Szent István University
Postgraduate School of Veterinary Science

Experiences and observations about the pathomorphology of avian influenza in mute swan, domestic goose, Pekin and mulard duck in Hungary

PhD dissertation thesis

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Introduction

The first wave of epidemic, caused by H5N1 HPAI strain reached Hungary in early 2006 and caused death in wild aquatic birds at the middle south site of the country.

Carcasses of 3300 wild and domestic birds, belonging to a wide range of species (including 165 mute swans), were examined between January and March 2006., within the scope of the avian influenza monitoring programme at Central Veterinary Institute (Central Agricultural Office, Veterinary Diagnostic Directorate). In this period HPAI infection was diagnosed in 67 mute swans. During the second and third wave of the epidemic (June-July 2006 and January 2007 respectively) diseases caused by highly pathogenic avian influenza strain (H5N1 subtype) were confirmed in 15 domestic goose and 15 domestic duck (11 Pekin duck and 4 mulard duck) flocks in a region of dense population of waterfowl in the Southern part of the Danube-Tisza interfluve region.

Diseases due to avian influenza infection were first reported in Hungary in the 1960th-70th.

Before 2002 AI strains of different pathogenicity caused disease and mortality mostly among hens and turkeys while waterfowl and wild aquatic birds were only slightly affected (mild disease or asymptomatic virus shedding).

The H5N1 AI strain killed about 6000 wild birds (including aquatic ones) in Western China in the surroundings of Qinghai lake. Considering the death occurred in these species, this strain was considered as aquatic birds-pathogen. This strain spread first in Eastern Asia, Middle East and recently to many countries of the European Union, including Hungary, where it caused acute illness and death in swans, geese, and ducks. Humans were not affected in Hungary.

The goal of my study was to examine the clinical signs, the pathological changes, the distribution of the viral antigen and the viral RNA in the tissues and cells in mute swans, domestic geese, mulard and Pekin ducks affected the H5N1 epidemic in Hungary. I wanted to compare the behaviour of the virus in two *Anserinae* and two *Anatinae* species.

Our observations and other examinations of the 2006 epidemic in the world refine the view that the waterfowls and wild aquatic birds are mere reservoir of HPAI viruses, they are less sensitive to the infection and they only carry the virus in great distances, in this way meaning the main source of infection of domestic fowls.

Material and method

Clinical samples

Between January and March 2006 (first wave of the epidemic) H5N1 HPAIV was isolated in 67 mute swans (*Cygnus olor*). Most of the positive samples originated from surroundings of Nagybaracska, South Hungary.
In June, July 2006 and January 2007 (second and third wave of the epidemic) the infection was confirmed in 15 domestic goose and 15 domestic duck (11 Pekin duck and 4 mulard duck) flocks in a region of dense population of waterfowl in the Southern part of the Danube-Tisza interfluve region (Bács-Kiskun county, Csongrád county respectively). 3-5 individuals from each flock affected (55 geese and 65 ducks) were examined. The animals under examination died in the first days of the epidemic or occasionally were moribund birds still alive.

We examined the tissue tropism of the virus strain on 10 mute swans (*Cygnus olor*), 6 domestic geese (*Anser anser var. domestica*), 6 mulard ducks (*Cairina moschata x Anas platyrhynchos*) and 5 Pekin ducks (*Anas platyrhynchos*). Tissue samples were collected for IHC from all of the above species, but only from domestic geese (n=3), mulard (n=4) and Pekin ducks (n=4) for qRRT-PCR.

