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Tamás Mató

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E-mail: cavanagh@metronet.co.uk URL: http://mc.manuscriptcentral.com/cavp

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Short beak and dwarfism syndrome of mule duck is caused by a distinct lineage of Goose parvovirus

Vilmos Palya^{1*}, Anna Zolnai¹, Zsófia Benyeda², Edit Kovács¹, Veronika Kardi¹ & Tamás Mató¹

 ¹CEVA-Phylaxia Veterinary Biologicals Co. Ltd., Szállás utca 5. 1107 Budapest, Hungary
 ²Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University, István u. 2., 1078 Budapest, Hungary

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*To whom correspondence should be addressed. Tel: + 36 1431 6103. Fax: + 36 1260 3889 E-mail: vilmos.palya@ceva.com

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Abstract

From the early 1970s until now numerous cases of short beak and dwarfism syndrome have been reported in mule ducks from France. The animals showed strong growth retardation with smaller beak and tarsus. It was suggested that the syndrome was caused by goose parvovirus on the basis of serological investigation, but the causative agent has not been isolated and the disease has not been reproduced by experimental infection so far.

The aim of the present study was to characterize the virus strains isolated from field cases of SBDS, and to reproduce the disease experimentally. Phylogenetic analysis proved that the parvovirus isolates obtained from SBDS of mule duck belonged to a distinct linage of goose parvovirus-related group of waterfowl parvoviruses. The authors carried out experimental infections of day-old, 2 and 3 weeks old mule ducks by oral route with 3 different parvovirus strains: strain D17/99 of goose parvovirus from Derzsy's disease, strain D176/02 isolated from SBDS of mule duck. The symptoms of short beak and dwarfism syndrome of mule duck could only be reproduced with the mule duck isolate (strain D176/02) following day-old inoculation. Infection with a genetically different strain of GPV isolated from classical Derzsy's disease (D17/99) or with MDPV strain (FM) did not cause any clinical symptoms or pathological lesions in mule ducks.

Introduction

Short beak and dwarfism syndrome (SBDS) was first reported in 1971-1972 in South-western France in mule duck (a sterile, intergenetic cross of Pekin and Muscovy ducks) flocks (Villatte, 1989). In the diseased flocks, about 10 to 30% of birds were affected showing strong growth retardation with smaller beak. On the basis of serological investigation the syndrome was suggested to be caused by goose parvovirus (GPV), but the causative agent has not been isolated and the disease has not been reproduced by experimental infection until now. The occurrence of the syndrome seemed to be controlled by vaccination of breeder flocks against Derzsy's disease. In 1989-1990 an acute disease with high morbidity and mortality (86-100 %) among different types of ducks was reported in Taiwan (Lu et al., 1993). The mortality rate depended on the age of the birds, but only young ducklings suffered from the disease. All breeds of ducklings (e.g. Muscovy ducks, pekin ducks, mule ducks) were affected showing the same clinical symptoms and similar severity of the disease. Surviving ducklings showed atrophic bills with protruding tongues and stunted growth. The condition was reported to have been caused by mixed infection with duck hepatitis virus and Muscovy duck parvovirus (MDPV). Experimental inoculation was carried out and only a duck parvovirus strain (isolate 902193) caused atrophic bills and stunting in SPF ducks. An inactivated bivalent (parvovirus and duck viral hepatitis virus) vaccine was used for the immunization of ducklings in the field and this vaccination had protective effect and resulted in preventing further outbreaks. SBDS was reported from Poland as well in 1995 (Samorek-Salamonowicz et al., 1995). The typical symptoms of SBDS were observed in mulard (mule) ducks at the first weeks of age and the morbidity was 15-25%.

The aim of our study was to isolate and characterize the causative agent of SBDS, and to reproduce the disease in mule ducks experimentally.

Materials and Methods

Virus isolation. GPV and MDPV. Antibody-free 12-day-old embryonated Muscovy duck eggs were inoculated with homogenates of selected internal organs (liver, spleen and heart) collected from mule ducks with clinical symptoms of SBDS originated from seven different

flocks. The inoculated eggs were incubated for 13 days and then, if no mortality occurred, two further passages were done using pooled samples of the allantoic fluids and homogenates of the embryos internal organs (liver, heart, intestine). If mortality occurred, the embryos were checked for pathological changes, and both the allantoic fluids and the clarified homogenates of the embryos internal organs were tested for parvovirus antigen by ELISA (Kardi and Szegletes, 1996). Primary goose embryo fibroblast (GEF) cell cultures were also inoculated with the organ homogenates, following the method described previously (Gough, 1998). The cell cultures were incubated at 37°C for 7 days and checked daily for cytopathic effect. Five blind passages were carried out in GEF cell cultures before a sample was considered negative for parvovirus.

