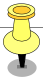

Tracking quality standard test for tissue culture pigeon pox virus Vaccine

تتبع معايير الجودة للقاح جدري الحمام النسيجي

By

Doaa I. Rady[1], Amany El Zieny[1], Zainab M. Ali[2], Ayatollah I. Ibrahim[3]

[1] Veterinary Serum and Vaccine Research Institute, Quality Control Laboratory (QCL), Agricultural Research Center (ARC), Cairo, Egypt

[2] Veterinary Serum and Vaccine Research Institute, New Castle Disease Virus Research Department, Agricultural Research Center (ARC), Cairo, Egypt

[3] Veterinary Serum and Vaccine Research Institute, Pox Vaccine Research Department, Agricultural Research Center (ARC), Cairo, Egypt

Doi: 10.21608/asajs.2024.386890

استلام البحث : ٧ / ٨ /٢٠٢٤

قبول النشـر : ٢٨ / ٨ / ٢٠٢٤

Rady, Doaa I. & El Zieny, Amany & Ali, Zainab M. & Ibrahim, Ayatollah I. (2024). Tracking quality standard test for tissue culture pigeon pox virus Vaccine. ***The Arab Journal of Agricultural Sciences***, Arab Institute for Education, Science and Arts, Egypt, 7 (24), 271 -288.

http://asajs.journals.ekb.eg

Tracking quality standard test for tissue culture pigeon pox virus Vaccine

Abstract :

The aim of the study is construction of a standard curve for molecular titration of the Pigeon Pox virus and uses it to quantify the virus concentrations in vaccine samples. The standard curve showed a linear relationship between cycle threshold values (Ct value) and Log10 virus titers, indicating the accuracy of this method. The amplification reactions of the Pigeon Pox vaccines samples and Fowl Pox virus exhibited specific amplification products without anomalies, as confirmed by melting curve analysis. The melting curve analysis also revealed the presence of two distinct viral strains or variants in the Pigeon Pox vaccine samples, each with a slightly different melting temperature, while the Fowl Pox virus sample contained a single viral strain or variant. The Ct values obtained from the rt-PCR analysis provided information about the amount of viral RNA present in the tested samples, with lower Ct values indicating higher concentrations of viral RNA. Additionally, the study highlights the need for further investigations about into the observed more than 100% efficiency in the standard curve to ensure result accuracy. Overall, these findings contribute to the understanding of virus concentrations in the tested vaccines and the specificity of the amplification reactions.

المستخلص:

ينتقل فيروس جدري الحمام في المقام الأول عن طريق الاتصال المباشر، مثل نتف الريش، أو بشكل غير مباشر من خلال لدغات البعوض. يمكن أن يسبب الفيروس عدوى موضعية على جسم الطائر، ولكنه يمكن أن ينتشر أيضًا من خلال الدم إلى الكبد ونخاع العظام. شكل تحصين القطعان التجارية دورًا حاسمًا في الحد من الخسائر الناجمة عن مرض جدري الحمام في أسراب الطيور. في صناعة الدواجن، يعد التطعيم بسلالات فيروس جدري الحمام الحية المنتجة في الغشاء المشيمي