Histologic and immunohistochemical examination

Following post-mortem examination of each bird, brain (including brain stem, cortex and cerebellum), heart, pancreas, and above this of 35 swans and 1-3 domestic birds from each flocks kidney, spleen, lung, liver, occasionally trachea, spinal cord, skeletal muscle, small intestine and bursa Fabricii samples were collected and fixed in 10 % neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin. IHC was performed to detect influenza virus antigen. Briefly, after digestion with 0.1 % pronase (Sigma Aldrich Co., St. Louis, Missouri, USA) sections were incubated overnight with nucleoprotein-specific monoclonal antibody for A type influenza virus (dilution 1:6,000; HYB 340-05, Statens Serum Institut, Copenhagen, Denmark). Antibody binding was detected by a horseradish peroxidase-labelled polymer (EnVision™ + anti-mouse HRP; Dako, Glostrup, Denmark). A brain sample from a naturally infected swan was used as positive control. For negative control the monoclonal antibody specific for A type influenza virus was replaced by a phosphate buffer solution on serial tissue sections.

Virus isolation, PCR

The presence of HPAI virus in individual birds was confirmed by inoculating homogenized multiple tissue samples (trachea, lung, liver and intestine) from each bird (diluted 10 % (w/v) in phosphate-buffered saline) into the allantoic cavity of five, 10 to 11-day-old antibody-free embryonated chicken eggs. Following embryonic death, a haemagglutination assay (HA) was performed and positive allantoic fluids were subtyped by haemagglutination-inhibition (HI) assay using monospecific antiserum to influenza A subtype H5. The detection and sequencing of H5 and N1 genes were carried out under the recommendation of the EU avian influenza laboratory (Avian Virology Laboratory, Veterinary Laboratories Agency, Weybridge, UK) using conventional and real time PCR.

Quantitative real-time reverse-transcriptase polymerase chain reaction (qRRT-PCR*)*

Goose, mulard and Pekin duck organ samples (brain, heart, pancreas, kidney, spleen, lung and trachea) were tested by qRRT-PCR detection kit (Taqman Influenza A Version 1.0, Applied Biosystems) in order to determine and compare their viral load. The 10-fold serial dilutions (10^2–10^8 copies) of the transcribed M gene positive control provided with the kit were used to create the standard curve for quantitative analysis. The standard curve was calculated automatically by the Rotor-Gene software (Corbett Robotics Pty. Ltd.) plotting the Ct values against each standard of known concentration and by extrapolating the linear regression line of this curve. Viral copy numbers were calculated comparing the threshold cycles of samples with the resulting standard curves. Results were expressed as log10 virus copies per reaction.

Results and conclusions

In the area where swans were originally found some individuals still alive have shown such signs of neurological disorders as circle swimming, tremor, incoordination, torticollis. Daily number of death increased dramatically in the infected goose and duck flocks. Due to the official measure (extermination) enacted any observation of the flock level course of the disease was not possible. Infected animals showed lethargy, anorexia, serous nasal discharge, lacrimation and neurological signs (deviation of the head, head-tremor, lateral deviation of the head, leg and wing paralysis). In some cases animals were found dead without any previous clinical signs. In the 55 geese and 65 ducks examined there were no skin lesions (cyanosis, oedema, haemorrhage, necrosis) observed.

All swans were in moderate to good body condition which implied to rapid course of the disease. Body condition of waterfowl was moderate in peracute, and weaker in subacute cases.

The most frequently observed macroscopic lesions included haemorrhages under the epicardium, in the proventricular and duodenal mucosa, pancreas and sometimes skeletal muscles; focal necrosis in the pancreas and liver; myocardial degeneration; congestion of spleen and lung and accumulation of sero-mucinous exudate in the body cavities.

After finding specific **histological** changes in the central nervous system, in the pancreas and in the heart we could presume the diagnosis of AI infection. Most typical histopathological lesions concerning all individuals examined comprised lymphocytic meningo-encephalomyelitis accompanied by gliosis and occasional perivascular haemorrhages. It was more marked in swans and geese than in the ducks. Pancreatitis with focal necrosis in the exocrine glands has affected all the swans and geese where there were more severe changes than in ducks.