Detection of parvovirus specific DNA by PCR. The detection of parvovirus specific DNA was attempted from samples obtained from diseased birds originated from seven different SBD cases between 2002 and 2006. Isolation of DNA was performed using QIAamp DNA mini kit (Qiagen) according to the manufacturer instruction, either directly from organ samples (generally bursa, spleen, liver and heart) or from the virus suspensions (allantoic fluids and embryo homogenates) obtained by the propagation of the isolates or reference strains of GPV and MDPV in embryonated Muscovy duck eggs. A 493 bp long (2562-3054) fragment of VP1 was amplified using a set of primers. The sequence of the primers was Gp3 5'-AGAAAACCCCAACGAAAAGA-3' (forward) and Gp4 5'-CTCCGCTTCCTCCTCTG-3' (reverse). The PCR reaction was carried out on the DNA samples (Tatár-Kis *et al.*, 2004).

Sequencing and phylogenetic analysis. PCR products were purified using QIAquick PCR purification kit (QIAGEN). The purified PCR products were sequenced using BigDye Terminator Cycle sequencing kit (Applied Biosystems) according to the supplier's instructions. Sequence of the PCR products was determined using dye terminator sequencing method and analysed by AB1 PRISM 310 autosequencer. Sequence differences were calculated by Kimura's method. Phylogenetic tree was constructed using the Neighbourjoining method by TREECON for Windows program (Van de Peer *et al.*, 1994). The sequence of a French MDPV strain (FM/Fr) was used as outgroup. Two nucleotide sequences (D146/3/02 and D176/02) of the seven isolates had already been published in the GenBank with common accession number: AY496906 (the two isolates were identical in the tested region). Sequences of the other parvovirus strains, isolated from outbreaks between 2004 and 2006 were deposited to the GenBank with the following accession numbers: EU938702-

EU938706. Sequence identity matrix was calculated by BioEdit sequence alignment editor software.

Experimental reproduction of the disease. Challenge experiment I. Two hundred and sixty, 1 day old female mule ducklings, free from parvovirus specific maternal antibodies were divided into seven groups. The ducklings were kept in isolated animal houses. Three groups (A, B and C), forty animals in each, were inoculated per os with one of the following parvovirus strains at one day of age. The virus strains used are indicated in Table 1: (i) strain D176/02 isolated from SBDS of mule duck; (ii) strain D17/99 (Bócsa/99Hu) a GPV isolate from Derzsy's disease; and (iii) strain FM a MDPV isolate from parvovirosis of Muscovy ducks. Another three groups (D, E and F) were infected with the same virus strains at 2 weeks of age. The last group of animals (K) served as uninfected controls (see Table 1 for study details). Birds were infected with 10^{4.0}-10^{4.3} TCID₅₀/EID₅₀ virus, each. All groups were examined daily for clinical signs (growth retardation, feathering disorders) for 6 weeks after infection. Body weight, as well as the width and length of the beak of 10 individually tagged animals in each group were measured weekly. Ratio of length and width of the beak was calculated. A bird was considered positive for SBDS if the relative body weight of the bird (body weight measured at the end of the post-infection observation period divided by the body weight measured on the day of infection) was lower than the average of the control (K), minus three times the standard deviation, and/or typical clinical signs of parvovirus infection were observed.

For the statistical analysis of the body weight and beak size data the ANOVA with repeated measurements test was used. In case of significant group-time interaction, the data were compared with one way ANOVA according to time points. The significant threshold was set at 5%. Statistical analysis was performed on MiniTabTM statistical software, release 13.20

Challenge experiment II. Two hundred, 1 day old female mule ducklings, originated from breeder flock (Pekin duck layers) previously vaccinated with live GPV vaccine were used. The experimental design was similar to experiment I, with the exception that thirty ducks were infected per groups and the second infection was carried out at 3 weeks of age when the maternally derived antibodies dropped to undetectable level. The observation period lasted till the birds reached 9 weeks of age (see Table 2 for study details).