ISSN: 2537-0804 eISSN: 2537- 0855

(CAM)لبيض الدجاج المخصب (ECE) أو الخلايا الليفية الجنينية للدجاج (CEF) الإجراء الوقائي الأساسي ضد فيروس جدري الحمام (PPV) . من الضروري التحقق من فعالية دفعة اللقاح، والتي تتضمن المعايرة قبل التطعيم. تتضمن الطرق التقليدية لمعايرة لقاح جدري الحمام استخدام زراعة الأنسجة لتقدير الجرعة، ولكن هذا النهج يمكن أن يكون مستهلكًا للوقت وشاقًا. تم استخدام تفاعل البلمرة المتسلسل الكمي في الوقت الحقيقي (rt-qPCR) للكشف عن وتوصيف ومعايرة فيروسات جدري الحمام المختلفة، مما يوفر الوقت والدقة مقارنة بالطرق التقليدية. تهدف هذه الدراسة إلى إنشاء اختبار RT-qPCR باستخدام Sybr green للكشف عن لقاح جدري الحمام وتحديد كميته بالإضافة إلى التمييز بين جدري الحمام وجدري الطيور. باختصار، تصف النتائج المقدمة في الدراسة الحالية المعايرة الجزيئية لفيروس جدري الحمام باستخدام تفاعل البلمرة المتسلسل العكسي وبناء منحنى قياسي. تم تحديد تركيزات الفيروس في لقاحات جدري الحمام وفيروس جدري الطيور باستخدام هذه الطريقة. وأكدت الدراسة أيضًا خصوصية تفاعلات البلمرة المتسلسل من خلال تحليل منحنى الانصهار، ومع ذلك، يجب التحقيق بدقة في ملاحظة كفاءة تزيد عن ١٠٠٪ في المنحنى القياسي لضمان دقة النتائج.

INTRODUCTION

Pox disease is an infectious condition affecting both domestic and wild birds of various ages, genders, and breeds. It is caused by a virus known as pox virus, which belongs to the Avipoxvirus genus within the Poxviridae family and the Chordopoxvirinae subfamily. The Avipoxvirus genus comprises 25 distinct members (**Weli and Tryland, 2011).** The disease is characterized by the presence of proliferative and nodular lesions on the skin's feather-free areas, or the formation of fibro-necrotic and proliferating regions in the mouth, esophagus, and mucous membrane of the upper respiratory tract **(Rebeka et al., 2019).**

Avian pox viruses (APVs) cause significant economic losses due to increased bird mortality, reduced growth, emaciation, decreased egg production, and the condemnation of affected bird carcasses with multiple nodular skin lesions, cachexia, and an unappealing appearance **(WOAH, 2023)**. Pigeon poxvirus is primarily transmitted through direct contact,

such as fighting or feather picking, or indirectly through mosquito bites. The virus can cause a localized infection at the entry point, but it can also spread systemically through the blood to the liver and bone marrow **(Doneley, 2006**). Vaccination of commercial flocks has played a crucial role in reducing losses caused by the disease in chicken populations **(Odoya, 2006)**.

For over half a century, fowl pox or pigeon pox viruses have been routinely used to prevent fowl pox in commercial poultry in endemic areas **(Siddique et al., 2011)**, or when previous flocks have been diagnosed with the virus (**OIE, 2019**). The virion of APVs consists of a centrally located biconcave core or nucleoid with two lateral bodies in each concavity, enclosed by an envelope. The viral DNA genome, approximately 260 kbp in size, encodes over 250 genes **(Tadese and Reed, 2003)**.

Polymerase chain reaction (PCR) is a valuable tool for detecting very low levels of viral nucleic acids. The orthologue of the vaccinia virus A3L gene, fpv167, which encodes the 4b core protein, has been successfully used as a diagnostic marker for APV infections. This marker not only detects the presence of the virus but also allows for the characterization of avian pox **viruses (Binns et al., 1989; Huw Lee and Hwa Lee, 1997; Tadese and Reed, 2003)**. Phylogenetic studies based on the P4b coding gene have distinguished APVs into five clades: A, B, C, A1-4, and B1-2, providing further insights into the genetic diversity of APVs **(Lüschow et al., 2004; Weli et al., 2005; Jarmin et al., 2006)**.

In the poultry industry, vaccination with live pigeon pox virus strains produced in chorioallantoic membrane (CAM) of embryonated chicken eggs (ECE) or chicken embryo fibroblast (CEF) cells is the primary prophylactic measure against pigeon

ISSN: 2537-0804 eISSN: 2537- 0855

pox virus (PPV) (**Tripathy and Reed, 2003**). Before production and field vaccination, it is essential to validate the efficacy of the vaccine batch, which involves titration. Traditional methods for pigeon pox vaccine titration involve using tissue culture to estimate TCID50, but this approach can be time-consuming and laborious. Real-time quantitative PCR (rt-qPCR) has been used to detect, characterize, and titrate different APVs, offering increased sensitivity compared to conventional PCR methods (**Farias et al., 2010; Davidson et al., 2015**).

