

EFFECT OF A PRELIMINARY INFECTION OF DUCKS WITH EDS-76 VIRUS ON THE DERZSY'S DISEASE PATHOLOGY

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Résumé

EFFET D'UNE PRÉ-INFECTION PAR UN ADÉNOVIRUS (EDS-76) SUR LA PATHOGÉNIE DE LA MALADIE DE DERZSY CHEZ LE CANARD DE BARBARIE. — Dans ce travail, nous nous sommes proposés d'étudier l'interaction de deux virus fréquents chez le canard de barbarie, l'un considéré comme non pathogène (EDS-76), et l'autre responsable d'une maladie aiguë, la maladie de Derzsy. Quatre lots expérimentaux ont été élevés en isolateurs pendant 6 semaines. Le lot 1 a été injecté le jour de l'éclosion avec un virus EDS-76 (A-127) puis 10 jours plus tard avec le virus de Derzsy. Les canards du lot 2 ont été injectés uniquement par le virus de Derzsy au 10^e jour. Le lot 3 n'a été injecté que par le virus EDS-76 le jour de l'éclosion ; le lot 4 de canards fut le lot témoin. La mortalité due à la maladie de Derzsy et les gains de poids ont été enregistrés. Le taux des anticorps contre les deux virus présents dans le sérum des différents groupes expérimentaux a été mesuré chaque semaine. Des essais *in vitro* de multiplication virale sur des cultures de fibroblastes d'embryons de canard ont été conduits. Les résultats obtenus montrent que la réponse humorale n'est pas influencée par l'injection concomitante des deux virus, l'intensité et le nombre des effets cytopathogènes sont identiques dans les cultures mono-contaminées et dans les cultures multipliant les deux virus. Par contre, *in vivo*, les canards ayant reçu les deux virus présentent une augmentation de la mortalité et une diminution significative du gain de poids par rapport aux canards mono-contaminés. Ce travail montre que le virus EDS-76, bien que peu pathogène par lui-même, peut entraîner un effet de synergie en cas d'association avec d'autres agents pathogènes, comme le virus de la maladie de Derzsy.

In the last few years, the prevalence of Egg Drop Syndrome virus (EDS-76) was demonstrated in a number of ducklings which exhibited high antibody levels to strain A-127 (Bouquet *et al* 1982, Marius *et al* 1983). This virus is not considered as so pathogenic when inoculated alone. However since it is frequently isolated in field conditions, its possible pathogenic role in interaction with other viruses needs to be investigated.

Derzsy's disease is a severe disease entity in growing Muscovy ducklings and goslings, caused by a parvovirus resistant to environmental conditions and horizontally transmitted. The acute form of disease results in the rapid death of young birds, but adults can be infected without any clinical sign of disease. Parvovirus was isolated from ducks and geese among other viruses namely adenoviruses (Bartha 1984, Bouquet *et al* 1982, Kisary 1974, Malkinson and Weisman 1980, Mac

Ferran and Adair 1977, Mac Ferran and Connor 1977, Zsak and Kisary 1984).

In this experiment, we were interested in the influence of an A-127 adenovirus infection on the course of Derzsy's disease in Muscovy ducklings. The *in vitro* interactions between these two viruses were also investigated.

Materials and Methods

1. Birds

Ninety-five male and female one day old Muscovy ducklings derived from a specific pathogen free (SPF) duck flock were used. This flock was maintained in isolated housing with a positive pressure ventilation system.

2. Virus strains

A virulent strain of Derzsy's disease virus (DDV) was

obtained from Dr Marius (Laboratoire de Pathologie Aviaire, Ploufragan, France). The LD50 of this strain for goslings was $10^{6.5}$

Strain B-38 of DDV adapted to goose embryo fibroblasts was kindly supplied by Dr Kisary (Academy of Sciences, Budapest, Hungary). This strain was passaged three times in Muscovy duck embryo fibroblasts (MDEF) and its Tissue Culture Infective Dose (TCID50) was $10^{5.0}$ in 0.1 ml.