Gross pathology, sampling for serology, PCR and histology. Five birds per group were exsanguinated at 1, 2, 3, 4 and 6 weeks of age in group A, B, and C and at 3, 4, 6 and 8 weeks of age in groups D, E, F (see Table 1) and blood sampled for serology in experiment I. After necropsy, spleen and liver samples were taken for PCR analysis. The remaining animals were exsanguinated at the end of the observation period and blood was collected for serology. Tissue samples from spleen, liver, kidney, duodenum and heart were taken and fixed in 10% buffered formalin for histology. Tissues were embedded in paraffin, cut at 3-5 µm and stained with haematoxylin and eosin. Samplings done in experiment II are shown in Table 2.

Serology. Serum samples collected from ducks were tested for antibodies to both GPV and MDPV by sandwich-blocking ELISA method (Kardi and Szegletes, 1996). Anti-GPV-goose IgG was used both as coating and detection antibodies. The detection anti-GPV goose IgG was labelled with biotin and it was reacted with extravidin-peroxidase (SIGMA). GPV and MDPV for antigens used in the ELISA test were propagated in embryonated Muscovy duck eggs and purified from the allantoic fluid/embryo homogenate by ultracentrifugation through a 30% (w/w) sucrose cushion at 140000xg for 33 hours at 4°C. The principle of the ELISA method was the same for both antibodies. For the measurement of GPV antibody GPV antigen, while for the MDPV antibody detection MDPV antigen was used.

Results

Isolation and propagation of the causative agent. Isolation of the virus from organ samples of mule ducks with clinical signs of SBDS, which were found positive for parvovirus specific DNA by PCR, was successful in five cases in embryonated Muscovy duck eggs following one or two blind passages. The virus killed the embryos with lesions characteristics of parvovirus infection after 6 to 10 days incubation. These isolates, however, did not cause cytopathic effect when inoculated onto primary goose embryo fibroblast cell cultures, even after five blind passages.

Phylogenetic analysis. The result of phylogenetic analysis is shown in Figure 1. Pair-wise sequence comparison revealed that all seven virus strains detected by PCR from mule duck with SBDS between 2002 and 2006 fell into the GPV-related waterfowl parvoviruses group. They were closely related to each other and clustered into the West-European linage of the

GPV-related group. Four (D146/02, D176/02, D479/12/04 and D697/3/06) of the seven mule duck isolates were identical with each other, and the remaining three isolates (D523/2/05, D657/3/06 and D518/3/05) differed from them only in two or three nucleotides. These nucleotide changes caused amino acid change only in the case of D657/3/06 isolate at the position of V140L. Homology among SBDS isolates ranged between 98.8 and 100%, while they shared only 95.9-96.8% homology with the classical Derzsy's disease viruses (Hungarian virulent isolates from geese). Comparison of the D176/2 mule duck isolate and the "classical Derzsy's disease" representative B strain revealed 15 nucleotide differences, which led to 4 amino acid changes. The other members of West-European group – SHM319 virulent field strain and Hoekstra vaccine strain- differed from the representative mule duck parvovirus strain (D176/02) in three or four nucleotides, but only the nucleotide change detected in the Hoekstra strain caused amino acid change at the position of S78N.

Experimental reproduction of the disease. *Experiment I. Clinical signs after challenge*. The clinical signs of SBDS could only be observed in birds challenged with the D176/02 strain. The morbidity in group inoculated at 1 day of age reached 70%, but the mortality remained low, only 2 out of 40 ducklings (5%) died due to the infection. When the infection was carried out at 2 weeks of age, only 20% of ducks showed the clinical signs of SBDS, and death did not occur. The main clinical signs observed were growth retardation, short beak - "goose beak-shape" - and feathering disorders (Figure 2 and 3).

The width of the beaks did not differ significantly among the groups, but the length of the beaks of the diseased animals was much shorter than those of the controls or the healthy group-mates, as a consequence the ratio between the length and the width of the beak was lower in ducks with clinical signs of SBDS. The ratio was significantly lower in group A (infected at one day of age) compared to the other groups (p<0.001). There was no significant difference among the groups challenged at 2 weeks of age and the controls (Table 3).

The birds infected with the D17/99 strain of GPV (isolate from classical Derzsy's disease) and the FM strain of MDPV did not show any clinical signs indicative of SBDS after infection at either of the two challenge dates.