This study aims to established a Sybr green RT-qPCR assay to detect and quantitatively titrate pigeon pox vaccine in addition to differentiate between PPV and FWPV.

MATERIAL AND METHODS

1- cell culture:

African green monkey kidney cell line (Vero cell line) was propagated in Hank's buffer-based MEM (Gibco, USA), supplemented with 10% FBS and 100 U/ml penicillin, 100 µg/ml streptomycin and 25 iu/ml mycostatin at 37°C provided by DPVR, VSVRI was used for determination of Fowl and Pigeon Pox virus infectivity by estimating the virus titer as 50% tissue culture infective dose ($TCID_{50}$) using standard methods (**Reed and Muench, 1938**).

2-Viruses:

2.1-Reference Pigeon Pox virus strain:

PPV namely PPLH, of 10^6 $TCID_{50}$/ml titer previously characterized (Ibrahim et al., 2015) was kindly provided by DPVR, VSVRI

2.2-Fowl pox virus:

A Fowl Pox virus strain, namely FPLH, characterized by **Ibrahim et al. (2015)** was used as a P4b core protein positive control Pox virus in the RT-qPCR assay.

3-Piegon pox vaccine samples:

ISSN: 2537-0804 eISSN: 2537- 0855

Two lyophilized Pigeon Pox vaccine samples representing two different produced patches, namely vaccines 1 and 2, of the same seed virus, that have $10^{4.5}$ and 10^{5} $TCID_{50}$/ml approximate infectivity titers, respectively were used to determine their virus titer using RT-qPCR.

4-Virus titration:

This test was test used for determination of pigeon pox virus titer on Vero tissue culture where 5 vaccine vials were reconstituted with 1 ml of sterile MEM /vial followed by pooling of the five reconstituted vaccine vials into one vial. The used method was the microtiter one carried out according to **(Rossiter and Jessette (1982))** and the virus titer was expressed as log10 $TCID_{50}$/ml according to **Reed and Muench (1938)**

5-Nucleic acid extraction:

DNA extraction from the tested samples was conducted using the QIAamp MinElute Virus Spin Kit (Qiagen, Germany, GmbH). In brief, 200 µl of the sample suspension (rehydrated lyophilized vaccine) was mixed with 25 µl of Qiagen protease and 200 µl of AL lysis buffer, followed by incubation at 56°C for 15 minutes. After incubation, 250 µl of 100% ethanol was added to the lysate and incubated at room temperature for 5 minutes. The lysates were then applied to QIAamp MinElute columns and centrifuged for 1 minute at 6000 x g, as per the manufacturer's instructions. Washing steps were performed using 500 µl of AW1 and AW2 buffers, followed by centrifugation. To ensure complete drying of the column silica membrane, high-speed centrifugation was applied for 3 minutes. Finally, the nucleic acid was eluted with 100 µl of AVE elution buffer..

6-Oligonucleotide Primer:

One primer pair flanking a 578 bp P4b protein-coding gene and supplied from Metabion (Germany) is listed in Table (1).