Multi-focal myocardial degeneration with lympho-histiocytic infiltration occurred less frequently and it was more marked in geese than in the other four species. Furthermore acute interstitial hepatitis with focal necrosis and haemorrhages were observed in all swans and geese while there were just mild focal lympho-histiocytic infiltration in three mulards. Liver samples of Pekin ducks remained negative. In about half of the lung samples of geese and ducks and only a single swan we found focal, perivascular lympho-histiocytic inflammation in the interstitium. There were oedema in most lungs and tracheas. We described focal skeletal muscle cell degeneration and mucous enteritis. There was lymphocyte depletion in the spleen and in bursa of Fabricius in young birds.

In the brain AIV antigen was detected by **IHC** in all birds of all the four species. All organs other than the brain remained negative in Pekin ducks.

In the brain there was more viral antigen detectable in swans and geese than in duck species. In the latter ones there were differences as well because in Pekin ducks only the ependyma was affected, while in mulard ducks all areas of the brain. As for the pancreas there were differences between swans, geese and ducks, the latters' samples being practically negative. In the myocardium we found stronger antigen coloration in geese and mulards compared to swans and the negative Pekin ducks. In the respiratory organs the highest amount of viral antigen was detectable in mulards' trachea and lung. In swans and geese only the lung was affected about at the same level. In the liver and spleen similar amount of antigen was visible in swans, geese and mulard ducks (**Table 1.**).

The highest antigen level was detected in the brain where in cerebrum, cerebellum and stem where neurons, oligodendro and microglial cells, astrocytes, ependymal cells, myocytes of blood vessels were affected. Antigen was more frequently detectable in the nucleus than in the cytoplasm. Huge amount of antigen was visible in the necrotic foci of the pancreas and liver in pancreatic exocrine acinar cells, hepatocytes and monocytes. In livers without necrosis the antigen was detected mostly in Kupffer cells. In the heart the myocytes and the epicardial cells contained the largest amount of antigen in the nucleus and in the cytoplasm as well. In the lung nucleoprotein antigen was detected in the respiratory epithelium and in macrophage cells of the interstitium. We found many infected cells in the spleen too in a focal pattern. Here the endothelium and smooth muscle cells of blood vessels, the reticulum cells, the smooth muscle cells of trabecules and macrophage cells contained viral antigen both in the cytoplasm and nucleus. In small intestine the *plexus submucosus*, the myocytes of *muscularis mucosae*, and MPS cells of the propria were infected. In some muscle cells and in the interstitial MPS cells of some kidney there were also positivity. However, viral antigen was frequently observed in areas without tissue lesions as well.

Table 1.: *Histological lesions and presence and amount of avian influenza A type nucleoprotein antigen in organs of four naturally infected bird species*

