Praca oryginalna *Original paper*

Prevalence of circovirus and adenovirus in pigeons in Dubai

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Summary

The purpose of this investigation was to study the prevalence of pigeon (Columba livia) circovirus as well as pigeon and fowl adenoviruses in domestic pigeons in Dubai and United Arab Emirates. Feather and cloacal swab samples were obtained from 132 clinically healthy pigeons from four Dubai zoological collections and seven clinically healthy free-living columbiformes. Feather samples were tested for pigeon circovirus with polymerase chain reaction (PCR), and 21% were positive. Cloacal swabs tested for pigeon and fowl adenoviruses were all negative. Eighteen feather samples and liver impression smears were obtained at post-mortem examination from birds derived from one flock. PCR tests revealed the presence of pigeon circovirus in 72% of liver impression smears and in 50% of feather samples. Although 44% of liver samples were positive for PiAdV in PCR, no clinical signs of adenoviral infection were noted in the flock nor were any typical lesions found in histopathological examinations. Fowl adenovirus was not detected by PCR.

This research is the first study of the prevalence of these viral infections of pigeons in the Arabian Peninsula.

Keywords: pigeon, circovirus, adenovirus, Dubai

Pigeon (*Columba livia*) adenovirosis and circovirosis are often associated with high morbidity and mortality in young pigeons (6, 7, 9, 10, 14, 16, 20, 23, 26).

Pigeon circovirus (PiCV) is a member of the genus *Circovirus* in the family *Circoviridae*. Infection with circovirus was first documented in Canada in 1986 (32). Circovirosis has been reported in many European countries, as well as in Australia and South Africa, and probably has a worldwide distribution (5, 32). Typically, circovirus infects young pigeons under one year of age (27). A comprehensive study demonstrated that PiCV plays an important role in a multifactorial disease called young pigeon disease syndrome (YPDS), by inducing immunosuppression in infected birds (20). In pigeons, as in other susceptible species, circoviruses have an affinity for the immune system. In parrots, and sometimes *Columbiformes*, the virus may cause feather loss and dystrophy (17, 24). Clinical signs depend mainly on secondary bacterial, fungal, parasitic or viral infections. Usually, the clinical course of circovirosis depends on secondary infections and is often associated with a high morbidity, but a low mortality (7, 13, 16, 23, 32).

Pigeon adenovirus (PiAdV) has been previously described as a cause of acute death in racing pigeons of all ages in Europe (10, 14). Two forms of adenovirosis have been identified in pigeons. The classical adenovirosis occurs almost exclusively in young pigeons. Clinical signs of this form are often compli-

cated by *E. coli* infection, which causes watery diarrhoea, vomiting and occasionally death. Microscopically, numerous large nuclear inclusion bodies are present in hepatocytes, but extensive hepatic necrosis is not observed. The other form of adenovirosis is described in pigeons of all ages and leads to sudden death. Macroscopic hepatic lesions are very typical with extensive hepatic necrosis (10).

The aim of the study was to investigate the prevalence of pigeon circovirus and pigeon and fowl adenovirus in captive domestic pigeons (*Columba livia*) bred to feed captive falcons in Dubai.

Material and methods

Samples. Feather samples and cloacal swabs were obtained for PCR analysis from 132 randomly selected, clinically healthy live pigeons from four flocks located in different sites in Dubai (tab. 1). These birds were mainly King and Racing pigeon breeds housed in wire mesh aviaries (12 m × 24 m × 2 m) and fed a standard seed diet. Only racing pigeons from site A were housed in open lofts typical of the European style of racing pigeon management. Pigeons from site D were vaccinated once, subcutaneously against pigeon paramyxovirus serotype 1 (PPMV-1) infection with Colombovac PMV (Fort Dodge) at 6 weeks of age. Six samples were obtained from captured free-living, clinically normal feral pigeons and one from a laughing dove (*Streptopelia senegalensis*) caught at site E (tab. 2) and released after feather samples and cloacal swab collection. Samples were also obtained from 18 pigeons examined post mortem at Dubai Falcon Hospital. Birds submitted for post-mortem examination were either found dead or euthanized by intravenous barbiturate injection because of poor condition, and often had neurological signs typical of pigeon paramyxovirosis. All birds examined post mortem originated from site D (tab. 3). Internal organ (liver, intestine, lungs) samples were obtained for bacteriology.

Impression smears were prepared from eighteen liver samples by blotting the cut surface with Whatman Filter paper No. 1 (Whatman International Ltd, Maidstone, England) (11).

Feather samples were also collected from these birds. Feather and filter paper samples were dried and stored in separate plastic bags.