ISSN: 2537-0804 eISSN: 2537- 0855

7-SYBR green RT-PCR:

In a 25-µl reaction volume, primers were used along with 12.5 µl of the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.5 µl of each primer at a concentration of 20 pmol, 8.5 µl of water, and 3 µl of DNA template. To establish a titration standard curve, a serial ten-fold dilution of DNA extracted from a standard pigeon pox virus with a titer of 10^6 TCID50/ml was prepared. Three dilutions corresponding to titers of 10^5, 10^3, and 10^1 TCID50/ml were used to generate the standard curve. Concurrently, DNA templates extracted from three samples, specifically fowl pox virus vaccines and two pigeon pox virus vaccines (vaccine 1 and vaccine 2), were analyzed using qrt-PCR reactions performed on a Stratagene MX3005P real-time PCR machine (Stratagene, USA). The SYBR Green quantitative real-time PCR targeting the 4b core protein gene, as described by Prukner Radović et al. (2006), was used for the detection and quantification of pigeon pox virus. Amplification curves, Ct values, and virus titers were determined using the Stratagene MX3005P software

8-Three points standard curve

Pigeon Pox virus vaccine previously titrated using Vero tissue culture cells and having a titer of 10^6 $TCID_{50}$/ml was used to perform real-time standard curve for molecular titration of the virus using SYBR green rt-PCR. Three ten-fold dilutions of the standard vaccine, designated as dilutions 1, 3, and 5, corresponding to 10^5, 10^3 and 10^1 $TCID_{50}$/ml, respectively, were selected to perform a three points standard curve using real time PCR.

ISSN: 2537-0804 eISSN: 2537- 0855

Table (1): Primer sequence and real-time qPCR thermal cycling used to amplify 578 bp of avian pox P4b core protein.

Primer Sequence	Amplified segment length	Amplification protocol (40 cycles)				Dissociation curve (1cycle)		
		Primary denaturation	Denaturation	Annealing (optics on)	Extension	Secondary Denaturation	Annealing	Final denaturation (optics on)
5'-CAG CAG GTG CTA AAC AAC AA-3' 5'-CGG TAG CCT TAA CGC CGA ATA-3'	578	94˚C 5 min.	94˚C 5 min.	53˚C 40 sec.	72˚C 40 sec.	94˚C 1 min.	53˚C 1 min.	60-94˚C in 0.5˚C increment (10 sec/step).

Melting curve of amplified products

To analyze specificity and identity of the amplified products, a melting curve thermal profile was implemented following real-time qPCR. The dissociation curve was generated by heating from 60°C to 94°C with 0.5°C increment and 10 seconds dwell time and sample read (optics on) at each temperature.

RESULTS

1- **Three points standard curve**

Three Ct values were obtained: 16.36, 22.17, and 27.85, which corresponded to viral dilutions 1, 3, and 5, respectively **(Table 2 and Figure 1**). A three-point linear standard curve was generated, correlating Ct values and Log10 virus TCID50 titer of the Pigeon pox virus, with an efficiency of over 100% (Figure 1). Virus titers can be determined using the following equation derived from the standard curve: $Y = -2.859 \times \text{Log}(X) + 30.44$,

where Y represents the Ct value and X represents the corresponding virus titer.

2. Determination of the virus concentration in Pigeon Pox virus vaccines 1 and 2 and Fowl Pox virus using rt-PCR

Using qrt-PCR**,** vaccines 1 and 2 amplified with 13.64 and 13.49 Ct values, respectively, corresponding to 7.561 x 10^5 and 8.533 x 10^5 $TCID_{50}$ titers. Fowl pox virus was amplified with 20.83 Ct value corresponding to 2.308 x 10^3 $TCID_{50}$ titer (Table 2).

3. Melting curve identity of rt-qPCR amplicons

The melting curve analysis of the rt-qPCR amplicons displayed distinct and singular peaks for each sample, indicating the absence of any irregularities. The melting curves for both pigeon pox vaccines 1 and 2 exhibited two distinct peaks at melting temperatures (Tm) of 85.75°C and 85.80°C, respectively. In contrast, the fowl pox virus melting curve displayed a single peak at a Tm of 87.65°C. **(Table 2).**

Table (2): Ct values, melting temperatures and virus titers obtained for Pigeon pox and fowl pox viruses.