		brain[1]		pancreas[2,4]		heart[2,3]		liver[2,4]		lung[2]		trachea[2]		spleen[6]		kidney[5]	
		hist	IHC	his.	IHC	hist	IHC	hist	IHC	hist	IHC	hist	IHC	hist	IHC	hist	IHC
mute swan	1.*	++	+++	+	++	+	-	+	+	-	-			++	+	-	-
	2.	++	++	++	+++	+	+	++	+	-	+			+	+	-	-
	3.	+++	+++	+	+++	-	-	+	-	+	-	+	-	+	+	-	+
	4.	++	+++	++	+++	+	+	+++	+++	-	++			+	+++	-	+
	5.	+++	+++	+++	+++	+	-	++	+	-	-			++	+	-	-
	6.	++	+++	++	+++	-	-	+	+	-	-	+	-	+	-	-	-
	7.	++	+++	++	+++	-	+			-	-			+	++	-	+
	8.	+++	+++	+	++	-	+									-	-
	9.	++	+++	+++	+++	-	+	+	++	-	+			++	++	-	+
	10.	+++	+++	+++	+++	+	+	+	+	-	+			++	+	-	-
	mean	**++ /+++**	**+++**	**++**	**+++**	**-/+**	**-/+**	**+/++**	**+**	**-**	**-/+**	**+**	**-**	**+/++**	**+/++**	**-**	**-/+**
goose	1.	++	+++	+++	+++	+	++	++	+							-	-
	2.	+	+++	++	+++			++	-	+	+	-	-				
	3.	+	+++	+++	+++	++	+			+	-	+	-				
	4.	+	+++	+++	+++	+	++									-	-
	5.	+++	+++	++	+++	+	++	++	++	-	+	-	-	++	++	-	-
	6.	+	+++	+	-	+	+	+	-	-	+	-	-	+	-	-	-
	mean	**+/++**	**+++**	**++ /+++**	**++ /+++**	**+**	**+/++**	**++**	**+**	**-/+**	**+**	**-**	**-**	**+/++**	**+**	**-**	**-**
mulard duck	1.	+	++	-	-	-	-	-	+	+	+	-	++	++	+	+	-
	2.	+	+	-	-	+	+	+	+	+	+	-	-	++	+	+	+
	3.	+	++	+	-	-	+	+	+	+	-	-	-	++	++	+	-
	4.	++	++	-	-	-	+	+	-	-	+	-	+			+	-
	5.	+	++	+	-	+	++			-	+					-	-
	6.	+	++	+	+	++	+++			-	+	-	+			-	+
	mean	**+**	**++**	**-/+**	**-**	**-/+**	**+ /++**	**+**	**+**	**-/+**	**+**	**-**	**+**	**++**	**+/++**	**+**	**-/+**
Pekin duck	1.	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
	2.	+	+	-	-	+	-	-	-	-	-	-	-	++	-	-	-
	3.	++	+	-	-	-	-	-	-	+	-	-	-	++	-	-	-
	4.	+	+	+	-	-	-	-	-	-	-	-	-	++	-	-	-
	5.	+	+	-	-	-	-	-	-	-	-	-	-	++	-	-	-
	mean	**+**	**+**	**-**	**-**	**-/+**	**-**	**-**	**-**	**-/+**	**-**	**-**	**-**	**++**	**-**	**-**	**-**

*: No of animal

Severity and extension of histological changes / amount of nucleoprotein antigen influenza A-type:

– = negative
\+ = focal, mild changes / few, scattered antigen positive cells
++ = focal but more severe, or multiplex-diffuse and mild changes / middle amount of antigen positive cells (scattered or in small areas)
+++ = multiplex or diffuse, marked changes / big amount of antigen positive cells (large areas)

[1]: lympho-histiocytic encephalitis
[2]: lympho-histiocytic inflammation
[3]: degeneration
[4]: focal necrosis
[5]: tubulonephrosis, gout
[6]: lymphocyte-depletion, lymphocyte-necrosis

The histopathological changes were in connection with the presence of nucleoprotein antigen in all species considering the brain, pancreas and liver. Examining the heart this statement was true in swans, but we found stronger antigen colorization than we had expected in geese and mulards considering the histological results. In Pekin ducks there were some histological alterations without any presence of antigen. In lungs there was good correlation between histopathological lesions and antigen presence in geese and mulards, but it was not true in swans and Pekin ducks. We have found the same in tracheas.

Having examined histologically and immunohisto-chemically the H5N1 AIV shows strong neurotropism in all species, but it was stronger in swans and geese than in duck species. In swans and geese there was marked epitheliotropism (pancreatotropism) as well. The virus replication was markedly in myocardium of geese and mulard ducks and moderate in swans (myotropism). In swans and geese we have found considerable affinity to liver (epitheliotropism). Affinity to respiratory organs has not been found in either species.

H5N1 HPAIV was detected by qRRT-PCR in 100% of goose in 25% of mulard duck and in 43% of Pekin duck tissue samples (**Table 2**.) The viral load of the different tissue samples ranged from 1.91 to 7.77 log10 copies/reaction, meaning a difference of six order of magnitude. The largest amount of viral RNA was detected in the brain tissue of all species, showing 3-4 order of magnitude higher average viral RNA load in domestic geese (7.53 log10 copies/reaction) compared to Pekin ducks (4.19 log10 copies/reaction) or mulard ducks (3.21 log10 copies/reaction). Among tissues other than the brain the virus RNA could be detected in 100% of geese but only in 13% of mulard duck and in 33% of Pekin duck organ samples.