Pathology, postmortem findings. In mule ducks with clinical signs, infected with the D176/02 isolate at 1-day of age, ascites, hydropericardium and fibrinous pseudomembrane on the surface of the liver could be observed 1 to 2 weeks after infection. By histology sero-fibrineous perihepatitis, enteritis, myocardial and muscular degeneration were the most

prominent findings. The challenge at 2 weeks of age with the D176/02 resulted in discrete hydropericardium in the diseased ducks between 1 and 2 weeks after infection and the histological lesions were similar to the previous ones, but they were less severe. In animals exsanguinated 3 or more weeks after infection the internal organs showed neither gross nor histological lesions. The internal organs of ducklings challenged with the D17/99 GPV strain and the MDPV FM strain were free from any pathological lesions at each of the sampling date.

PCR. GPV-specific DNA could be detected from all birds infected with the D176/02 or D17/99 virus strains from 1 or 2 weeks, respectively, until 6 weeks post-infection, regardless to the age of the ducklings at infection. The only difference between the two isolates was that following the day-old infection, GPV-specific DNA could be detected one week later (2 weeks p.i.) from the animals infected with the D17/99 (Derzsy's disease isolate). MDPV-specific DNA could not be detected from any of the birds infected with the MDPV strain. Samples from non-infected ducks remained negative during the whole observation period.

Serology. The ELISA titres of parvovirus specific antibodies in the groups infected with the different parvovirus strains are shown in Table 4. Significant level of antibody to GPV was detected in the groups infected with D176/02 strain (mule duck isolate). The immune response was more rapid in ducks infected at 2 weeks of age (the antibody level raised high already 1 week after infection). In animals infected with the GPV strain (D17/99) considerable level of immune-response to goose parvovirus could be detected, but somehow the immune-response was delayed compared to the one obtained in the birds infected with the mule duck isolate. Negligible immune response to MDPV could be measured, only in a smaller portion of the birds infected with MDPV.

Experiment II. *Clinical signs after challenge*. The clinical signs of SBDS could be observed only in animals challenged with the mule duck isolate (D176/02) at 1 day of age. The morbidity reached 33%, but the mortality remained low, only 1 out of 30 ducklings (3%) died at 14 days after the infection. The dead bird showed growth retardation without any gross pathological lesions indicative of parvovirus infection. The birds infected with the mule duck parvovirus strain (D176/02) at older age (3 weeks) or at any of the two challenge dates with the GPV strain from classical Derzsy's disease (D17/99) or with the MDPV strain (FM) did

not show any specific clinical signs (short beak or growth retardation) indicative of parvovirus infection.

Pathology The internal organs of animals with clinical signs had no gross pathological lesions, only mild reticulum cell proliferation in the spleen, myocardial and muscular degeneration, and focal myocarditis could be observed by histology.

PCR Parvovirus-specific nucleic acid could be detected only from those animals which were infected with the D176/02 mule duck parvovirus isolate. In those animals the virus could be detected from the spleen and liver even 5 weeks after infection. In birds infected with the D17/99 and FM strain there were no detectable parvovirus specific nucleic acid.

Serology. Significant level of antibody to GPV was detected in the birds infected with the mule duck parvovirus strain (D176/02). Peak of antibody levels were measured at 4 and 5 weeks after infection. Only moderate humoral immune response was induced in the birds infected with GPV strain of classical Derzsy's disease (D17/99) at one day of age. The ELISA titres measured 2, 3, 5, 7 and 9 weeks after infection were 462, 1827, 45, 1.6 and 0. No antibodies to parvoviruses could be detected in the animals which were infected with this virus strain at 3 weeks of age and in ducks infected with the MDPV at either one day or 3 weeks of age.

Discussion

The isolation of the causative virus from birds showing characteristic signs of "short beak and dwarfism syndrome" was successfully achieved in parvovirus antibody-free embryonated Muscovy duck eggs. The strains isolated so far from mule ducks with SBDS could be efficiently propagated only in Muscovy duck eggs. The isolation and propagation of the mule duck virus in primary goose embryo-fibroblast cell culture was not successful, the virus did not cause cytopathic effect on this cell substrate. On the other hand, the detection of goose parvovirus specific DNA by PCR from samples obtained from ducks with clinical signs was always successful; therefore the use of PCR seems to be the preferred choice of method to confirm the presumptive clinical diagnosis of SBDS.

With the characterisation of the isolates obtained from cases of SBDS and with experimental infection of mule ducks we could demonstrate that the "short beak and dwarfism syndrome" could be reproduced only with specific goose parvovirus strain. This isolate together with other isolates from SBDS cases are clustered together with the West-European linage of goose parvoviruses and they are genetically very closely related.