Sample No	Sample ID.	SYBR green Pox 4b core Ct	Tm (°C)	Titer ($TCID_{50}$/ml)
1	Fowl pox vaccine	20.83	87.65	2.308 x 10^3
2	Pigeon pox virus dil. 1	16.36	85.83	**10^5**
3	Pigeon pox virus dil. 3	22.17	85.83	**10^3**
4	Pigeon pox virus dil. 5	27.85	85.83	**10^1**
5	Pigeon pox Vaccine 1	13.64	85.75	7.561 x 10^5
6	Pigeon pox Vaccine 2	13.49	85.80	8.533 x 10^5

ISSN: 2537-0804 eISSN: 2537- 0855

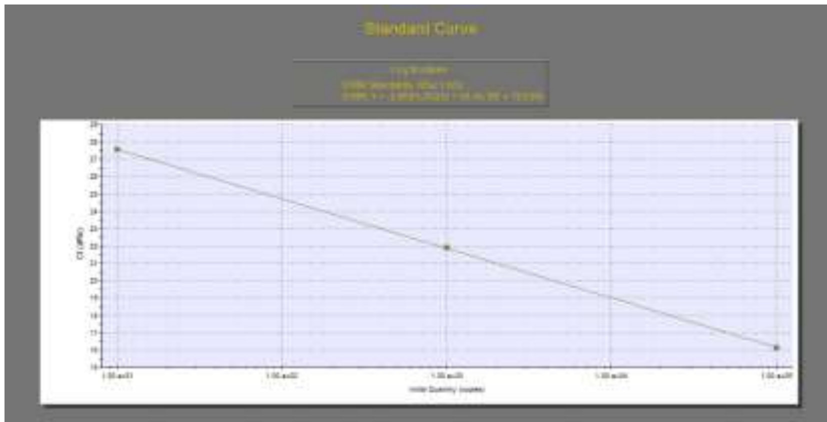

Figure (1): A three-point linear standard curve was established using three tenfold dilutions of Pigeon Pox virus vaccine, which corresponded to virus concentrations of 101, 103, and 105 TCID50

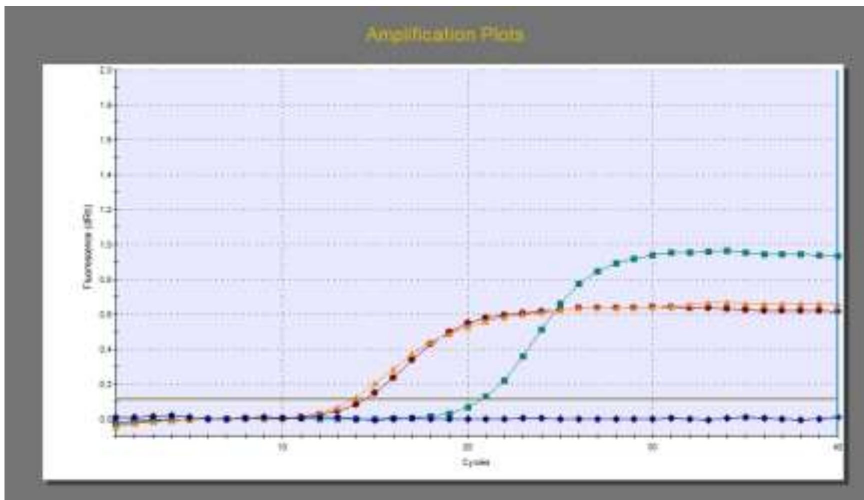

Figure (2): Amplification plots of three samples, Fowl pox virus (green squares line), pigeon pox vaccine 2 (orange triangles line), and pigeon pox vaccine 1 (brown circles line). While amplicons fluorescence of Fowl pox virus sample detected at cycle 20, amplicons of both pigeon pox vaccines 1 and 2 started

to fluoresce at cycle 13. No fluorescence obtained for negative control sample (dd H_2O) (blue diamond's line).