Table 2.:. *Amount of H5N1 AIV RNA in three waterfowl species naturally infected (log10 copy/reaction)*

	No. of animal	brain	pancreas	heart	lung	trachea	spleen	kidney
goose	3.	5,93	4,56	3,67	1,91		2,31	
	5.	7,77	2,25	4,31	3,22	2,58	3,73	3,62
	6.	7,62	2,93	5,53	2,49	3,08	3,05	3,62
	mean	**7,53**	**4,09**	**5,08**	**2,83**	**2,89**	**3,35**	**3,62**
mulard duck	1.	2,84	0	0	2,05	0	0	0
	2.	3,71	0	0	0	0	0	0
	3.	2,14	0	0	2,01	0	0	0
	4.	2,75	0	0	0	0	2,29	0
	mean	**3,21**	**0**	**0**	**1,73**	**0**	**1,68**	**0**
Pekin duck	2.	2,35	0	0	0	0	0	0
	3.	3,45	0	0	3,31	3,11	0	0
	4.	3,45	0	2,72	2,21	2,06	0	0
	5.	4,75	0	3,67	3,75	0	0	2,39
	mean	**4,19**	**0**	**3,11**	**3,29**	**2,55**	**0**	**1,78**

We found 3 and 4 orders of magnitude higher viral RNA load in goose organ samples (6,68 lg copy/reaction) compared to Pekin duck (3,43 lg copy/reaction) and mulard duck (2,39 lg copy/reaction), respectively

We have scored PCR results so that we can compare its results to histological and IHC examinations. (see **Table 3**.)

Table 3.: *The results of histological, immunohistochemical and qRRT-PCR examinations on three waterfowl species naturally infected with H5N1 HPAIV (histological lesions in detail in Table 1.)*

		brain			pancreas			heart			lung			trachea			spleen			kidney		
		hist	IHC	PCR	hist	IHC	PCR	hist	IHC	PCR	hist	IHC	PCR	hist	IHC	PCR	hist	IHC	PCR	hist	IHC	PCR
goose	3*	+	+++	+++	+++	+++	+++	++	+	++	+	-	+	+	-				+			
	5.	+++	+++	+++	++	+++	+	+	++	++	-	+	+	-	-	+	++	++	++	-	-	++
	6.	+	+++	+++	+	-	+	+	+	+++	-	+	+	-	-	+	+	-	+	-	-	++
	mean	**++**	**+++**	**+++**	**++**	**++/ +++**	**++**	**+**	**+/ ++**	**++**	**-/+**	**+**	**+**	**-/+**	**-**	**+**	**+/ ++**	**+**	**+**	**-**	**-**	**++**
mulard duck	1.	+	++	+	-	-	-	-	-	-	+	+	+	-	++	-	++	+	-	+	-	-
	2.	+	+	++	-	-	-	+	+	-	+	+	-	-	-	-	++	+	-	+	+	-
	3.	+	++	+	+	-	-	-	+	-	+	-	+	-	-	-	++	++	-	+	-	-
	4.	++	++	+	-	-	-	-	+	-	-	+	-	-	+	-			+	+	-	-
	mean	**+**	**++**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**+**	**-/+**	**-**	**+**	**-**	**++**	**+**	**-**	**+**	**-**	**-**
Pekin duck	2.	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	++	-	-	-	-	-
	3.	++	+	+	-	-	-	-	-	-	+	-	+	-	-	+	++	-	-	-	-	-
	4.	+	+	+	+	-	-	-	-	+	-	-	+	-	-	+	++	-	-	-	-	-
	5.	+	+	+++	-	-	-	-	-	++	-	-	++	-	-	-	++	-	-	-	-	+
	mean	**+**	**+**	**+/ ++**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**+**	**-**	**-**	**-/+**	**++**	**-**	**-**	**-**	**-**	**-**

