NOTE *Virology*

Genetic Characterization of a Potentially Novel Goose Parvovirus Circulating in Muscovy Duck Flocks in Fujian Province, China

Shao WANG[1,2)#], Xiao-Xia CHENG[1,2)#], Shao-Ying CHEN[1,2)]*, Xiao-Li ZHU[1)], Shi-Long CHEN[1,2)], Feng-Qiang LIN[1,2)] and Zhao-Long LI[1)]

[1)]*Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agriculture Sciences, Wu Si Road, Fuzhou, Fujian 350003, China*
[2)]*Fujian Animal Diseases Control Technology Development Center, Pu Dan Road, Fuzhou, Fujian 350013, China*

(Received 3 December 2012/Accepted 25 March 2013/Published online in J-STAGE 8 April 2013)

ABSTRACT. We report a novel goose parvovirus (MDGPV/PT) isolated from an affected Muscovy duck in Fujian Province, China. In this study, the NS1 sequence analyses indicated a close genetic relationship between MDGPV/PT and Muscovy duck parvovirus (MDPV) strains, although MDGPV/DY, which was isolated from a Muscovy duck in 2006 in Sichuan Province, could be divided into GPV-related groups. Phylogenetic analysis showed that except for differences in the NS1 gene, MDGPV strains PT and DY are closely related to a parvovirus that infects domestic waterfowls. This is the first demonstration of recombination between goose and Muscovy duck parvoviruses in nature, and MDGPV/PT might have led to the generation of a novel waterfowl parvovirus strain circulating in Muscovy duck flocks in China.
KEY WORDS: Derzsy's Disease, goose parvovirus, Muscovy duck, recombination.

doi: 10.1292/jvms.12-0527; *J. Vet. Med. Sci.* 75(8): 1127–1130, 2013

Waterfowl parvoviruses, part of the Dependovirus genus and Parvoviridae family, can be divided into goose parvoviruses (GPVs) and Muscovy duck parvoviruses (MDPVs) [21]. The waterfowl parvoviruses are nonenveloped and contain a single-stranded DNA genome of approximately 5.1 kb in length, including two large nonoverlapping open reading frameworks (ORF). The left ORF (LORF) encodes one nonstructural protein (Rep), namely regulatory protein NS1; the right ORF (RORF) encodes three structural proteins (VPs), namely VP1, VP2, and VP3. VP1 and VP2/VP3 are identical except for an additional 145 amino acids at the amino terminus of the VP1 protein called the VP1-unique region (VP1u). The coding overlapping architecture of the gene is characterized as a "basket structure" with an identical 3' end for VP proteins, and a small noncoding gap of 18 nucleotides can also be found between the two ORFs [14, 19].

GPV infection (also known as Derzsy's Disease, DD) is a highly contagious gastrointestinal disease of geese and ducks that is similar to the infectious characterization of a three-week disease (also known as 3-w disease) among Muscovy ducks (*Cairina moschata*) caused by MDPV in China [3, 20]. Since DD is prevalent in Muscovy duck flocks in the coastal city of Putian, Fujian Province, the waterfowl industry in these areas has been suffering significant economic losses. To prevent waterfowl parvovirus infection, vaccination is commercially available against GPV and MDPV in China. Many attenuated or killed vaccines against DD have been recommended to be used in commercial Muscovy ducks, and an attenuated MDPV vaccine designated as P1 has been used in the field for the control of 3-w disease on Muscovy ducks farms in China. Recently, several cases of Muscovy ducks infected with variant waterfowl parvoviruses currently thought to be circulating in goose and Muscovy duck flocks have been reported [2, 10, 11]. In 1997, one Chinese prevalent virulent parvovirus strain was isolated from a large-scale Muscovy duck farm, which contained an extremely large number of young Muscovies, and the breeder duck flock was maintained at a high density and exposed intensively to live vaccines. The etiologic agent was antigenically identified as a GPV-like virus, based on serologic tests including agar gel immunoprecipitation, an indirect fluorescent antibody test, a monoclonal antibody latex agglutination test and virus neutralization (Tables 1 and 2). This GPV strain of Muscovy duck origin was designated as MDGPV/PT [1, 7, 22].