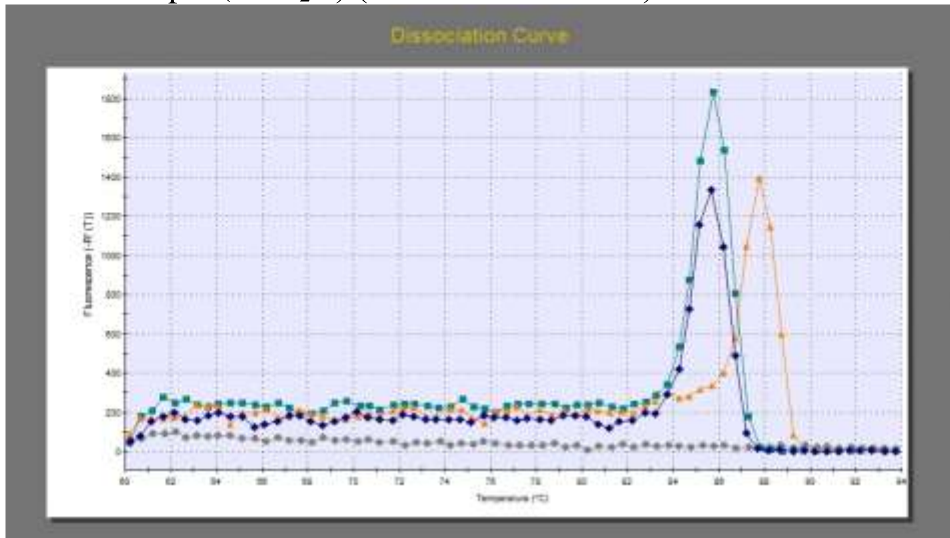

Figure (3): Dissociation (melting) curve of fowl pox virus (orange triangles line), pigeon pox vaccines 1 and 2 (blue diamonds line- , and green squares line), respectively. Grey circles line - represents negative control.

DISCUSSION

The provided results describe the determination of virus concentrations in Pigeon Pox virus vaccines and Fowl Pox virus using real-time polymerase chain reaction (rt-PCR) and the construction of a standard curve for molecular titration of the Pigeon Pox virus.

A three-point standard curve was constructed using real-time PCR and three ten-fold dilutions of the Pigeon Pox virus vaccine. The dilutions (10^{-1}, 10^{-3} and 10^{-5}) corresponded to virus titers of 10^{1}, 10^{3}, and 10^{5} $TCID_{50}$/ml, respectively. Ct values of 16.36, 22.17, and 27.85 were obtained for the dilutions 10^{-1}, 10^{-3} and 10^{-5}, respectively.

ISSN: 2537-0804 eISSN: 2537- 0855

The standard curve showed a linear relationship between Ct values and Log10 virus titers. Additionally, it demonstrated more than 100% efficiency.

Using rt-PCR, Pigeon Pox vaccines 1 and 2 were amplified with Ct values of 13.64 and 13.49, corresponding to virus titers of 7.561 x 10^5 and 8.533 x 105 $TCID_{50}$, respectively. Fowl Pox virus was amplified with a Ct value of 20.83, indicating a virus titer of 2.308 x 10^3 $TCID_{50}$. **(Iskra et al., 2023; Weli et al., 2011).**

These results demonstrate the quantification of virus concentrations in the Pigeon Pox vaccines and Fowl Pox virus using rt-PCR. The Ct values obtained from the amplification reactions can be correlated with the virus titers using the standard curve equation as stated by **(Gelaye et al.,2013)**

Melting curve analysis was performed to assess the specificity and identity of the rt-qPCR amplicons. Each sample showed a defined single peak without anomalies, indicating the presence of specific amplification products.
The melting curves of Pigeon Pox vaccines 1 and 2 displayed two single peaks at Tm (melting temperature) values of 85.75 and 85.80°C, respectively. The Fowl Pox virus melting curve exhibited a single peak at a Tm value at 87.65°C similar findings were reported by **fahmy et al. (2009).**

Based on the results obtained from rt-PCR analysis, it can be determined that Pigeon Pox virus vaccines 1 and 2 have virus concentrations corresponding to 7.561 x 10^5 and 8.533 x 10^5 $TCID_{50}$ titers, respectively. Fowl Pox virus has a concentration corresponding to a 2.308 x 10^3 $TCID_{50}$ titer.