*: No of animal

Severity and extension of histological lesions / amount of nucleoprotein antigen influenza A-type (IHC):

– = negative
\+ = focal, mild changes / few, scattered antigen positive cells
++ = focal but more severe, or multiplex-diffuse and mild changes / middle amount of antigen positive cells (scattered or in small areas)
+++ = multiplex or diffuse, marked changes / big amount of antigen positive cells (in large areas)

qRRT-PCR:
\- = 0 log10 copy/reaction
\+ = 0-3,5 log10 copy/reaction
++ = 3,5-4,5 log10 copy/reaction
+++ = >4,5 log10 copy/reaction

Table 3. shows that all three diagnostical methods sign the infection in goose surely. Pancreas, lung and spleen gave about the same results by all of the three methods, but examining the brain and the heart IHC and PCR was more sensitive compared to histology.

Mulard ducks' brains were the only samples that gave positive results by all of the methods and the stronger reaction was found by IHC. In heart samples of mulards only IHC gave positive results while histological lesion was found in only a single heart. Pancreas remained negative by all of the methods. As for all mulard duck organs the PCR positivity drop behind even more compare to IHC (spleen, lung, trachea). A possible explanation to this phenomenon might be the difference between the peace of organs examined by PCR and IHC, respectively. It seems more useful to take more samples from a single organs and to homogenise them (PCR), or to cut the tissue samples into more plane (histology, IHC). Kidney lesions are not in connection with virus multiplication.

As for Pekin ducks only the brain was positive by all of the methods as well, from which PCR proved to be the most sensitive one. Pancreas remained negative and only PCR could detect the infection in heart and in respiratory apparatus.

In all of the three waterfowl species which has been examined by all of the three methods the brain is the organ by which examination we could suspect (histology) or establish (IHC, PCR) the diagnosis of AI infection.

Our results by the three methods confirm the neurotropic behaviour of the H5N1 HPAI in three waterfowl species. The epitheliotrop (pancreatotrop) attribute of the strain was pronounced in goose, myotropic attribute was stronger in goose than in ducks. Considerable affinity to respiratory organs has not been found in either species. The typical but nonspecific lesions in spleen were possible to explain with virus replication only in case of goose and mulard duck. In kidneys of geese we could detect the virus without any histological lesions, in mulards however, despite of lesions we could not detect it except one IHC positive sample. Explanation for PCR positivity in kidneys of geese and one Pekin duck could be the fact that at these individuals the respiratory organs and possibly the air sacs were PCR positive and by sampling the kidney it is not possible not to contaminate the sample with air sac covering the kidney. During experimental infections this possibility is mentioned as well.

New scientific results

1. Description of pathological and histopathological lesions in mute swans infected with H5N1 HPAIV (pathogen to aquatic birds, and waterfowls) during the 2006 Hungarian epidemic, interpretation of the epidemic.
2. Description of pathological and histopathological lesions in Hungarian geese, mulard ducks and Pekin ducks.
3. Comparing the histological lesions and the amount of H5N1 AIV by immunohisto-chemical and quantitative PCR methods in organ samples of three domesticated waterfowl species (goose, Pekin and mulard duck) infected naturally.
4. The highest viral load could have been detected in the brain of swan, goose, mulard and Pekin duck, which confirms the strong neurotropic character of H5N1 highly pathogenic avian influenza virus.
5. The histological changes and the amount of viral nucleoprotein in the brain and in other organs were substantially more serious and higher, respectively in mute swan and domestic goose than in the two duck species examined.
6. There was many orders of magnitude higher viral RNA load detectable in organ samples of goose compared to the two duck species