Histopathology. Samples collected from 14 of the 18 dead birds were submitted for histopathology. Samples included organs with lesions, as well as the liver, spleen and bursa of Fabricius (if present), and brain if neurological signs were observed. Selected tissues were fixed in 10% formalin, dehydrated through graded alcohol and embedded in paraffin wax. Sections (3 µm) were cut and stained with haematoxylin and eosin (HE), and with periodic acid Schiff (PAS) (2).

Bacteriology. Bacteriology and mycology investigations were conducted at Dubai Falcon Hospital (DFH) (1, 22). Internal organ (liver, intestine, lungs) samples were cultured onto blood agar and MacConkey agar. All plates were incubated aerobically at 37°C. After 48 hours, bacteria and fungi (yeasts and *Aspergillus*) were identified using Trek, Sensititre, USA. Samples from pigeons with enteritis were also incubated anaerobically on Columbia Agar at 37°C for 72 h.

Tab. 1. Pigeon circovirus PCR results from feather DNA samples

Pigeons' origin sites with GPS co-ordinates	Age	Number of samples	Number of PiCV PCR positive results	Percentage (%)
Site A N 24°50.045' E 55°38.243'	Y*	12	–	0
	A**	13	–	0
Site B N 25°10.666' E 55°33.452'	Y	7	1	14
	A	6	–	0
Site C N 25°73.477' E 55°17.362'	A	17	4	23.5
Site D N 25°05.326' E 55°16.948'	Y	40	11	27.5
	A	37	10	27.0
Free living caught in site E N 25°13.240' E 55°18.451'	A	7	3	43
	Total	139	29	21%

Explanations: * Y < 6 months; ** A ≥ 6 months

Tab. 2. Primers used in this study

Target	Primers		Product	Source
PiCV	PiCV- s	ggtaactgaatgcgagcccatagt	206 bp	Raue et al., 2005
	PiCV- as	tgacggagccagaaaatgggat		
PiAdV	F1	atcaactacgacaacgaaggc	977 bp	Raue et al., 2002
	F2	cggtagagttacggggaaatt		
FAdV	H3	aacgt caaccccttcaaccacc	1319 bp	Raue and Hess, 1998
	H4	ttgcc tgtggcgaaaggcg		

Virology. Virus isolation to exclude any cytopathogenic virus (eg: paramyxo-, influenza- and herpesvirus) was performed on necropsy samples from 5 pigeons (tab. 3). Virus isolation was conducted at Central Veterinary Research Laboratory (CVRL) at Dubai on chicken embryo fibroblasts (CEF) as described by Wernery and others (30).

Molecular analysis. DNA isolations and PCR tests were performed at the Avian Disease Division of the Faculty of Veterinary Medicine in Warsaw University of Life Sciences (Poland). DNA from feathers and swabs was isolated by the 5% Chelex method (31). Isolation of DNA from impression smears was done with Sherlock AX, DNA isolation kit (DNA-Gdansk, Poland). For all PCR reactions, premixes were prepared containing 25 µl of PCR Master Mix (Fermentas International Inc, Canada), 100 pmol of each primer, 4 µl template DNA in a total volume of 50 µl. The pigeon circovirus PCR test was performed according to Raue et al. (20) whose primers sequences are presented in Tab. 2. PCR commenced with an initial denaturation step of 5 min at 95°C, followed by 40 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. The reaction was terminated after a final elongation step of 5 min at 72°C.

Tab. 3. Comparison of different examination results including PCR tests for PiCV and PAdV. All pigeons examined post-mortem originated from site D