The Ct values obtained from qrt-PCR analysis provide an indication of the amount of viral RNA present in the samples. Lower Ct values indicate a higher concentration of viral RNA. In this case, vaccine 1 had a slightly lower Ct value (13.64)

ISSN: 2537-0804 eISSN: 2537- 0855

compared to vaccine 2 (13.49), suggesting a slightly higher concentration of Pigeon Pox virus in vaccine 1.

The melting curve analysis of the rt-qPCR amplicons provides information about the specificity and purity of the amplified products. Each sample produced a defined single peak without anomalies, indicating that the amplification was specific to the target viruses.

The melting curves of both Pigeon Pox vaccines 1 and 2 showed two single peaks at 85.75 and 85.80°C Tm. This suggests the presence of two distinct viral strains or variants in the vaccines, each with a slightly different melting temperature.

On the other hand, the Fowl Pox virus melting curve revealed a single peak at 87.65°C Tm, indicating the presence of a single viral strain or variant in the sample.

Conclusion:

Overall, these results provide information about the virus concentrations in the Pigeon Pox vaccines and Fowl Pox virus sample, as well as the specificity and purity of the amplification products.

These melting curve results confirm the specificity of the PCR amplification for the respective viruses. The distinct Tm values observed for each virus suggest differences in the nucleotide sequences or amplicon characteristics, allowing their differentiation based on melting temperature.

In summary, the results presented in the current study describe the molecular titration of Pigeon Pox virus using rt-PCR and the construction of a standard curve. The virus concentrations in Pigeon Pox vaccines and Fowl Pox virus were determined using this method. The study also confirmed the specificity of the amplification reactions through melting curve

ISSN: 2537-0804 **eISSN: 2537- 0855**

analysis. However, the observation of more than 100% efficiency in the standard curve should be thoroughly investigated to ensure the accuracy of the results.

ISSN: 2537-0804 eISSN: 2537- 0855

REFERENCES

Binns M.M., Boursnell M.E.G. Tomley F.M. and Campbell J. (1989). Analysis of the Fowl pox virus gene encoding the 4b core polypeptide and demonstration that it possesses efficient promoter sequences. Virol., 170 (1): 288-291.

Davidson I, Raibstein, I and Altory A (2015): Differential diagnosis of fowl pox and infectious laryngotracheitis viruses in chicken diphtheritic manifestations by mono and duplex real-time polymerase chain reaction. Avian Pathology, 44 (1): 1–4.

Doneley B (2006). Pigeon medicine and surgery. North Am. Vet. Conf., 20: 1525-1530.

Fahmy, H. A., Arafa, A., Kanawaty, Z. R., & Mahmoud, A. H. (2009). Molecular detection of pox virus in pigeon. *Veterinary Medical Journal Giza*, *57*(3), 253-262.

Farias M, LaPointe D, Atkinson C, Czerwonka C, Shrestha R and Jarvi S (2010): Taqman Real-Time PCR Detects Avipoxvirus DNA in Blood of Hawai`i `Amakihi (Hemignathus virens). PLoS ONE, 5 (5): e10745.

Gelaye, E., Lamien, C. E., Silber, R., Tuppurainen, E. S., Grabherr, R., & Diallo, A. (2013). Development of a cost-effective method for capripoxvirus genotyping using snapback primer and dsDNA intercalating dye. *PloS one*, *8*(10), e75971.

Huw Lee, L. and Hwa Lee, K. (1997). Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. J Virol Methods, 63: 113–119.

