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Standardisation of a multiplex PCR for detection of circovirus and adenovirus in pigeons#

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Abstract

Young Pigeon Disease Syndrome (YPDS) is an emerging condition in pigeons characterised by high morbidity and mortality, with multiple viral agents implicated in its aetiology. Among these, pigeon circovirus (PiCV) and fowl adenovirus (FAdV) are considered important contributors, but reports from India remain limited. This study investigated their presence in YPDS suspect pigeons and standardised a multiplex PCR (mPCR) assay for their simultaneous detection. A total of 75 clinical samples, including tissues (liver, spleen, bursa of Fabricius, intestine, lungs, kidney and brain), feathers and cloacal swabs from 27 affected pigeons, were screened using uniplex PCR assays targeting the PiCV capsid and FAdV hexon genes. Representative amplicons were sequenced, and BLAST analysis confirmed their identity with reference PiCV and FAdV sequences. Uniplex PCR detected PiCV DNA in 4 samples (5.3%) and FAdV DNA in 5 tissue samples (23.8%). Sequencing of representative amplicons confirmed their identity, showing nucleotide similarities of 95.7-99.5% with published PiCV and FAdV reference strains. Based on these findings, a multiplex PCR (mPCR) assay was standardised with optimised primer concentrations and cycling conditions, which yielded distinct, reproducible amplicons for both viruses without cross-reactivity. The detection of PiCV and FAdV in YPDS suspected pigeons highlighted their role in the syndrome, while the standardised mPCR provided a rapid, reliable and cost-effective diagnostic tool for simultaneous identification of these agents. This method offers clear advantages over uniplex PCR by reducing time and reagent use, and it can support routine diagnosis and epidemiological surveillance of pigeon populations affected by YPDS.

Key words: Pigeon circovirus, Fowl adenovirus, Young pigeon disease syndrome, Multiplex PCR, Kerala

Pigeon farming is emerging as a sustainable microenterprise in Kerala, mainly for ornamental and sporting purposes, with most owners maintaining 10-100 birds (Prasad *et al*., 2017). However, the sector's growth is hindered by infectious diseases, particularly Young Pigeon Disease Syndrome (YPDS), which affects birds aged 4-12 weeks and causes 20-50% morbidity and mortality (Franciosini *et al*., 2005; Duchatel *et al*., 2006; Mengfan *et al*., 2023). Clinical signs are non-specific including anorexia, diarrhoea, vomiting, crop distension, lethargy and poor performance, which complicates early diagnosis and control.

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The aetiology of YPDS is multifactorial involving a range of viral, bacterial and protozoal agents. Among these, pigeon circovirus (PiCV) is recognised as a key pathogen due to its immunosuppressive effects. PiCV, a small single-stranded DNA virus of the family *Circoviridae*, targets lymphoid tissues, causing lymphoid depletion and B-cell apoptosis that predispose birds to secondary infections (Schmidt *et al*., 2008; Stenzel *et al*., 2020). It is transmitted via faecal-oral, respiratory and vertical routes (Duchatel *et al*., 2006, 2009). PCR assays targeting the *Cap* or *Rep* genes are routinely used for detection, while advanced molecular tools such as qPCR and next-generation sequencing provide improved strain characterisation (Stenzel *et al*., 2020; Kong *et al*., 2021). Fowl adenoviruses (FAdVs), non-enveloped double-stranded DNA viruses of the genus *Aviadenovirus*, are widespread among avian species including pigeons (Davison *et al*., 2003; Mo, 2021). They are classified into five species (A-E) with 12 serotypes (Marek *et al*., 2013). In pigeons, adenoviral infections have been described as adenovirosis type I (enteric form) and type II (hepatic form) (Coussement *et al*., 1984; De Herdt *et al*., 1995). Molecular assays targeting the conserved *hexon* gene have replaced older diagnostic methods, offering rapid and specific detection (Meulemans *et al*., 2001; Gunes *et al*., 2012; Ugwu *et al*., 2023).