Strain A-127 of avian adenovirus was received from Dr Vielitz (Lohmann, Cuxhaven, West Germany). This strain was adapted to chick embryo kidney cell culture and passaged five times in MDEF culture ; this hemagglutination (HA) titre was $4 \log_2$. The same strain, before being used for infection of ducklings, was propagated in duckling embryo liver cells ; the TCID50 of this strain was $10^{5.0}$ in 0.1 ml.

3. Muscovy duck embryo fibroblast culture (MDEF)

The cultures were prepared from 17 day-old embryos. The cells were grown in a mixture of minimum essential medium (MEM) with 10 % calf serum and 1 % bactotryptose-phosphate (BTP).

4. Determination of virus titres

TCID50 of Derzsy's disease virus. Tenfold dilutions of the virus in maintenance medium were made. Each dilution was pipetted into five replicate wells on micro-culture plates. MDEF suspension was added to each well. The plates were placed in a humidified incubator with 5 % CO₂ at 37 °C. Cell monolayers were examined with an inverted microscope to detect any cytopathic effect (CPE). The virus titre was calculated using the Reed-Muench method.

HA titre of A-127 strain. The test was made according to McFerran *et al* (1978), using a 1 % suspension of chicken erythrocytes in PBS and twofold dilutions of virus materials (microtitre system).

5. Serological (tests)

A haemagglutination inhibition test (HI) against A-127 was performed according to McFerran *et al* (1978) using 1 % chicken red blood cells, double dilutions of serum (initial dilution 1:2) and 4 HA-units of the virus. All sera titres higher than $3 \log_2$ were considered as positive.

A. virus neutralization test (VN) against DDV was made according to Marius *et al* (1983) and Kisary (1974). Twofold serial dilutions of sera diluted 1:10 were mixed with equal volumes of 100 TCID50 of the virus and incubated at 37 °C for 1 h. Five wells of micro-culture plates per dilution were inoculated with the serum-virus mixture. Cell cultures infected only with virus or serum served as a control. The highest dilution of serum neutralizing 50 % of the virus was considered to be SND₅₀.

All experiments *in vitro* were repeated three times and the results were expressed as mean geometrical values.

6. Experimental design

In vivo experiment : The effect of A-127 adenovirus infection in Muscovy ducklings previously inoculated with DDV was studied. One day-old ducklings were divided into three groups.

The thirty-nine birds of group 1 were inoculated at first day of life *per os* and intratracheally with an A-127 virus dose of 10^5 TCID50 per bird, and then on 10th day of life infected intramuscularly with 0.5 ml of DDV ($10^{8.5}$ LD50, diluted 1:300 000).

Group 2 was constituted of 30 ducklings, infected intramuscularly with the same dose of DDV, on 10th day of life.

Group 3 was formed of 13 ducklings, infected on the first day of life with the same dose of A-127 virus as group 1.

Group 4 was a control group of 13 uninfected ducklings.

Table 1. - Losses among ducklings infected with EDS-76 (strain A-127) and Derzsy's disease (DDV) viruses

	Experimental groups			
	1	2	3	4
virus	EDS-76+DDV	DDV	EDS-76	...
age of infection (days)				
virus				
EDS-76	1	0	1	0
DDV	10	10	0	0
Number of ducklings (50% male, 50% female)	39	30	13	13
Mortality (no. ducklings dead per week)				
		age (weeks)		
1	1	0	0	0
2	2	0	1	0
3	2	7	0	0
4	2	2	0	0
5	5	2	0	0
6	6	1	0	0
total (%)	18 (46)	12 (40)	1 (7)	0

All birds were observed for 6 weeks. The clinical symptoms and mortality due to DDV were observed in ducklings of groups 1 and 2. After 6 weeks, all ducklings were weighed and the mean weights in each group were calculated.

The sera from ducklings of each group were collected at weekly intervals and the VN test with DDV and the HI test with A-127 virus were performed.

In vitro experiment : The interaction between DDV and A-127 adenovirus in MDEF was investigated.

In the first trial, the MDEF cultures were inoculated simultaneously with 10^{2.83} TCID₅₀ of DDV strain B-38 and with various doses of A-127 strain (from 10^{1.3} to 10^{5.3} TCID₅₀).