List of publications

Pálmai Nimród, Deim Zoltán, Erdélyi Károly, Bálint Ádám, Dán Ádám, Márton Lázár, Glávits Róbert: **A madárinfluenza erősen virulens (H5N1 altípusú) törzse okozta kórbonctani és kórszövettani elváltozások bütykös hattyúban (*Cygnus olor*). / Gross, and histopathological lesions caused by highly pathogenic avian influenza virus (H5N1 in mute swans (*Cygnus olor*)**, preliminary report. Magyar Állatorvosok Lapja, 128. 265-272, 2006. (the whole text in Hungarian and in English)

Pálmai Nimród, Erdélyi Károly, Bálint Ádám, Márton Lázár, Dán Ádám, Deim Zoltán, Ursu Krisztina, Brandon Z. Löndt, Ian H. Brown, Glávits Róbert: **Pathobiology of highly pathogenic avian influenza virus (H5N1) infection in mute swans (*Cygnus olor*)**, Avian Pathology, 36. 245-249, 2007.

Szeredi Levente, Pálmai Nimród, Erdélyi Károly, Deim Zoltán, Márton Lázár, Glávits Róbert: **A madárinfluenza-vírus erősen patogén, H5N1 altípusának kimutatása immunhisztokémiai módszerrel bütykös hattyúból (*Cygnus olor*), (Detection of highly pathogenic avian influenza subtype H5N1 with immunochisto-chemical method in mute swan [*Cygnus olor*])** Magyar Állatorvosok Lapja, 129. 98-102, 2007. (in Hungarian)

Ivanics Éva, Bálint Ádám, Pálmai Nimród, Márton Lázár, Dán Ádám, Ursu Krisztina, Szeredi Levente, Deim Zoltán, Rigó Dóra,Tekes Lajos, Zarka Péter, Nagy Eörsné, Dobos-Kovács Mihály és Glávits Róbert: **A madárinfluenza erősen virulens (H5N1 altípusú) vírustörzse okozta megbetegedések hazai liba- és kacsaállományokban. / Diseases in Hungarian goose and duck flocks caused by highly pathogenic avian influenza strain (H5N1 subtype)**, Magyar Állatorvosok Lapja, 129. 387-399, 2007. (the whole text in Hungarian and in English)

Ivanics Éva, Glávits Róbert, Bálint Ádám, Palya Vilmos, Márton Lázár, Dán Ádám, Ursu Krisztina, Szeredi Levente, Rigó Dóra, Pálmai Nimród, Tekes Lajos, Dobos-Kovács Mihály és Kovács Zoltán: **Tömegesen előforduló vesekárosodás és köszvény H5N1 altípusú madárinfluenza vírussal fertőzött mulard kacsa állományban**, **(Kidney damage and gout occurring frequently in a mulard duck flock naturally infected with avian influenza virus subtype H5N1)** Magyar Állatorvosok Lapja, 129. 535-541, 2007. (in Hungarian)

Szeredi Levente, Dán Ádám, Pálmai Nimród, Ursu Krisztina, Bálint Ádám, Szeleczky Zsófia, Ivanics Éva, Erdélyi Károly, Rigó Dóra, Tekes Lajos, Glávits Róbert: **Tissue tropism of high-pathogenicity avian influenza virus subtype H5N1 in naturally infected mute swans (*Cygnus olor*), domestic geese (*Anser anser var. domestica*), Pekin duck (*Anas platyrhynchos*) and mallard duck (*Cairina moschata x Anas platyrhynchos*),** Acta Veterinaria Hungarica, 58. 133-145, 2010.

Rigó Dóra, Pálmai Nimród, Dán Ádám, Ursu Krisztina, Szeredi Levente, Szeleczky Zsófia, Bálint Ádám, Glávits Róbert: **Experimental study of avian influenza virus strains of different pathogenicity (H5N1, H5N2, H3N8, H10N4) in chicken embryos,** Acta Veterinaria Hungarica, 2010. Ready for publishing

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