Polymerase chain reaction (PCR) remains the most sensitive and specific method for viral detection. Although uniplex PCR is effective for single agents, multiplex PCR (mPCR) allows simultaneous amplification of multiple targets, making it a faster and more economical diagnostic tool (Frieck *et al*., 2008). This study aimed to detect PiCV and FAdV in YPDS-suspect pigeons and standardise a mPCR assay for their concurrent detection.

Materials and methods

Ethical statement

All sample collection and laboratory procedures were conducted in accordance with institutional ethical guidelines approved by the College of Veterinary and Animal Sciences (CVAS), Mannuthy, Kerala. The study was approved by the Institutional Biosafety Committee (IBSC), College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy (Order No. Acad (3)999/2012, dated 21/03/2025).

Sample collection and DNA extraction

A total of 75 clinical samples were collected from 27 pigeons, comprising 21 dead birds and 6 ailing birds, presented to the Departments of Veterinary Microbiology and Veterinary Pathology, CVAS, Mannuthy, during the period from January 2024 to August 2025 (Table.1). All birds showed clinical signs suggestive of YPDS such as diarrhoea, anorexia and ruffled feathers. From the dead birds, tissue samples were collected during necropsy, including the liver, spleen, bursa of Fabricius, intestine, lungs, kidney and brain. In addition, cloacal swabs and feather samples were collected from both dead and ailing birds. All tissue samples were stored in sterile phosphate-buffered saline (PBS, pH 7.2) at -20°C, while feather and cloacal swab samples were placed in separate sterile containers prior to further processing. The DNA was extracted from tissues and feather samples using the DNeasy blood and tissue kit (Qiagen, Germany) and from cloacal swabs using the QIAamp fast DNA stool mini kit (Qiagen, Germany), following the respective manufacturer's protocols.

Uniplex PCR detection of PiCV and FAdV

Uniplex PCR assays were performed to detect PiCV and FAdV separately using primers designed in this study. For PiCV, primers targeting the *capsid* gene (forward: 5′- ATTTGAAAGGTTTTCAGCCTGGCA-3′ and reverse: 5′- TAGGAGACGAAGGACACGCCTCTA-3′) were used whereas for FAdV, primers targeting the *hexon* gene (forward: 5′- GGAGGTCGCTCTGAATTAAGGAAT -3′ and reverse: 5′- AGTGAAGACGSCACAACAT -3′) were used. Each PCR reaction was carried out in a total volume of 20 µL containing 10 µL of 2X EmeraldAmp GT PCR Master Mix (Takara Bio Inc., Japan), 1 µL each of forward and reverse primers (10 pmol/µL), 2 µL of template DNA and nuclease-free water (NFW) to make up the final volume. The annealing temperature was standardised at 60.5 °C, based on the calculated melting temperature (Tm) of the primers to ensure specific amplification. Thermal cycling was performed in an MJ Mini thermal cycler (Bio-Rad, USA) with an initial denaturation at 95°C for 15 min, followed by 39 cycles of denaturation at 94°C for 30 sec, annealing at 60.5°C for 90 sec and extension at 72°C for 90 sec with a final extension at 72°C for 10 min. For FAdV, a known positive control maintained at the Department of Veterinary Microbiology, CVAS, Mannuthy was used. As no

Table 1. Details of samples collected

Source of sample collection	Bird condition	Sample type	Number of samples
Dept. of Veterinary Microbiology/ Veterinary Pathology	Dead	Tissues	21
		Feather	21
		Cloacal swab	21
	Ailing	Feather	6
		Cloacal swab	6
Total			75

PiCV-positive control was available, field-positive samples confirmed by sequencing were used for validation. NFW served as the negative control. The PCR amplicons were separated by electrophoresis on 1.5 % agarose gel containing ethidium bromide at 80 V for 45 min. and visualised under ultraviolet (UV) transillumination.

Sequencing and BLAST analysis

Representative PCR amplicons from PiCV and FAdV positive samples were submitted for bidirectional Sanger sequencing (GeneSpec Pvt. Ltd., India). The forward and reverse reads were assembled using the EMBOSS Merger tool to generate consensus sequences. Sequence identity was confirmed through BLASTn analysis against reference sequences available in the NCBI GenBank database.