In the second trial, the MDEF cultures were inoculated with 10^{2.83} TCID₅₀ of DDV and after 24 hours they were infected with several doses of adenovirus ranging from 10^{1.3} to 10^{5.3} TCID₅₀.

In the third trial, the MDEF cultures were inoculated with several doses ranging from 10^{1.3} to 10^{5.3} TCID₅₀ of A-127 strain and after 24 hours they were infected with DDV at a dose of 10^{2.83} TCID₅₀. As controls, the MDEF cultures inoculated only with 10^{2.83} TCID₅₀ of DDV or only with A-127 strain were used in several doses, as above.

All inoculated cultures were incubated for 7 days in a humidified incubator with 5% CO₂ at 37 °C. After 7 days the virus materials of each trial were collected and determinations of TCID₅₀ for DDV or HA titre for A-127 were conducted.

7. Statistical analysis

A chi-square test was performed to compare mortality between group 1 and group 2 of ducklings.

The significance of differences between means was calculated by Student's t-test.

Serological mean titers was calculated by expression of titration end points as tube or dilution numbers (Brugh 1978).

Table 2. - Mean weight of ducklings infected with EDS-76 (strain A-127) and Derzsy's disease (DDV) viruses

Experimental group virus	age of infection (d)	mean weight ± se (g) at 6 weeks of age
group 1 EDS-76 + DDV	1+10	814±99 (a,b)
group 2 DDV	10	1032±78 (a)
group 3 EDS-76	1	1750±111
Group 4 uninfected	...	1752±119

a : significantly different from control (P < 0.001)
b : significantly different from group 2 (P < 0.02)

Table 3. - Neutralizing antibodies againsts Derzsy's disease virus (DDV) in sera of Muscovy ducklings

	age (weeks)				
	2	3	4	5	6
log ₂ seroneutralizing antibody titer (no. of ducklings)					
<i>Group 1 : A-127 (1d) ± DDV (10 d)</i>					
	0 (11)	4.3 (4)	6.3 (3)	6.3 (1)	7.3 (3)
	4.3 (9)	5.3 (9)	7.3 (3)	7.3 (2)	8.3 (2)
	5.3 (1)	6.3 (3)	8.3 (6)	8.3 (6)	9.3 (2)
	...	7.3 (3)	9.3 (3)	9.3 (4)	11.3 (1)
	...	8.3 (2)	11.3 (1)	11.3 (5)	...
geometric mean titer ± sd	2.09 ± 5.1	5.82 ± 1.5	8.11 ± 1.8	9.13 ± 2.5	8.55±1.9
	y	y	y	y	y
<i>Group 2 : DDV (10 d)</i>					
	0 (11)	5.3 (2)	6.3 (2)	7.3 (10)	8.3 (4)
	4.3 (5)	6.3 (4)	7.3 (5)	8.3 (7)	9.3 (6)
	5.3 (2)	7.3 (8)	8.3 (4)	9.3 (6)	10.3 (4)
	...	8.3 (2)	9.3 (4)	10.3 (1)	11.3 (1)
geometric mean titer ± sd	1.78 ± 5.4	6.82 ± 0.8	7.97 ± 1.1	8.77 ± 0.6	9.43 ± 0.8
	y	z	y	y	y

means with a different letter are significantly different (P < 0.01)

Results and Discussion

Mortality due to DDV was higher but non significant (Chi-square test) in group 1, infected with DDV and A-127 virus than in group 2, infected with DDV alone since 18 ducklings were dead in group 1 and only 12 ducklings died in group 2 (table 1). Furthermore, the mean weight of uninfected ducklings was 1752 g whereas the mean weight of ducklings in group 1 infected with A-127 and DDV was 814 g. The difference between these two groups was 938 g and was highly significant (table 2). The ducklings of group 2 inoculated with DDV only weighed 1032 g and their mean weight differed significantly from the mean weight of ducklings with dual infection by about 218 g (table 2). No significant difference was observed between group 3 inoculated with A-127 and control group (table 2).