As part of our ongoing program for GPV-associated emerging infections, we postulated that a new group of GPVs among waterfowl parvovirus strains might be responsible for outbreaks of disease. In a previous paper, we reported that MDGPV strains PT and DY differed in the VP1u gene, resulting in them belonging to different waterfowl parvovirus groups. Phylogenetic analyses also confirmed that MDGPV strains PT and DY represent a distantly related lineage to other GPVs in the VP2 and VP3 gene phylogenies [18] (Figs. 1–4).

However, little is known about the LORF nucleotide sequences of MDGPV strains isolated from cases in China. The aim of the present study was to generate new sequence information for the MDGPV/PT NS1 gene and examine sequence diversity among GPV- and MDPV-related groups. These data represents the first report of the complete NS1

*CORRESPONDENCE TO: CHEN, S.-Y., Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agriculture Sciences, 247 Wusi Road, Fuzhou, Fujian 350003, China.
e-mail: chensy58@163.com

#These authors contributed equally to the authorship of this work.

Table 1. Virus neutralization assay titers between strains MDPV-P, MDGPV-PT and GPV-GD

Antiserum	Virus strains		
	MDPV-P	MDGPV-PT	GPV-GD[a)]
MDPV-P	14.25*	7.25	8
MDGPV-PT	7.25	11.25	9.25
GPV-GD	5.25	8	9.75

a) The GPV-GD strain was purchased from China Veterinary Culture Collection (CVCC) and was the Chinese standard virulent GPV strain. *Neutralization titer (NT) was obtained in a reciprocal beta (β) VN assay (diluted serum, constant virus) using parvovirus-free fertile embryonated Muscovy duck eggs and represents the logarithm (log_2) difference in titer between virus alone and virus-antiserum mixtures.

Table 2. Antigenic relatedness values (*R* values) (%) between strains MDPV-P, MDGPV-PT and GPV-GD

Virus strains	MDPV-P	MDGPV-PT	GPV-GD
MDPV-P	100		
MDGPV-PT	2	100	
GPV-GD	2	27	100

NT values in Table 1. *R* values were calculated by the formula of Archetti and Horsfall. *R*=80–100%, same serotype; *R*=25–80%, different subtype; *R*=0–25%, different serotype.

gene sequence of MDGPV from Southern China. In this study, the NS1 gene was cloned by PCR from strain MDGPV/PT. The nucleotide and deduced amino acid sequences of the NS1 gene were compared with those of 10 other references strains whose NS1 gene sequences were already available in GenBank (listed in Table 3). A phylogenetic tree was then constructed and analyzed according to the NS1 gene sequences. Sequences were aligned using the Mega v4.0 program by using the ClustalW method. Phylogenetic and molecular evolutionary analyses were conducted by using the Mega v4.0 program, and the phylogenetic tree was built with the neighbor-joining (NJ) method and 1,000 replications of bootstrap values.

The viral genome was extracted as previously described [19] and then subjected to PCR using one pair of primers. The primer sequences were as follows: forward primer

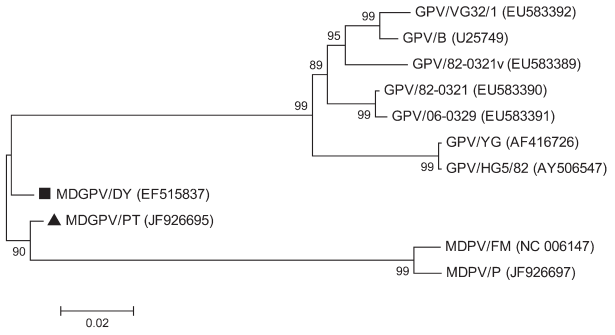

Fig. 1. Phylogenetic tree of waterfowl parvovirus strains based on the nucleotide sequences of the VP1 gene. The tree was constructed using the neighbor-joining algorithm with bootstrap values calculated for 1,000 replicates (square, MDGPV/DY; triangle, MDGPV/PT).