Multiplex PCR standardisation

Based on the uniplex PCR results, a mPCR assay was standardised for the simultaneous detection of PiCV and FAdV. The primers designed in this study for PiCV (*capsid* gene, 327 bp) and FAdV (*hexon* gene, 181 bp) were tested in combination for multiplexing. Primer concentrations and annealing temperatures were optimised by gradient PCR using a temperature range of 52-62 °C to yield clear, distinct amplicons without cross-reactivity. The annealing temperature was standardised at 60.5 °C for subsequent reactions.

The optimised mPCR was carried out in a total volume of 25 µL containing 12.5 µL of 2X EmeraldAmp GT PCR Master Mix (Takara Bio Inc., Japan), 1 µL each of forward and reverse primers (10 pmol/µL) for both PiCV and FAdV, 2 µL of template DNA and NFW to make up the final volume. Thermal cycling was performed in an MJ Mini thermal cycler (Bio-Rad, USA) with an initial denaturation at 95 °C for 15 min, followed by 39 cycles of denaturation at 94 °C for 30 sec, annealing at 60.5 °C for 90 sec and extension at 72 °C for 90 sec, with a final extension at 72 °C for 10 min. NFW served as the negative control. The PCR amplicons were separated by electrophoresis on 1.5 % agarose gels containing ethidium bromide at 80 V for 45 min and visualised under UV transillumination. Distinct bands of 327 bp (PiCV) and 181 bp (FAdV) confirmed the specificity of the multiplex assay.

Representative mPCR products were further submitted for bidirectional Sanger sequencing at GeneSpec Pvt. Ltd., India and consensus sequences generated from forward and reverse reads were analysed using BLASTn against the NCBI GenBank database to confirm their identity.

Results and discussion

Uniplex PCR targeting the *capsid* gene of PiCV generated amplicons of approximately 327 bp. Among tissue samples from dead pigeons, 2 out of 21 tested positive, corresponding to a positivity rate of 9.5 per cent. Additionally, one cloacal swab (4.8%) and one feather sample (4.8%) from dead birds were positive. All samples collected from ailing pigeons, including tissues, cloacal swabs and feathers were negative for PiCV. Overall, the PiCV was detected in 4 of 75 samples (5.3%), with higher positivity in tissue specimens compared to cloacal swabs and feather samples. The choice of the *capsid* gene as a target was appropriate given its variability and epidemiological relevance (Zhang *et al*., 2011; Stenzel *et al*., 2014).

The predominance of detection in tissues reflects the viral predilection for lymphoid and visceral organs, where higher viral loads accumulate (Duchatel *et al*., 2006). Similar findings have been reported by Franciosini *et al*. (2005) and Ledwon *et al*. (2011), with consistent detection of PiCV in visceral organs, particularly in young and stressed pigeons. The lower positivity in cloacal and feather samples is consistent with intermittent shedding of PiCV, which has been described as a common feature of natural infections (Stenzel *et al*., 2014; Stenzel and Pestka, 2014). Nevertheless, the detection of viral DNA in these sample types supports their role in horizontal transmission via faecal-oral or respiratory routes (Duchatel *et al*., 2006). The absence of PiCV in samples from ailing birds suggests that the observed clinical signs may not have been directly caused by the virus, but could reflect co-infections or stress-related factors, as highlighted by Franciosini *et al*. (2005) and Yamamoto *et al*. (2015). While cloacal swabs and feathers are practical for non-invasive field surveillance (Bougiouklis, 2007; Ledwon *et al*., 2011), their limited sensitivity compared to tissues underscores the importance of necropsy samples for definitive diagnosis.