ISSN: 2537-0804 eISSN: 2537- 0855

Ibrahim, S. M., Abd El-Razek. B. Abd El-Razek . Hanan. M. El-Zahed Amal. A. Fatouh and Ayatollah. I. Ibrahim (2015). Comparative molecular study on Avian pox viruses. Egyptian J. Virol., Vol. 12: 12-21.

Iskra, S., Kolyovska V, Ilieva I, Markova T, Dimitrova-Dikanarova D, Hadjiolova R. (2023). The Development of Methods for the Production of New Molecular Vaccines and Appropriate RNA Fragments to Counteract Unwanted Genes: A Pilot Study. *Vaccines*. 11(7):1226. https://doi.org/10.3390/vaccines11071226

Jarmin S., Manvell R., Gough R.E., Laidlaw S.M. and Skinner M.A. (2006): Avipoxvirus phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades. J. Gen. Virol., 87 (Pt 8): 2191-2201.

Lüschow D., Hoffmann T. and Hafez H.M. (2004): Differentiation of avian poxvirus strains on the basis of nucleotide sequences of *4b* gene fragment. Avian Dis., 48 (3): 453-462.

Odoya EM, Abegunde A, Omotainse SO, Gwankat E, and Okapara UG (2006): Outbreak of turkey pox disease in fowl pox-vaccinated poults in Vom Plateau State, Nigeria Afr J Clin Exp Microbiol, 7: 136–8; https://doi.org/10.4314/ajcem.v7i2.7443

OIE. 2019. OIE Terrestrial manual, chapter 3.3.10 Fowl pox. 906-913.

Prukner-Radovčić, E., Lüschow, D., Grozdanić, I. C., Tišljar, M., Mazija, H., Vranešić, Ł., & Hafez, H. M. (2006). Isolation and molecular biological investigations of avian

ISSN: 2537-0804 eISSN: 2537- 0855

poxviruses from chickens, a turkey, and a pigeon in Croatia. Avian Diseases, 50(3), 440-444.

Rebeka, S., Nazir, K. N. H., Rahman, M. T., Nipa, S. A., Rahman, M. M., Soma, S. S., & Rahman, M. B. (2019). Isolation and molecular detection of fowl pox and pigeon pox viruses for the development of live attenuated vaccine seeds from the local isolates. *Journal of the Bangladesh Agricultural University*, *17*(2), 211-219.

Reed LJ and Muench H. (1938): A simple method of estimating fifty per cent endpoints. Am. J. Hyg., 27: 493–497.

Rossitter, P.B. and Jessett, D.M. (1982): Microtiter techniques for the assay of rinderpest virus and neutralizing antibody; Res. Vet. Sci.,3 (2): 253

Siddique, A. B., Hossain, F. M. A., and Zinnah, M. A. (2011). Determination of host specificity of pigeon pox and fowl pox viruses isolated from a field outbreak. *Bulgarian J. Vet. Med*, *14*(4), 209-214.

Tadese, T. and Reed, W. M. (2003): Detection of specific reticuloendotheliosis virus sequence and protein from REV-integrated fowlpox virus strains. J Virol Methods, 110: 99–104.

Tripathy D.N. and Reed W.M. (2003): ‘Pox’, in Saif Y.M., Barnes H.J., Glisson J.R., Fadly A.M., McDougald L.R. & Swayne D.E. (eds.), *Diseases of poultry*, 11th edn., pp. 253–269, Iowa State University Press, Ames.

Weli, S. C., & Tryland, M. (2011). Avipoxviruses: infection biology and their use as vaccine vectors. Virology Journal, 8(1), 1-15.

ISSN: 2537-0804 eISSN: 2537- 0855

Weli, S. C., Nilssen, Ø., and Traavik, T. (2005). Avipoxvirus multiplication in a mammalian cell line. Virus research, 109(1), 39-49.

WOAH 2023. (World Organization of Animal Health) Chapter 3.3.10 Pigeon Pox.

ISSN: 2537-0804 eISSN: 2537- 0855