SBDS could not be reproduced in mule duck with classical goose parvovirus strain of Derzsy's disease. The virus could, however, be detected following the experimental inoculation of mule ducks from clinically healthy birds, but infection was established only in a smaller portion of the inoculated birds. The resistance of mule duck to infection with GPV of classical Derzsy's disease was confirmed also by the delay or the lack of immune response in the inoculated mule ducks. These findings may indicate that the GPV strains, which are able to cause SBDS, have specifically been adapted to mule duck that happened under unknown circumstances. Hypotheses for the emergence of this host variant virus, which can be put forward, include a direct mutation from a field GPV of the West-European linage or a mutation from a live GPV vaccine. It is interesting to note that in Hungary, where a different linage of goose parvovirus is endemic, SBDS in mule ducks has never been reported.

The morbidity of birds after experimental inoculation at one day of age was lower than in the study carried out by Lu *et al.* (1993) in Taiwan, however the breeds of ducks used in the two experiments were different. The rate of animals with clinical signs in the experiment carried out in mule ducks with maternally derived antibodies was similar to the one recorded in France and Poland in field cases. This may suggest that the causative agent of SBDS in Europe is different, in terms of pathogenicity, from the one reported in Taiwan, although the field cases in Taiwan were caused by a mixed infection of MDPV and duck hepatitis.

The main clinical signs observed in field cases are growth retardation and short beak with low morbidity rate. It is the bad weight gain and the heterogeneity within the flock that causes the economical losses. Parvovirus infection is usually associated with high morbidity and mortality rates in susceptible young goose and Muscovy duck flocks (Derzsy 1967, Woolcock *et al.*, 2000) and infection causes severe pathological lesions in the internal organs of these species (Glávits *et al.*, 2005). In our studies the experimental infection of susceptible day-old mule ducks with a specific isolate of goose parvovirus obtained from field case of SBDS caused relatively high morbidity, but the mortality remained low. The age of mule ducks at infection has also influence on the development of clinical signs. According to the results of our studies, mule ducks did not exhibit clinical disease when the infection was done at 3 weeks of age. The moderate clinical signs and the negligible mortality, even in case of

early infection, suggest a relatively high resistance of mule duck to infection with parvoviruses, moreover infection with MDPV was abortive in mule ducklings.

The mule duck parvovirus strain did not cause serious lesions in the internal organs, however, the development of tarsus and beak was influenced greatly by the infection. The shortening of the beak is suggested as a consequence of virus effect on beak morphogenesis. The beak is made up of multiple facial prominences. During development, these prominences are proportionally coordinated to compose a unique beak. There are two proliferative zones in the frontonasal mass of beak in ducks. These growth zones are associated with bone morphogenetic protein 4 (BMP4) activity (Wu *et al.*, 2004) that might be affected during parvovirus infection which in turn can result in the modulation of the beak shape. The mechanism of parvovirus infection and its effect on these cells however needs further investigation.

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Group	No of animals	Age at infection	Challenge virus strain	Weighing	Beak measurement	Sampling for serology, PCR and histology
А	40	1-day-old	D176			
В	40	1-day-old	D17/99	1, 2. 3, 4, 6, weeks of	1, 2. 3, 4, 6, weeks of age	$ \begin{array}{c} 1 (5)^*, 2 (5), 3 \\ (5), 4 (5), 6 (5) \end{array} $
С	40	1-day-old	FM	age		weeks of age
D	40	2-week-old	D176	22469		2(5) A(5) E(5)
E	40	2-week-old	D17/99	2, 3, 4, 0, 8 weeks of	2, 3, 4, 6, 8	8(5), $4(5)$, $0(5)8(5) weeks of$
F	40	2-week-old	FM	age	weeks of age	age
K	20	9	-	1, 2. 3, 4, 6, 8 weeks of	1, 2. 3, 4, 6, 8 weeks of age	1, 2. 3, 4, 6, 8 (2) weeks of age

Table 1. Challenge experiment I (parvovirus maternal antibody-free ducklings)