FAdV detection using uniplex PCR targeting the *hexon* gene produced 181 bp amplicons and was observed in 5 out of 21 tissue samples from dead pigeons (23.8%). The *hexon* gene, a highly conserved region, is widely employed for adenovirus detection and typing (Meulemans *et al*., 2001). No viral DNA was amplified from cloacal swabs (n=27) or feather samples (n=27) collected from either dead or ailing birds, emphasising the tissue-specific tropism of FAdV. Earlier reports have shown that adenoviruses preferentially replicate in internal organs, particularly the liver and intestine, where they are associated with necrotising hepatitis and enteric lesions (Coussement *et al*., 1984; Vereecken *et al*., 1998). McFerran and Adair (1977) also highlighted that adenovirus detection is rarely successful in non-invasive samples, reinforcing the value of necropsy tissues as the most reliable diagnostic material for FAdV identification.

The higher detection rate of FAdV compared to PiCV suggests that adenoviruses may play a more prominent role in YPDS-like clinical manifestations in the affected population. However, it is also possible that FAdV

functions as a secondary pathogen, aggravating disease in immunocompromised pigeons, particularly those previously or concurrently infected with PiCV (Vereecken *et al*., 1998; Stenzel *et al*., 2014). In the present study, PiCV and FAdV were detected independently in different birds and no co-infections between these viruses were observed. This finding differs from some earlier reports where PiCV was consistently identified in YPDS-affected flocks, often with sporadic detection of other viruses, such as PiHV or pigeon adenoviruses, which together were thought to exacerbate clinical outcomes (Raue *et al*., 2005; Frieck *et al*., 2008; Sahindokuyucu *et al*., 2022). The absence of co-infections in the present study may be attributed to the relatively small sample size or flock-level differences in circulating pathogens. Importantly, the independent detection of PiCV and FAdV in clinically affected pigeons indicates that either virus alone is capable of producing YPDS-like manifestations, reinforcing their significance as key viral contributors to the syndrome. The distribution of virus detection across different sample types and bird conditions is summarised in Table 2.

Table 2. Detection of FAdV, PiCV by PCR in samples collected from dead and ailing birds

Sample type	Bird condition	No. of samples tested	FAdV positive	PiCV positive
Tissue	Dead	21	5	2
Feather	Dead	21	0	1
Cloacal swab	Dead	21	0	1
Feather	Ailing	6	0	0
Cloacal swab	Ailing	6	0	0

Representative PCR products from both PiCV and FAdV were subjected to bidirectional Sanger sequencing. The consensus sequences obtained from PiCV-positive samples showed 95.73% and 97.05% nucleotide identity with published PiCV sequences in GenBank (KC691686.1 and KU593626.1, respectively), while FAdV-positive samples exhibited 99.45% identity with reference sequences DQ314840.2, KM217572.1 and FN394664.1. These results confirmed the specificity and reliability of the uniplex PCR assays. The Kerala PiCV isolates exhibited high homology with both Asian and European reference sequences, indicating the widespread circulation and genetic conservation of the virus across regions. This observation is consistent with earlier reports (Yamamoto *et al*., 2015; Stenzel *et al*., 2014) that demonstrated high similarity among PiCV strains with moderate diversity, largely driven by immune selection pressure on the *capsid* gene. These findings further suggested that international pigeon movement through trade, migration, or racing might facilitate its dissemination. Similarly, the high sequence similarity of FAdV positive samples from Kerala to global reference strains across the Middle East and South Asia suggests possible inter-regional transmission and viral adaptation to new avian hosts, as reported by Harrach *et al*. (2019) and Hess (2020). To the best of our knowledge, this is the first molecular report of PiCV and FAdV from Kerala and these sequences therefore represent the first baseline genomic data for these viruses in the state.