Pigeons ID#	FAV liver	PiCV liver	PiCV feathers	PiAV liver	Bacteriology/Virology	Endoparasites	Clinico-pathological findings
1 Y**** R*	–	+	–	+	–/–	*Trichomonas sp.* ++++; *Ascaridia sp.* ++++; *Capillaria sp.* +++	Disseminated trichomoniasis, lung aspergillosis
2 YK**	–	–	–	–	–/–	–	Head tremor, hepatitis, enteritis
3 YK	–	+	–	–	–/NE***	–	Hepatitis, enteritis, nephritis, air sac aspergillosis
4 AK	–	+	–	+	–/–	*Ascaridia sp.*	Liver congestion, spleen with active follicles
5 YR	–	+	–	–	NE/–	*Eimeria sp.* ++; *Hexamita columbae* ++	Skin abrasions, cervical airsac rupture, nephritis, liver hemosiderosis, pneumonia
6 YK	–	+	–	–	–/–	–	Head tremor, hepatitis, airsacculitis, mild gliosis
7 YR	–	+	+	–	Mix growth (contaminants)/NE	–	Hepatitis, enteritis, lung aspergillosis
8 YK	–	+	+	+	*Salmonella sp.*, *Cl. perfr.*/NE	–	Pneumonia (fig. 1), enteritis, airsacculitis, feather dystrophy
9 YW	–	–	+	+	–/NE	–	Pox, pneumonia, airsacculitis, liver hyperaemia
10 YK	–	–	–	+	–/NE	*Trichomonas sp.* +	Head tremor, hepatitis, enteritis, nephritis
11 YK	–	+	+	+	–/NE	*Trichomonas sp.* +++; *Eimeria sp.* ++	Head tremor, feather abnormalities (fig. 2), swollen liver, enteritis, airsacculitis
12 YK	–	+	+	–	NE/NE	*Trichomonas sp.* +++; *Eimeria sp.* +	Head tremor, enteritis, kidney oedema
13 YK	–	+	+	+	NE/NE		Hepatitis, enteritis, pneumonia
14 YK	–	+	+	–	NE/NE	NE	NE
15 YK	–	–	–	–	NE/NE	NE	NE
16 YK	–	+	+	–	NE/NE	NE	NE
17 YK	–	+	+	+	NE/NE	NE	NE
18 Dove	–	–	–	–	NE/NE	NE	NE

Explanations: * R: Racing pigeon; ** K: King pigeon; *** NE: not examined; **** age: Y < 6 months, A ≥ 6 months; –/ negative result

Pigeon adenovirus (PiAdV) PCR was performed according to Raue et al. (18). PCR conditions were as follows: 5 min initial denaturation at 94°C 30 cycles each with 1 min denaturation at 94°C, 1 min annealing at 60°C and 1.5 min elongation at 72°C. Final elongation was 10 minutes at 72°C. Fowl adenovirus (FAdV) was tested by PCR according to Raue and Hess (19).

Feathers and liver impression smears were tested with a pigeon circovirus (PiCV) test; cloacal swabs, as well as liver impression smears, were tested by PCR for both PiAdV and FAdV.

Results and discussion

Out of 139 feather DNA samples tested, 29 (21%) were positive for PiCV (tab. 1, 4) with most cases occurring at sites C and D. No positive cases were detected at site A, and only one positive case at site B.

All cloacal swabs and liver impression smears were negative for FAdV-PCR (tab. 3, 4). All cloacal swabs were PiAdV-PCR negative.

In pigeons from site D examined post mortem, eight (44%) liver impression smears were positive for PiAdV-PCR despite the absence of typical inclusion bodies in histopathology examination. Pathological findings from necropsied pigeons are presented in tab. 3.

Pigeon circovirosis or adenovirosis has not been previously reported in pigeons in the United Arab Emirates. Clinical and pathological lesions for circovirus infection are not very specific (10, 26). Affected

Fig. 1. Feather dystrophy in a young king pigeon with circovirus infection

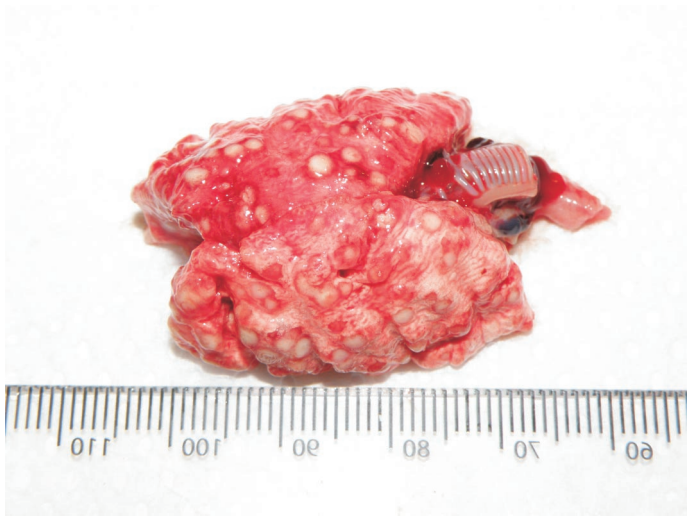

Fig. 2. Pulmonary salmonellosis in a PiCV-positive young king pigeon

pigeons are more susceptible to secondary parasitic and bacterial (fig. 2) infections. In rare cases feather abnormalities may also be present. There are no reports of cicrcovirosis or pigeon-originated adenovirosis in raptors, but parasitic and bacterial infections, as well paramyxovirus virus, may be easily transmitted. It is known that affected young pigeons have concurrent infections, which suggests an ineffective immune response (23). In birds from site D examined in this study, neurological signs typical of PMV-1 infection and pox-like pustules were observed in spite of vaccination against these infections. Aspergillosis, parasite infestations and severe salmonellosis were confirmed in some cases, which can also be signs of immunosuppresion (13, 19). No clinical or pathological signs consistent with adenovirosis were observed in pigeons examined at site D despite positive PCR results.