The results of serological investigations are reported in tables 3 and 4. Titers of neutralizing antibodies against DDV did not differ significantly in groups 1 and 2 of ducklings, except at 3 weeks of age. Geometric mean titers of neutralizing antibodies in group 1 with dual infection reached a peak at 5 weeks of age and decreased slightly at 6 weeks of age. The highest individual titer was observed at 4 weeks of age and it was equal to 11.3 log₂. On the other hand, in group 2 of

ducklings inoculated only with DDV, geometric mean titers increased until the end of the experiment. The highest individual titer was observed only at 6 weeks of age and it was equal to 11.3 log₂.

The HI antibody titers against A-127 virus differ significantly in group 1 and 3 of ducklings, at 1 and 3 weeks of age (table 4). Individual HI titers above 3 log₂ are noted earlier in the sera of ducklings from group 3, inoculated only with A-127 virus, whereas in group 1 of ducklings with dual infection, the first reactions above 3 log₂ were shown in the second week of age. Furthermore, in group 1 of ducklings, geometric mean titers were slightly lower than in group 3, except at 3 weeks of age. The highest individual HI titer in group 3 was equal to 6 log₂ and observed at one week of age, whereas in group 1 it was equal to 7 log₂ and observed later, at 3 weeks of age. After 3 weeks of age, HI antibodies decreased in the two groups of ducklings until the end of the experiment.

The observed differences in mortality and depressed weight in ducklings infected with the two viruses induced us to carry out an additional *in vitro* experiment.

The results are shown in tables 5, 6 and 7. Non significant differences in TCID₅₀ of DDV propagated in MDEF cultures in the absence or presence

Table 4. — Hemagglutination inhibition antibodies against adenovirus A-127 in sera of Muscovy ducklings

	age (weeks)					
	1	2	3	4	5	6
log ₂ hemagglutination inhibition antibody titer (no. of ducklings)						
<i>Group 1 : A-127 (1 d) + DDV (10 d)^a</i>						
0 (6)	0 (3)	0 (2)	0 (12)	0 (19)	0 (13)	0 (13)
1 (2)	1 (1)	2 (7)	1 (3)	1 (3)	1 (5)	1 (5)
3 (1)	2 (4)	3 (6)	3 (4)	2 (2)	2 (2)	2 (2)
...	3 (2)	4 (4)	4 (3)	3 (1)
...	4 (2)	5 (5)	5 (2)	5 (2)
...	...	6 (7)
...	...	7 (1)
geometric mean titer ± sd						
0.55 ± 1.02	1.92 ± 2.08	3.81 ± 3.44	1.64 ± 3.57	0.74 ± 2.10	0.45 ± 0.47	
y	y	y	y	y	y	y
<i>Group 2 : A-127 (1 d)</i>						
0 (3)	2 (3)	1 (3)	1 (4)	1 (6)	0 (6)	0 (6)
2 (1)	3 (1)	2 (8)	2 (4)	2 (4)	1 (5)	1 (5)
3 (5)	4 (2)	4 (1)	3 (2)	0 (2)	4 (1)	4 (1)
4 (2)	...	5 (1)	5 (1)
6 (1)
geometric mean titer ± sd						
2.58 ± 3.35	2.83 ± 0.97	2.15 ± 1.30	2.09 ± 1.49	1.16 ± 0.51	0.75 ± 1.29	
z	y	z	y	y	y	y

Means with a different letter are significantly different (P < 0.01)
a : virus (age of inoculation, days)

Table 5. – Propagation of Derzsy's disease virus (DDV) in the presence of A-127 virus in MDEF cultures

INFECTION	PROPAGATION			
	<i>virus DDV</i> mean TCID50		<i>Virus A-127</i> mean HA titer ^b	
<i>virus DDV</i> 2.83 (log ₁₀ TCID50) ^a	<i>virus A-127</i>		<i>virus DDV</i>	
+ <i>virus A-127</i> dose	present	absent	present	absent
log ₁₀ TCID50	log ₁₀ TCID50		log ₂ HA titer	
5.3	4.5	4.7	4	4
4.3	4.7	4.7	4	3
3.3	4.5	4.5	3	4
2.3	4.7	4.7	3	3
1.3	4.3	4.5	2	3
0	...	5.0

a : simultaneous infection with DDV and A-127 virus
b : hemagglutination titer

Table 6. – Propagation of Derzsy's disease virus (DDV) in the presence of A-127 virus in MDEF cultures