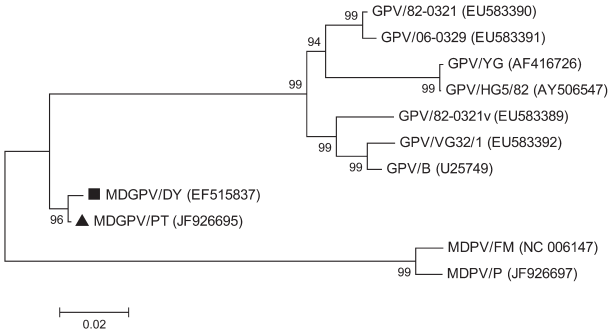

Fig. 3. Phylogenetic tree of waterfowl parvovirus strains based on the nucleotide sequences of the VP2 gene. The tree was constructed using the neighbor-joining algorithm with bootstrap values calculated for 1,000 replicates (square, MDGPV/DY; triangle, MDGPV/PT).

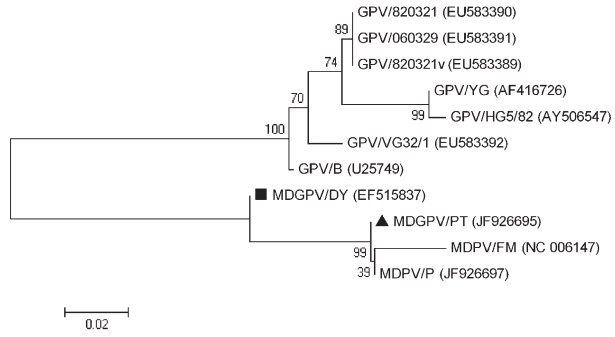

Fig. 2. Phylogenetic tree of waterfowl parvovirus strains based on the nucleotide sequences of the VP1u gene. The tree was constructed using the neighbor-joining algorithm with bootstrap values calculated for 1,000 replicates (square, MDGPV/DY; triangle, MDGPV/PT).

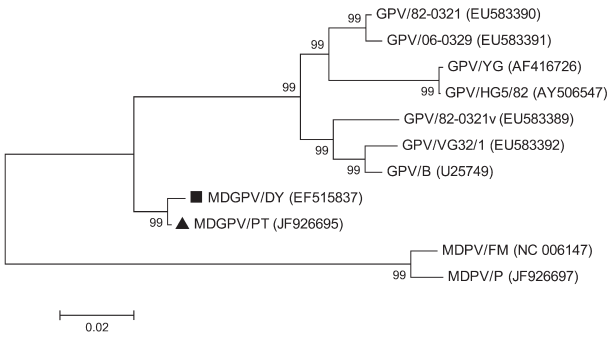

Fig. 4. Phylogenetic tree of waterfowl parvovirus strains based on the nucleotide sequences of the VP3 gene. The tree was constructed using the neighbor-joining algorithm with bootstrap values calculated for 1,000 replicates (square, MDGPV/DY; triangle, MDGPV/PT).

Table 3. Source of waterfowl parvovirus (GPV and MDPV) NS1 sequences used in the analyses

Strain name	Place of origin	Host	Gene name (GenBank accession number)
DY	Sichuan Province, China	Muscovy duck	NS1 (EF515837)
PT	Fujian Province, China	Muscovy duck	NS1 (JF926695)
82-0321	Taiwan	Goose	NS1 (EU583390)
82-0321v	Taiwan	Goose	NS1 (EU583389)
06-0329	Taiwan	Goose	NS1 (EU583391)
VG32/1	Germany	Goose	NS1 (EU583392)
YG	Shanghai, China	Goose	NS1 (AF416726)
B	Hungary	Goose	NS1 (U25749)
FM	Hungary	Muscovy duck	NS1 (NC_006147)
P	Fujian Province, China	Muscovy duck	NS1 (JF926697)
HG5/82	Heilongjiang Province, China	Goose	NS1 (AY506546)

