Reed W. Avian pox: Infection and immunity with quail, psittacine, fowl and pigeon pox viruses. Poult Sci. 1985;64:65-70.
34. Reed WM, Fatunmbi OO. Pathogenicity and immunological relationship of quail and mynah poxviruses to fowl and pigeon poxviruses. Avian Pathol. 1993;22:395-400.
35. Oxfam. Tanzania Agricultural Scale-Up Programme. Local Chicken Production for Local Markets: Mid-term Report, May to September; 2009.
Available:www.tanzaniafund.org/files/tz_fund_midterm_report_2009.pdf
(Accessed 11 April 2014)
36. Rural Livelihood Development Company. Transforming traditional poultry keeping into a commercial venture: The experience of Rural Livelihood Development Company with the comprehensive poultry rearing model; 2012.
Available:www.rldc.org/capex/poultry-keeping-capex.pdf
(Accessed 11 April 2014)
37. Nyaga PN, Bebora LC, Mbuthia PG, Njagi LW, Gathumbi PK. Diagnostic poultry post-mortem examination in Avian Medicine; 2014.
Available:www.cavs.uonbi.ac.ke/sites/default/files/cavs/vetmed/vetpathology/PPT
(Accessed 11 February 2015)
38. Huw Lee L, Hwa Lee K. Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. J Virol Methods. 1997;63:113-119.
39. Nguhiu-Mwangi J, Mbithi PMF. Prevalence of sole haemorrhages and its correlation with subclinical and chronic laminitis in dairy cows. Bull Anim Hlth Prod Afr. 2007;55:232-242.
40. Payne LN, Venugopal, K. Neoplastic diseases: Marek's disease, avian leukosis and reticuloendotheliosis. Rev Sci Tech Off Int Epiz. 2000;19(2):544-564.
41. Xue M, Shi X, Zhao Y, Cui H, Hu S, Cui E, et al. Effects of reticuloendotheliosis virus infection on cytokine production in SPF chickens. PLoS ONE. 2013;8(12):e83918. DOI:10.1371/journal.pone.0083918.
42. Wang G, Wang Y, Yu L, Jiang Y, Liu J, Cheng Z. New pathogenic characters of reticuloendotheliosis virus isolated from Chinese partridge in specific-pathogen-free chickens. Microb Pathog. 2012;53:57-63.

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