INFECTION	PROPAGATION			
	<i>virus DDV</i> mean TCID50		<i>Virus A-127</i> mean HA titer ^b	
<i>virus DDV</i> 2.83 (log ₁₀ TCID50) ^a	<i>virus A-127</i>		<i>virus DDV</i>	
+ <i>virus A-127</i> dose	present	absent	present	absent
log ₁₀ TCID50	log ₁₀ TCID50		log ₂ HA titer	
5.3	4.5	4.5	4	4
4.3	4.2	4.5	3	4
3.3	4.5	4.7	4	2
2.3	4.5	4.9	2	2
1.3	4.7	4.9	2	2
0	...	4.9

a : inoculation of DDV virus, 24 h before A-127 virus infection
b : HA titers : hemagglutination titer

Table 7. – Propagation of Derzsy's disease virus (DDV) in the presence of A-127 virus in MDEF cultures

INFECTION	PROPAGATION			
	<i>virus DDV</i> mean TCID50		<i>Virus A-127</i> mean HA titer ^b	
<i>virus DDV</i> 2.83 (log ₁₀ TCID50) ^a	<i>virus A-127</i>		<i>virus DDV</i>	
+ <i>virus A-127</i> dose	present	absent	present	absent
log ₁₀ TCID50	log ₁₀ TCID50		log ₂ HA titer	
5.3	4.5	4.5	4	4
4.3	4.7	4.7	3	4
3.3	4.7	5.0	3	3
2.3	4.5	4.8	2	2
1.3	4.7	4.7	1	1
0	...	5.0

a : inoculation of A-127 virus 24 h before DDV infection
b : HA titer : hemagglutination titer

of A-127 virus were exhibited. Also no differences were found in CPE in cells when they were infected either with DDV only or with DDV and A-127 strain. Independently of the inoculation time of MDEF with A-127 virus either simultaneously, prior to or after infection with DDV, the differences between TCID₅₀ of DDV did not exceed 0.2-0.3 log. Furthermore, the dose of inoculated A-127 virus had no influence on TCID₅₀ of DDV.

The results of *in vitro* investigations did not reveal an interaction between DDV and the adenovirus strain A-127. No significant differences were observed in the serological responses of infected ducklings.

On the other hand, *in vivo* results showed that EDS-76 (A-127) virus, non pathogen by itself

could induce an increase of other diseases, as the Derzsy's disease. Considering the high frequency of EDS-76 (A-127) virus in field conditions, it should be of interest to investigate the interaction of this virus with other pathogen or non pathogen agents (viruses or bacteria) and to determine the benefit of detecting quickly the presence of such a virus in ducklings flocks.

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Abstract

The aim of this work was to investigate the role of a non pathogenic adenovirus (EDS-76, strain A-127), frequently isolated from ducklings on the infection by a pathogen agent (such as the parvovirus responsible for Derzsy's disease, DDV). Three groups of ducklings were respectively infected with A-127 alone, at day old, DDV alone at day 10th, A-127 at day old plus DDV at day 10th. The infected groups and a control one were raised under similar conditions. Pathological signs were observed and body weights recorded. Sera were collected weekly and analysed for DDV and A-127 antibodies. In addition, *in vitro* assays of the multiplication of these viruses and studies on cytopathic effects on duck embryo fibroblasts were conducted. The humoral response did not appear to be influenced by dual infection. No differences in cytopathogenic effects were detected *in vitro* between single and dual infection. On the other hand, in ducklings, dual infection increased the mortality rate and depressed the body weight gain, as compared to infection with either virus alone. The drastic effect on the body weight gain of the EDS-76 virus in the presence of DDV clearly shows a synergic role of this virus on the course of Derzsy's disease.

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