Gradient PCR (52-62°C) was used to optimise primer annealing conditions for simultaneous detection of PiCV and FAdV. The annealing temperature was

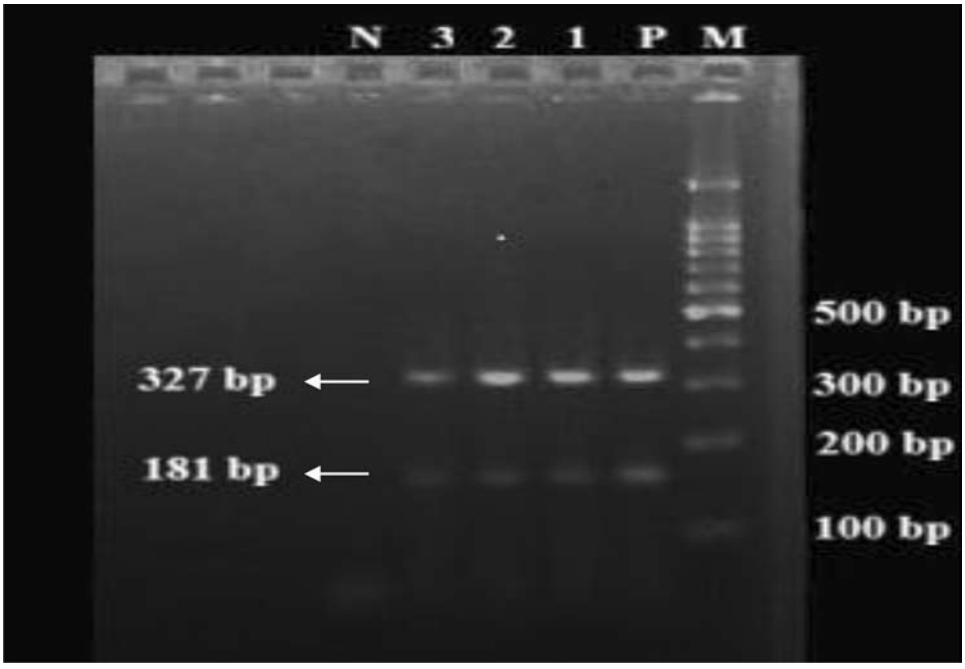

Fig. 1. Agarose gel electrophoresis showing multiplex PCR amplification of PiCV and FAdV. Distinct amplicons of ~327 bp (PiCV, capsid gene) and ~181 bp (FAdV, hexon gene) are visible. Lane M: 100 bp DNA ladder; Lane P: Positive control (PiCV and FAdV); Lanes 1-3: representative field samples; Lane N: Negative control

standardised at 60.5 °C with clear and distinct amplicons for both targets without cross-reactivity. The optimised mPCR generated two discrete bands of 327 bp (PiCV) and 181 bp (FAdV) (Fig. 1). Representative mPCR amplicons were sequenced (GeneSpec Pvt. Ltd., India) and their identity with reference sequences confirmed by BLAST analysis. The standardised mPCR successfully detected both viruses in positive samples identified by uniplex PCR with comparable sensitivity. The assay reduced reagent consumption and processing time compared to performing separate uniplex PCRs. Compared to conventional uniplex PCR, the mPCR reduced both reagent usage and processing time without compromising sensitivity or specificity. This aligns with earlier work highlighting the value of multiplex assays for cost-effective diagnosis and surveillance of avian pathogens (Freick *et al*., 2008). The assay standardised here can therefore serve as a useful diagnostic tool for field samples where multiple agents are suspected, thereby supporting timely management of YPDS outbreaks. A limitation of the present study was the relatively small number of birds examined, which might have the prevalence of PiCV and FAdV in the region. Additionally, the constraint of nonavailability of a PiCV positive control was overcome by using positive field samples confirmed by sequence analysis for the validation of the assay. Furthermore, only molecular detection was performed without concurrent serological confirmation. Future studies incorporating larger sample sizes, serological assays and longitudinal surveillance are essential to further understand the epidemiological role of these viruses in YPDS.

Conclusion

The present study demonstrates the occurrence of both PiCV and FAdV in pigeons exhibiting signs of YPDS in Kerala and reports the successful standardisation of a multiplex PCR assay for their simultaneous detection. While PiCV was detected at a lower frequency, FAdV showed a higher tissue prevalence among affected birds. Although the overall frequency of YPDS in Kerala could not be determined within the scope of this study, the detection of PiCV and adenovirus highlights their role in clinically affected pigeons. The standardised mPCR assay offers a rapid, sensitive and cost-effective diagnostic alternative to conventional uniplex PCR, with potential application in routine diagnostics and epidemiological surveillance. Adoption of such molecular tools will aid in the early detection and management of viral infections in pigeons, thereby contributing to improved flock health and productivity.