According to the literature, PiAV and Fowl Adenoviruses (FAV) are widely distributed in pigeons, causing only sporadic cases of inclusion body hepatitis (6, 8, 9, 18). In 8 of 18 liver impression smears, PiAdV PCR tests were positive, but virus culture was negative and changes consistent with adenovirus infection were not observed in histopathology investigations. Also all cloacal swab samples examined were negative. This indicates that there was no virus shedding at the time of sampling. Isolation of adenoviruses from pigeons in susceptible cell cultures is difficult (26). This is also the case with pigeon adenovirosis type II, which is a more sporadic disease affecting birds at all ages. In pigeon adenovirosis type II, inclusion bodies are less numerous and smaller than in classical adenovirosis type I (10, 19, 29).

There is no information in the literature about the potential transmission of pigeon circovirus or adenoviruses from pigeons to raptors. Circoviral infection has not been described in falcons. It is known that fowl adenoviruses, which were not identified in presented research, can also infect pigeons (8, 9, 18). It has been

Tab. 4. Summary of the results

Pigeons origin	Nr of examined pigeons	Health status	PiCV +	PiADV +	FAV +
Site A	25	healthy	–	–	–
Site B	13	healthy	2	–	–
Site C	17	healthy	4	–	–
Site D	77	healthy	21	–	–
Site E	7	healthy	3	–	–
Site D	18	sick and dead	16	8	–
Total	157		46	8	0

hypothesised that poultry adenoviruses can also affect some species of falcons (3, 4). However, the anticipated affinity between falcon and fowl isolates had not been corroborated and in the light of latest studies falcon adenovirus is now reckon a new species in the genus Aviadenovirus with closest similarity to the group I members FAV-1 and FAV-4 (15, 21, 28).

Recent European studies indicated an increased prevalence of infections with circoviruses at a decreased number of adenovirosis cases. The spread of circoviroses appeared particularly evident among pigeons in Germany and Poland (12, 25).

These studies shows that the prevalence of pigeon circovirus and adenovirus in pigeons is very similar in United Arab Emirates and Europe.

References

1. *Aloia M. A. D., Bailey T. A., Samour J. H., Naldo J., Howlett J. C.*: Bacterial flora of captive Houbara (Chlamydotis undulata), Kori (Ardeotis kori), and Rufous-crested (Eupodotis ruficrista) Bustards. Avian Path. 1996, 25, 459--468.
2. *Bancroft J. D., Stevens A.*: Theory and Practice of Histological Techniques. Churchill Livingstone, Hongkong 1996.
3. *Forbes N. A., Simpson G. N.*: A review of viruses affecting raptors. Vet. Rec. 1997, 141, 123-126.
4. *Forbes N. A., Simpson G. N., Higgins R. J., Gough R. E.*: Adenovirus infection in Mauritius kestrels (Falco punctatus). J. Avian Med. Surg. 1997, 11, 31-33.