NS1f, 5'-GAGAGCTTTTTCCGGTTGCATTCATTCG-3', and reverse primer NS1r, 5'-AGTCTTCAAATTTCTC-TAAAAAAGTAG-3' (targeting a 1,884 bp fragment). The primers were designed from multiple alignments of the nucleotide sequences of NS1 regions of GPV and MDPV. The polymerase chain reaction (PCR) contained 5 μl extracted DNA, 2 μl primer pairs and 50 μl 2 ×GoTaq® Green Master Mix (Promega Corporation, Madison, WI, U.S.A.) in a total volume of 100 μl. The amplification was carried out under the following conditions: initial pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min and extension at 72°C for 2 min (depending on the amplified fragment length). Amplified DNA fragments were visualized following electrophoresis on 0.8% agarose gel. PCR products were purified using a PCR purification kit (Qiagen, Hilden, Germany) and directly sequenced from both directions by using an ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit. The complete nucleotide sequence of the MDGPV/PT NS1 gene has been deposited in the GenBank database and assigned the accession number JF926695.

Sequence analysis indicated that the NS1 gene of MDGPV/PT consisted of 1,884 bases and coded for a protein of 627 amino acids and that MDGPV/PT was highly homologous to MDPV strains rather than to GPV strains. MDGPV/PT possessed 81.2–83.0 and 90.0–90.8% identity with seven GPV reference strains at the nucleotide and protein levels, respectively. However, the NS1 coding regions of MDGPV/PT and MDPV/FM shared the highest nt (99.1%) and aa (98.4%) identities, and together with another strain, MDPV/P, these strains formed a strongly supported cluster within the phylogenetic tree (Fig. 5). Interestingly, the NS1 gene shared low identity to MDGPV/DY (nt, 82.6%; aa, 90.3%). Phylogenetic analysis indicated that strain MDGPV/DY was closely related to GPV-related parvoviruses, while strain MDGPV/PT was an MDPV-related virus, indicating that Chinese MDGPV strains have genetic diversity with respect to the NS1 genes (Fig. 5).

Muscovy ducks are raised in large numbers in several parts of China, especially in Fujian Province. GPV and MDPV infections are two highly pathogenic waterfowl infectious diseases that infect geese and Muscovy ducks. In China, GPV

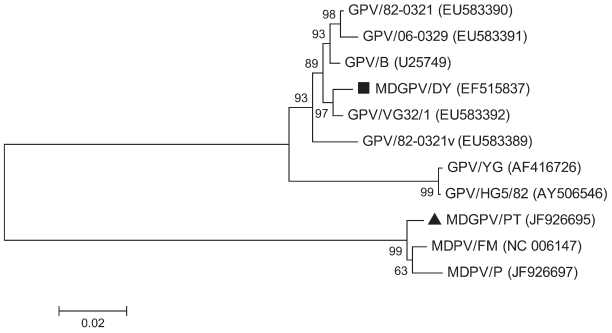

Fig. 5. Phylogenetic tree of waterfowl parvovirus strains based on the nucleotide sequences of the NS1 gene. The tree was constructed using the neighbor-joining algorithm with bootstrap values calculated for 1,000 replicates (square, MDGPV/DY; triangle, MDGPV/PT).

has been previously isolated from sick and dead Muscovy ducks, but there are few reports on the molecular characteristics of pandemic strains in GPV-affected Muscovy duck flocks. Prior to the emergence of the Chinese GPV strains of Muscovy duck origin, no recombination events had been identified in the genomes of GPV and MDPV [15, 16]. In the current study, we sequenced the NS1 gene of the previously isolated MDGPV/PT strain and analyzed its phylogenetic relationship with published strains. To our knowledge, only one other sequence of the coding region of NS1 of strain MDGPV/DY isolated in Sichuan Province of China has been determined. Now, we strongly suggest that genetic recombination between waterfowl parvovirus strains isolated from the same species or related species from different geographic regions at different time periods must be monitored.