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Conflicts of interest

The authors declare that they have no conflict of interest.

References

Bougiouklis, P.A. 2007. Avian circoviruses of the genus Circovirus: a potential trigger in Pigeon breeder's lung (PBL)/Bird fancier's lung (BFL). *Med. Hypotheses*. **68**(2): 320-323.

Coussement, W., Ducatelle, R., Lehmahieu, P., Froyman, R., Devriese, L.A. and Hoorens, J. 1984. Pathologie van adenovirus infekties bij duiven. Vlaamsdiergeneeskundig Tijdschrift. **53**: 277-283.

Davison, A.J., Benko, M. and Harrach, B. 2003. Genetic content and evolution of adenoviruses. *J. Gen. Virol.* **84**(11): 2895-2908.

De Herdt, P., Ducatelle, R., Lepoudre, C., Charlier, G. and Nauwynck, H. 1995. An epidemic of fatal hepatic necrosis of viral origin in racing pigeons (*Columba livia*). *Avian Pathol.* **24**: 475-483.

Duchatel, J.P., Todd, D., Smyth, J.A., Bustin, J.C. and Vindevogel, H. 2006. Observations on detection, excretion and transmission of pigeon circovirus in adult, young and embryonic pigeons. *Avian Pathol.* **35**(1): 30-34.

Duchatel, J.P., Todd, D., Willeman, C. and Losson, B. 2009. Quantification of pigeon circovirus in serum, blood, semen and different tissues of naturally infected pigeons using a real-time polymerase chain reaction. *Avian Pathol.* **38**(2): 143-148.

Franciosini, M. P., Fringuelli, E., Tarhuni, O., Guelfi, G., Todd, D., Proietti, P. C. and Asdrubali, G. 2005. Development of a polymerase chain reaction-based in vivo method in the diagnosis of subclinical pigeon circovirus infection. *Avian Dis.* **49**(3), 340-343.

Freick, M., Muller, H. and Raue, R. 2008. Rapid detection of pigeon herpesvirus, fowl adenovirus and pigeon circovirus in young racing pigeons by multiplex PCR. *J. Virol. Methods*. **148**(1-2): 226-231.

Gunes, A., Marek, A., Grafl, B., Berger, E. and Hess, M. 2012. Real-time PCR assay for universal detection and quantitation of all five species of fowl adenoviruses (FAdV-A to FAdV-E). *J. Virol. Methods*. **183**(2): 147-153.

Harrach, B., Tarjan, Z.L. and Benko, M. 2019. Adenoviruses across the animal kingdom: a walk in the zoo. *FEBS Lett.* **593**(24): 3660-3673.

Hess, M. 2020. Aviadenovirus infections. In D.E. Swayne, M. Bouliann, C.M. Logue, L.R. McDougald, V. Nair, D.L. Suarez, S. de Wit, T. Grimes, D. Johnson, M. Kromm, T.Y. Prajitno, I. Rubinoff and G. Zavala (Eds.), Diseases of poultry (14th ed., pp. 322-332). Hoboken, NJ: Wiley-Blackwell.

Kong, Y., Yan, C., Zhang, G., Cai, Y., He, B. and Li, Y. 2021. Detection of pigeon circoviruses in ticks of sheep and camels in Inner Mongolia, China. *BioRxiv*. 2021-06.

Ledwon, A., Bailey, T., O`Donovan, D., Mckeown, S., Lloyd, C., Wieckowski, T., Kinne, J., Silvanose, C., Szeleszczuk, P. and Wernery, U. 2011. Prevalence of circovirus and adenovirus in pigeons in Dubai. *Med. Weter.* **67**: 752-756.

