Susceptibility of domestic pigeon (*Columba livia* L.) to West Nile virus lineage 2 infection

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- **Domestic pigeons (Rock dove, *Columba livia* L.) were experimentally infected with a lineage 2 strain of West Nile virus, circulating in Central Europe**
- **The birds stayed clinically healthy within the three-weeks period of the experiment and developed antibody response within one to two weeks**
- **Low dose (<100 $TCID_{50}$) WNV (*s.c.*) inoculations resulted in abortive infections**
- **Viraemia and shedding of virus / viral RNA was detected between DPI 3 and 12**
- **WNV RNA was detected in the spleens of the birds even at the end of the experiment (DPI 21)**
- **Direct transmission to contact pigeons was not recorded**
- **Pigeons may play a role in the non-vectorial (alimentary) transmission of WNV to predatory birds**

Figure 1: Experimental infection in BSL-3 conditions

Background and Objectives:

Wild birds are the natural hosts of West Nile virus (WNV, family *Flaviviridae*, genus *Flavivirus*) (1). In Europe, WNV strains belonging to lineage 1 are endemic in the Mediterranean region (1, 2). An exotic, lineage 2 strain of WNV emerged in Hungary in 2004, and has caused outbreaks of West Nile encephalitis in wild birds, horses and humans since (3-5). In 2008, increased number of West Nile encephalitis cases and considerable geographic spread of the infection was recorded; when the virus dispersed all around the country (5) and emerged in the eastern region of the neighbouring Austria as well (6). Since then the virus became endemic in the area and has been further spreading southwards, to northern Greece (7), Italy (8-10), and Serbia (11), were it caused significant outbreaks in humans and animals. The natural transmission cycle of WNV is based on mosquito vectors (1, 2, 12). However, fatal WNV infections due to the lineage 2 strain were frequently observed in birds of prey in Hungary and in Austria (3-5), therefore an alternative route of infection via consumption of infected prey carcasses were hypothesized (12). Because WNV-affected captive raptors were often fed with pigeons, this species was suspected as a potential source of infection. To estimate the host competence of domestic pigeons to WNV lineage 2 we have performed experimental infections.

Results:

Within both experiments all pigeons remained clinically healthy. In the first experiment (Table 1), birds infected with 1.000–10.000 $TCID_{50}$ of WNV became seropositive until day 7; those infected with 100 $TCID_{50}$ became seropositive between day 8 and 14; while the ones infected with 1–10 $TCID_{50}$ remained seronegative on day 21. Birds infected with higher doses of WNV have been shedding the virus by oral discharges and / or faeces between days three and eleven after infection. Virus RNA was detected in the spleen of infected birds even 21 days post infection (DPI). In the second experiment (Table 2) 6 out of 12 pigeons inoculated with 1.000 $TCID_{50}$ of WNV have been shedding the virus by pharyngeal discharge and / or faeces between day 3 and 12. Viraemia was detected in 4 birds (DPI 4-12). Viral RNA was detected in the spleen (n=7, DPI 2-12), liver (n=5, DPI 2-8), kidney (n=4, DPI 2-12), intestine (n=4, DPI 4-12), and lung (n=2, DPI 6-8) samples. The virus was detected only in one brain sample (DPI 12). WNV was back-isolated from the cloacal swab sample of one bird (DPI 5) and the spleen sample of another one (DPI 12). Anti-WNV antibodies were detected in three birds (DPI 10-12). Three contact control birds were kept together with the infected ones for 12 days. Within this period they remained WNV-free and seronegative.

Table 1.: Experiment 1: Inoculations of pigeons with different WNV doses. Red figures indicate Ct values of the real-time RT-PCRs (45 cycles of amplification). Viral RNA was not detected in the animals infected with 1 and 10 $TCID_{50}$ of WNV, and any of the pharyngeal and cloacal swab samples collected after DPI 11.

Virus $TCID_{50}$		Day 3		Day 4		Day 5		Day 6		Day 7			Day 8		Day 9		Day 10		Day 11		Day 21
	pigeon	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	blood	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	spleen
	1	negative	32.81	negative	32.21	negative	33.50	negative	29.58	negative	30.65	negative	negative	34.00	negative	34.04	negative	35.40	negative	negative	35.88
	2	negative	negative	negative	negative	43.23	negative	41.92	29.99	negative	28.65	negative	negative	negative	negative	negative	negative	negative	negative	negative	35.01
10.000	3	negative	negative	negative	negative	38.55	negative	36.08	35.02	negative	35.04	negative	negative	25.50	33.96	41.81	negative	negative	negative	negative	35.00
	4	negative	negative	negative	36.64	34.10	32.52	34.50	negative	27.89	32.17	39.76	32.18	37.41	negative	32.55	negative	negative	negative	negative	42.98
	5	negative	negative	35.45	31.74	36.62	26.84	35.31	27.12	negative	31.22	negative	negative	39.48	negative	negative	negative	negative	negative	negative	32.99
1.000	6	32.43	negative	33.19	33.89	33.30	negative	34.41	28.30	35.33	30.95	negative	negative	negative	negative	negative	29.23	negative	negative	negative	40.24
	7	negative	negative	35.03	negative	35.37	28.98	30.13	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	40.94
	8	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	28.59	negative	34.80
100	9	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative

Table 2.: Experiment 2: Inoculations of pigeons with 1.000 $TCID_{50}$ of WNV. Red figures indicate Ct values of the real-time RT-PCRs (45 cycles of amplification). Viral RNA was not detected in the contact control birds (number 9 / cage 3, number 12 / cage 4 and number 15 / cage 5). Back-isolations of WNV in Vero cells and in suckling mouse brain was successful from the samples highlighted with yellow background. NA: Not applicable, sample not available

Virus $TCID_{50}$	pigeon / cage	Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Blood	Organs						ELISA
		pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca	pharynx	cloaca		Spleen	Brain	Liver	Kidney	Intestine	Lung	
	1 / 1	negative	negative	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	negative	negative	negative	31.83	36.32	negative	negative	negative
	2 / 1	negative	negative	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	negative	33.45	negative	35.35	negative	negative	negative	negative
	3 / 1	negative	negative	negative	negative	negative	negative	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	negative	29.28	negative	40.21	negative	negative	negative	negative
	4 / 2	negative	negative	39.85	negative	negative	negative	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35.82	29.74	negative	negative	negative	33.34	negative	negative
	5 / 2	negative	negative	negative	negative	negative	negative	negative	31.06	33.62	37.42	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	37.82	31.01	negative	32.53	31.49	33.99	38.07	negative
	6 / 2	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	negative	negative	negative	negative	negative	negative	negative	negative
1.000	7 / 3	negative	negative	negative	negative	37.01	31.61	34.29	25.29	31.66	27.68	29.43	26.57	28.23	29.89	NA	NA	NA	NA	NA	NA	NA	NA	29.31	34.75	negative	33.26	31.14	28.08	34.75	negative
	8 / 3	negative	negative	negative	negative	negative	36.91	negative	negative	negative	37.29	negative	26.48	negative	26.59	negative	31.49	negative	negative	NA	NA	NA	NA	negative	negative	negative	negative	negative	negative	negative	positive
	10 / 4	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	NA	NA	NA	NA	NA	NA	NA	NA	negative	negative	negative	negative	negative	negative	negative	negative
	11 / 4	negative	negative	negative	38.77	27.72	30.98	26.05	30.98	27.12	27.26	26.81	28.23	31.92	31.84	negative	33.17	negative	negative	negative	negative	negative	34.71	29.88	27.52	negative	negative	33.91	28.62	negative	positive
	13 / 5	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	NA	NA	NA	NA	negative	negative	negative	negative	negative	negative	negative	negative
	14 / 5	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	34.83	negative	negative	negative	negative	negative	negative	negative	negative	negative	29.89	34.28	negative	negative	negative	negative	positive

Materials and Methods:

Commercially reared homing pigeons (Rock dove, *Columba livia* L.) were quarantined in mosquito-free environment for two weeks. Seronegative birds were placed in BSL-3 animal house facilities and were experimentally infected with a field isolate of WNV lineage 2 (strain 578/10, horse, Hungary, 2010). In a first experiment pigeons were subcutaneously (*s.c.*) infected with 1 to 10.000 $TCID_{50}$ of WNV and were observed for 21 days. Oral and faecal samples were collected individually every day, blood samples were collected weekly. Animals were put down on day 21, carcasses were necropsied and organ samples were collected. In a second experiment pigeons were infected with 1.000 $TCID_{50}$ of WNV. On every second day oral and faecal / cloacal swabs were collected, two birds were sacrificed, necropsied and organ samples were collected. Swab and organ samples were tested for the presence of WNV RNA with quantitative real-time RT-PCR method. Virus back-isolations from selected, PCR-positive samples were performed in Vero cell cultures and by suckling mouse brain inoculation. Anti-flavivirus antibodies were detected by competitive, WNV ELISA method.

Conclusions:

The results of the study indicate that pigeons are susceptible host of the lineage 2 WNV strain which is circulating in central Europe. The birds carried virus RNA for at least three weeks and have been shedding it through saliva and faeces. However, the viraemic phase was relatively short and no direct transmission to contact control pigeons was recorded. Experimental infections of pigeons with the New York 1999 (lineage 1) strain indicated that Rock doves develop strong immune response after a short, non-infectious viraemic phase; therefore they do not play a significant role in the transmission cycle of WNV (13). The results of our study revealed similar situation with the lineage 2 WNV strain circulating in Central Europe. Nevertheless, pigeons might be sources of non-vectorial transmission of WNV to predatory birds, because infectious virus was detected in the visceral organs of clinically healthy prey birds even 12 days after infection. Further studies could reveal the virus doses necessary for alimentary infection of birds of prey with WNV.

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