The genetic analyses also indicated that recombination of the mammalian parvoviruses genes could occur under natural circumstances. Human bocavirus 3 (HBoV3) is probably a progeny form of a recombination event between HBoV1 and 4, and these recombination points are located close to VP1u. Recombination events among HBoV2 variants have also been observed, as recently reported for animal parvoviruses [5, 6, 12]. On the other hand, the NS ORF of Gorilla Bocavirus species 1 (GBoV1) is more similar in length to

the NS ORF found in canine parvovirus (CPV) and bovine parvovirus (BPV) than in HBoV, although phylogenetic analysis revealed that the complete protein-coding region sequence of GBoV1 is most closely related to HBoV [4]. In addition, Wang *et al.* reported that the genome of mink enteritis virus (MEV) strain LN-10 is composed of the NS1 gene originating from CPV, while the VP1 gene is of MEV origin [17]. Ohshima *et al.* also reported that the genome of feline panleukopenia virus (FPLV) strain XJ-1 is generally composed of the NS1 gene of CPV origin and the VP1 gene of FPLV origin [8]. These results possibly indicate that cross-species transmission events have been quite frequent and also show that the NS1 and capsid proteins together determine the host range of parvovirus [9, 13].

In conclusion, MDGPV/PT might be a novel Muscovy duck-infecting GPV circulating in China, and it appears that variant waterfowl parvoviruses do exist in nature. The high prevalence of GPV and MDPV infection does provide the opportunity for coinfection, the first step in generating recombinant viruses. Genetic data indicate that the MDGPV/PT strain has adapted to infect Muscovy ducks, and antigenic variation has resulted in inconsistent responses to traditional GPV vaccines. So, it is important to have a vaccine against MDGPV in China. At present, an attenuated MDGPV vaccine designated as D has been used experimentally in the laboratory for the control of MDGPV. Future studies will be aimed at characterizing the remaining MDGPV sequence regions, molecular epidemiology of MDGPV infections and pathogenesis studies.

ACKNOWLEDGMENTS. We thank Prof. Shao Liangping, Fujian Agriculture and Forestry University, China, for revision of the manuscript. This work was funded by grants from the Chinese National Special Fund for Agro-scientific Research in the Public Interest (No. 201003012), the Chinese modern agricultural industry technology system (CARS-43) and the Fujian Natural Sciences Foundation (2012J01110).