Marek, A., Kosiol, C., Harrach, B., Kajan, G.L., Schlotterer, C. and Hess, M. 2013. The first whole genome sequence of a Fowl adenovirus B strain enables interspecies comparisons within the genus Aviadenovirus. *Vet. Microbiol.* **166**(1-2): 250-256.

McFerran, J. B. and Adair, B. M. 1977. Avian adenoviruses-a review. *Avian Pathol*. **6**(3), 189-217.

Mengfan, L.I.U., Junhong, K.E., Hanyu, J.I.A.N.G., Huihu, Y.A.N.G., Zimin, X.I.E., Rui, L.U.O., Shujian, H.U.A.N.G. and Kun, M.E.I. 2023. Recent Advances in Etiological Analysis and Diagnosis of Young Pigeon Disease Syndrome. *Guangdong Journal of Animal and Veterinary Sciences.* **48**(5): 69.

Meulemans, G., Boschmans, M., Van den Berg, T.P. and Decaesstecker, M. 2001. Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. *Avian Pathol.* **30**(6): 655-660.

Mo, J. 2021. Historical investigation of fowl adenovirus outbreaks in South Korea from 2007 to 2021: a comprehensive review. *Viruses*. **13**(11): 2256.

Prasad, K., Barman, D., Asif, M.M. and Abraham, J. 2017. Pigeon farming practices and constraints in Kerala. *Global Journal of Bio-science and Biotechnology*. **6**(1): 86-88.

Raue, R., Schmidt, V., Freick, M., Reinhardt, B., Johne, R., Kamphausen, L., Kaleta, E.F., Muller, H. and Krautwald-Junghanns, M.E. 2005. A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. *Avian Pathol.* **34**: 418-425.

Sahindokuyucu, I., Yazici, Z. and Barry, G. 2022. A retrospective molecular investigation of selected pigeon viruses between 2018-2021 in Turkey. *PLoS One*. **17**(8): e0268052.

Schmidt, V., Schlomer, J., Luken, C., Johne, R., Biere, B., Muller, H. and Krautwald-Junghanns, M.E. 2008. Experimental infection of domestic pigeons with pigeon circovirus. *Avian Dis.* **52**(3): 380-386.

Stenzel, T. and Pestka, D. 2014. Occurrence and genetic diversity of pigeon circovirus strains in Poland. *Acta Vet. Hung.* **62**(2): 274-283.

Stenzel, T., Piasecki, T., Chrzastek, K., Julian, L., Muhire, B.M., Golden, M., Martin, D.P. and Varsani, A. 2014. Pigeon circoviruses display patterns of recombination, genomic secondary structure and selection similar to those of beak and feather disease viruses. *J. Gen. Virol.* **95**(6): 1338-1351.

Stenzel, T., Dziewulska, D., Tykalowski, B. and Koncicki, A. 2020. The clinical infection with pigeon circovirus (PiCV) leads to lymphocyte B apoptosis but has no effect on lymphocyte T subpopulation. *Pathogens*. **9**(8): 632.

Ugwu, C. C., Hair-Bejo, M., Omar, A. R., Nurulfiza, M. I. and Aini, I. 2023. TaqMan probe-based qPCR method for specific detection and quantification of fowl adenovirus 8b challenge from chickens inoculated with live attenuated or inactivated virus. *Open Vet. J*. **13**(2), 171-178.

Vereecken, M., de Herdt, P. and Ducatelle, R. 1998. Adenovirus infections in pigeons: a review. *Avian Pathol*. **27**: 333-338.

Yamamoto, E., Ito, H., Kitamoto, E., Morinishi, K., Yano, A., Miyoshi, S. and Ito, T. 2015. Complete genome sequence of pigeon circovirus detected in racing pigeons in western Japan. *Virus Genes*. **51**: 140-143.

Zhang, Z., Lu, C., Wang, Y., Wang, S., Dai, D., Chen, Z. and Fan, H. 2011. Molecular characterization and epidemiological investigation of Pigeon circovirus isolated in eastern China. *J. Vet. Diagn. Investig.* **23**(4): 665-672. ■