5. *Gerdes G. H.*: Two very small viruses – a presumptive identification. J. S. Afr. Vet. Assoc. 1993, 64, 2.
6. *Goryo M., Ueda Y., Umemura T., Haruna A., Itakura C.*: Inclusion body hepatitis due to adenovirus in pigeons. Avian Path. 1988, 17, 391-401.
7. *Gough R., Drury S. E. N.*: Circovirus-like particles in the bursae of young racing pigeons. Vet. Rec. 1996, 138, 167.
8. *Hess M., Prusas C., Monreal G.*: Growth analysis of adenoviruses isolated from pigeons in chicken cells and serological characterization of the isolates. Avian Path. 1998, 27, 196-199.
9. *Hess M., Prusas C., Vereecken M., De Herdt P.*: Isolation of fowl adenoviruses serotype 4 from pigeons with hepatic necrosis. Berl. Münch. Tierärztl. Wochenschr. 1998, 111, 140-142.
10. *Herdt P., Ducatelle R., Lepoudre C., Charlier G., Nauwynck H.*: An epidemic of fatal hepatic necrosis of viral origin in racing pigeons (Columba livia). Avian Path. 1995, 24, 475-483.
11. *Kailash U., Hedau S., Gopalkrishna V., Katiyar S., Das B. C.*: A simple „paper smear" method for dry collection, transport and storage of cervical cytological specimens for rapid screening of HPV infection by PCR. J. Med. Microbiol. 2002, 51, 606-683.
12. *Kamphausen L.*: Pigeon health: Current problems in Germany. Proc. 3rd Olympic Jamboree of Polish Columbopathologists. Veterinary problems in racing pigeon breeding. Poznań 2011, pp. 13-14.
13. *Marlier D., Vindevogel H.*: Viral infections in pigeons. Vet. J. 2006, 172, 40-51.
14. *McFerran J., McCracken R., Connor T., Evans R.*: Isolation of viruses from clinical outbreaks of inclusion body hepatitis. Avian Path. 1976, 5, 315-324.
15. *Oaks J. L., Schrenzel M., Rideout B., Sandfort C.*: Isolation and epidemiology of falcon adenovirus. J. Clin. Microbiol. 2005, 43, 3414-3420.
16. *Pare J. A., Brash M. L., Hunter D. B., Hampson M. J.*: Observations on pigeon circovirus infection in Ontario. Can. Vet. J. 1999, 40, 659-662.
17. *Raidal S. R., Riddoch P. A.*: A feather disease in Senegal doves (Streptopelia senegalensis) morphologically similar to psittacine beak and feather disease. Avian Path. 1997, 26, 829-836.
18. *Raue R., Hafez H. M., Hess M.*: A fiber gene-based polymerase chain reaction for specific detection of pigeon adenovirus. Avian Path. 2002, 31, 95-99.
19. *Raue R., Hess M.*: Hexon based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. J. Virol. Methods 1998, 73, 211-217.
20. *Raue R., Schmidt V., Freick M., Reinhardt B., Johne R., Kamphausen L., Kaleta E. F., Müller H., Krautwald-Junghanns M. E.*: A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. Avian Path. 2005, 34, 418-425.
21. *Schrenzel M., Snook E., Gagneux P.*: Molecular assays for detection of falcon adenovirus. J. Vet. Diagn. Invest. 2007, 19, 479-485.
22. *Silvanose C., Bailey T., Di Somma A.*: Antifungal susceptibility testing of fungi isolated from the respiratory tract of falcons in the United Arab Emirates. Vet. Rec. 2006, 159, 282-284.
23. *Smyth J. A., Carroll B. P.*: Circovirus infection in European racing pigeons. Vet. Rec. 1995, 136, 173-174.
24. *St. Leger J.*: Feather dystrophy associated with circovirus infection in columbiformes. Proc. 47th Western Poultry Dis. Conference, March 8-10, 1998, 47, 18.
25. *Szeleszczuk P., Hałun A., Koralewski A., Lamacha R., Ledwoń A., Olczyk B., Piasecki T., Szczepańczyk L., Stenzel T., Wawrzyniak M., Wawrzyniak S., Weiler A., Zieliński K.*: Pigeon health evaluation in Poland: Retrospective study. Proc. Olympic Jamboree of Polish Columbopathologists. Veterinary problems in racing pigeon breeding. Poznań 2011, pp. 1-7.
26. *Taras L., Kubíček O., Juranová R., Jurajda V.*: The first demonstration of pigeon circovirus infection in the Czech Republic based on histology and nested PCR. Acta Vet. Brno 2003, 72, 577-582.
27. *Todd D.*: Avian circovirus diseases: lessons for the study of PMWS. Vet. Microbiol. 2004, 98, 169-174.
28. *Tomaszewski E. K., Phalen D. N.*: Falcon adenovirus in an American kestrel (Falco sparverius). J. Avian Med. Surg. 2007, 21, 135-139.
29. *Vereecken M., De Herdt P., Ducatelle R.*: Adenovirus infections in pigeons: A review. Avian Path. 1998, 27, 333-338.
30. *Wernery U., Remple J. D., Neumann U., Alexander D. J., Manvell R. J., Kaaden O. R.*: Avian paramyxovirus serotype 1 (Newcastle disease virus) infections in falcons. Zbl. Vet. Med. B 1992, 39, 153-158.
31. *Willard J. M., Lee D. A., Holland M. M.*: Recovery of DNA for PCR amplification from blood and forensic samples using a chelating resin. Method Mol. Cell Biol. 1998, 98, 9-18.
32. *Woods L. W., Latimer K. S., Niagro F. D., Riddell A. M., Crowley B. M., Anderson B. M., Daft D. D., Moore R. P., Campagnoli R. W., Nordhausen R. W.*: A retrospective study of circovirus infection in pigeons; nine cases (1986-1993). J. Vet. Diagn. Invest. 1994, 6, 156-164.

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