REFERENCES

1. Cheng, X. X., Chen, S. Y., Zhu, X. L., Chen, S. L., Lin, F. Q., Wang, S., Cheng, Y. Q. and Lin, T. L. 2008. Isolation and identification of goose parvovirus from Muscovy ducks. *Fujian J. Agric. Sci.* **23**: 355–358.
2. Chu, C. Y., Pan, M. J. and Cheng, J. T. 2001. Genetic variation of the nucleocapsid genes of waterfowl parvovirus. *J. Vet. Med. Sci.* **63**: 1165–1170. [Medline] [CrossRef]
3. Ji, J., Xie, Q. M., Chen, C. Y., Bai, S. W., Zou, L. S., Zuo, K. J., Cao, Y. C., Xue, C. Y., Ma, J. Y. and Bi, Y. Z. 2010. Molecular detection of Muscovyduck parvovirus by loop-mediated isothermal amplification assay. *Poult. Sci.* **89**: 477–483. [Medline] [CrossRef]
4. Kapoor, A., Mehta, N., Esper, F., Poljsak-Prijatelj, M., Quan, P. L., Qaisar, N., Delwart, E. and Lipkin, W. I. 2010. Identification and characterization of a new bocavirus species in gorillas. *PLoS One* **5**: e11948. [Medline] [CrossRef]
5. Kapoor, A., Simmonds, P., Slikas, E., Li, L., Bodhidatta, L., Sethabutr, O., Triki, H., Bahri, O., Oderinde, B. S., Baba, M. M., Bukbuk, D. N., Besser, J., Bartkus, J. and Delwart, E. 2010. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J. Infect. Dis.* **201**: 1633–1643. [Medline] [CrossRef]
6. Kapoor, A., Slikas, E., Simmonds, P., Chieochansin, T., Naeem, A., Shaukat, S., Alam, M. M., Sharif, S., Angez, M., Zaidi, S. and Delwart, E. 2009. A newly identified bocavirus species in human stool. *J. Infect. Dis.* **199**: 196–200. [Medline] [CrossRef]
7. Mengeling, W. L., Paul, P. S., Bunn, T. O. and Ridpath, J. F. 1986. Antigenic relationships among autonomous parvoviruses. *J. Gen. Virol.* **67**: 2839–2844. [Medline] [CrossRef]
8. Ohshima, T. and Mochizuki, M. 2009. Evidence for recombination between feline panleukopenia virus and canine parvovirus type 2. *J. Vet. Med. Sci.* **71**: 403–408. [Medline] [CrossRef]
9. Palermo, L. M., Hafenstein, S. L. and Parrish, C. R. 2006. Purified feline and canine transferrin receptors reveal complex interactions with the capsids of canine and feline parvoviruses that correspond to their host ranges. *J. Virol.* **80**: 8482–8492. [Medline] [CrossRef]
10. Palya, V., Zolnai, A., Benyeda, Z., Kovács, E., Kardi, V. and Mató, T. 2009. Short beak and dwarfism syndrome of mule duck is caused by a distinct lineage of goose parvovirus. *Avian Pathol.* **38**: 175–180. [Medline] [CrossRef]
11. Poonia, B., Dunn, P. A., Lu, H., Jarosinski, K. W. and Schat, K. A. 2006. Isolation and molecular characterization of new Muscovy duck parvovirus from Muscovy ducks in the USA. *Avian Pathol.* **35**: 435–441. [Medline] [CrossRef]
12. Schildgen, O., Qiu, J. and Söderlund-Venermo, M. 2012. Genomic features of the human bocaviruses. *Future Virol.* **7**: 31–39. [Medline] [CrossRef]
13. Shackelton, L. A., Hoelzer, K., Parrish, C. R. and Holmes, E. C. 2007. Comparative analysis reveals frequent recombination in the parvoviruses. *J. Gen. Virol.* **88**: 3294–3301. [Medline] [CrossRef]
14. Smith, D. H., Ward, P., Linden, R. M. and Linden, M. 1999. Comparative characterization of rep proteins from the helper-dependent adeno-associated virus type 2 and the autonomous goose parvovrius. *J. Virol.* **73**: 2930–2937. [Medline]
15. Tatár-Kis, T., Mató, T., Markos, B. and Palya, V. 2004. Phylogenetic analysis of Hungarian goose parvovirus isolates and vaccine strains. *Avian Pathol.* **33**: 438–444. [Medline] [CrossRef]
16. Tsai, H. J., Tseng, C. H., Chang, P. C., Mei, K. and Wang, S. C. 2004. Genetic variation of viral protein 1 genes of field strains of waterfowl parvoviruses and their attenuated derivatives. *Avian Dis.* **48**: 512–521. [Medline] [CrossRef]
17. Wang, J., Cheng, S., Yi, L., Cheng, Y., Yang, S., Xu, H., Zhao, H., Yan, X. and Wu, H. 2012. Evidence for natural recombination between mink enteritis virus and canine parvovirus. *Virol. J.* **9**: 252. [CrossRef]
18. Wang, S., Zhu, X. L., Ling, F. Q., Cheng, X. X., Chen, S. L., Li, Z. L. and Chen, S. Y. 2012. Cloning and sequence analysis of VP gene of goose parvovirus PT strain isolated from Muscovy duck origin. *Chin. J. Agr. Biotechnol.* **20**: 79–84.
19. Wozniakowski, G., Kozdrun, W. and Samorek-Salamonowicz, E. 2009. Genetic variance of Derzsy's disease strains isolated in Poland. *J. Mol. Genet. Med.* **3**: 210–216. [Medline]
20. Yang, J., Yang, R., Cheng, A., Wang, M., Fu, L., Yang, S., Zhang, S., Yang, L. and Xu, Z. 2010. A simple and rapid method for detection of Goose Parvovirus in the field by loop-mediated isothermal amplification. *Virol. J.* **7**: 14.
21. Zádori, Z., Stefancsik, R., Rauch, T. and Kisary, J. 1995. Analysis of the complete nucleotide sequences of goose and Muscovyduck parvoviruses indicates common ancestral origin with adeno-associated virus 2. *Virology* **212**: 562–573. [Medline] [CrossRef]
22. Zhu, X. L., Chen, S. Y., Lin, F. Q., Cheng, X. X., Chen, S. L., Wang, S. and Li, Z. L. 2012. Development of the latex agglutination diagnostic method of muscovy duck gosling plague. *Chin. J. Prev. Vet. Med.* **34**: 715–718.