* No of tested animals are in parenthesis

esis

Group	No of	Age at	Challenge	Weighing Beak		Sampling for	
	animals	infection	virus strain		measurement	serology, PCR	
						and mistology	
А	30	1-day-old	D176				
		•		1, 2, 3, 4, 5,	2570	2 (2), 3 (2), 5 (3),	
В	30	1-day-old	D17/99	7, 9 weeks 5, 5, 7, 9		7 (3), 9 (3) weeks	
a	20			of age	weeks of age	of age	
С	30	l-day-old	FM	8		8-	
р	20	2 wook old	D176				
D	30	J-WEEK-OIU	D170	2 4 5 7 0			
F	30	3-week-old	D17/99	5, 4, 5, 7, 9	3, 5, 7, 9	5 (2), 7 (3), 9 (3)	
L	50	5 WEEK Old	DIN	weeks of	weeks of age	weeks of age	
F	30	3-week-old	FM	age	0	0	
-	20						
Κ	20	-	-	1, 2. 3, 4, 5,	2 5 7 0	0(5) weaks of	
				7, 9 weeks	3, 5, 7, 9	9 (3) WEEKS OI	
				of age	weeks of age	age	

Table 2. Challenge experiment II (ducklings from parvovirus vaccinated parents)

* No of tested animals are in parenthesis

.desis

			Mean length/width of beaks mm (mean ratio ±SD)				
Group		at 1 week of age	at 2 weeks of age	at 3 weeks of age	at 4 weeks of age	at 6 weeks of age	at 8 weeks of age
	А	22.31/12.93	26.39/15.08	30.44/17.46	35.63/19.67	42.35/22.65	
at 1 ge	D176/2	(1.73±0.04)	(1.75±0.09)	(1.73±0.16)	(1.8±0.14)	(1.85±0.16)	-
on of a	B D17/99 (GPV)	26.27/14.55	34.4/17.98	40.81/21.18	45.84/23.98	54.18/26.65	
ecti ay c		(1.81±0.08)	(1.91±0.06)	(1.92±0.13)	(1.91±0.16)	(2.03±0.18)	
Infe dâ	C FM (MDPV)	24.53/13.89	32.38/17.32	41.05/20.42	46.76/22.95	54.03/25.83	
		(1.77±0.11)	(1.87±0.08)	(2.01±0.11)	(2.04±0.09)	(2.09±0.10)	
Infection at 2 weeks of age	D	n m	33.55/15.7	41.57/21.07	45.82/23.0	51.64/25.65	55.91/26.78
	D176/2	11.111.	(2.14±006)	(1.97±0.05)	(1.99±0.06)	(2.01±0.06)	(2.09±0.09)
	E D17/99 (GPV)	nm	32.15/15.1	43.77/21.77	48.11/22.68	56.66/25.98	60.79/27.98
		11.111.	(2.13±0.14)	(2.01±0.09)	(2.12±0.09)	(2.18±0.17)	(2.17±0.14)
	F		32.85/15.45	42.89/21.76	49.48/24.25	58.22/27.05	63.78/27.72
	FM (MDPV)	n.m.	(2.13±0.09)	(1.97±0.06)	(204±0.06)	(2.16±0.08)	(2.31±0.14)
	Κ	25.05/13.94	33.88/16.8	42.6/21.14	47.6/23.09	54.53/24.98	61.31/26.21
non-infected control		(1.8±0.04)	(2.02±0.10)	(2.02±0.07)	(2.06±0.08)	(2.18±0.07)	(2.35±0184)

Table 3. Measurement of beaks of mule ducklings

n.m.: not measured

γ) τ 5.8 42. 0.10) (2.02.

Table 4. Mean ELISA titres of goose parvovirus specific antibodies (after infection with
D176/2 and D17/99 strains) and titres of MDPV specific antibodies (after challenge with FM
strain) in experiment I

Groups		Postinfection time (week)						
		1	2	3	4	6		
Infection at 1 day of age	A D176/2	90	4181	20617	23085	25374		
	B D17/99 (GPV)	-ve	1346	14889	13068	4167		
	C FM (MDPV)				27 (2/5)	23 (8/20)		
		-ve	-ve	-ve	(430-4097)	(40-12071)		
Infection at 2 weeks of age	D D176/2	1059	12010	ND	37331	19344		
	E D17/99 (GPV)	7 (2/5) (73-205)	1717	ND	8954	65850		
	F FM (MDPV)				4 (1/5)	4 (4/24)		
		-ve	-ve	ND	(1213)	(56-12500)		
	K non-infected control	-ve	-ve	ND	-ve	-ve		

In parenthesis: No. of seropositive animals/No. of tested animals (min.-max. titres of seropositive animals)

ND: not done

Figure legends

Figure 1. Phylogenetic analysis of goose parvovirus strains

Figure 2. Uneven-growth, feathering disorder

Figure 3. Short beak

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