

# Influenza D Virus in Animal Species in Guangdong Province, Southern China

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Molecular tests revealed influenza D viruses of D/OK lineage widely circulating in farmed animal species in Guangdong Province, southern China. In particular, we found high levels of influenza D virus infection in goats and pigs. We also detected viral RNA in serum specimens and feces of animals with certain severe diseases.

Four types of influenza viruses (A–D) have been confirmed (<https://www.cdc.gov/flu/about/viruses/types.htm>). The recently discovered influenza D virus is thought to cause respiratory diseases primarily in cattle and to a lesser extent in pigs (1–4). Moreover, serologic evidence for influenza D virus infection in small ruminants and humans has been established (5,6). Since the initial influenza D virus isolation in the United States in 2011 (1), the virus has been reported in China, Mexico, France, Italy, and Japan (7–11). Genetic analysis of the hemagglutinin-esterase-fusion gene demonstrated that these viruses had 2 distinct lineages, represented by D/OK and D/660 (12). Recently, a novel influenza D virus that emerged in Japan has been proposed as the third lineage (11). D/OK lineage-related viruses were previously identified in native Luxi yellow cattle in Shandong Province, northern China (7). Despite good progress in identifying domestic cattle as the primary reservoir of influenza D virus, we know little about prevalence in other animals. We conducted a study to clarify the origin and transmission dynamics of influenza D virus in goats, buffalo, and pigs as well as farmed cattle.

## The Study

In 2016, we collected 607 clinical samples from 4 species of animals with different clinical diseases and 250 nasal swab samples from asymptomatic animals (Table) from 16 farms in 4 cities of Guangdong Province: Guangzhou,

Qingyuan, Heyuan, and Jiangmen (Figure 1). In addition, we randomly chose 200 archived Holstein dairy cattle serum samples, 40 per year, from 2011–2015 to investigate possible early RNA distribution of influenza D virus in this region. We used the reverse transcription PCR method and subcloning protocol (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/8/17-0059-Techapp.pdf>). We performed sequence alignment using ClustalW implemented in DNASTar software (DNASTar, Madison, WI, USA), and we conducted phylogenetic analyses based on our obtained sequences and reference truncated sequences (496-bp) of influenza D viruses from GenBank by using MEGA 5.1 software (<http://www.megasoftware.net>; online Technical Appendix Table).

After testing by reverse transcription PCR with further sequencing confirmation, we found influenza D virus–positive rates in 230 total nasal swab samples of 12.8% (20/156) for dairy cattle, 7.3% (4/55) for native yellow cattle, and 36.8% (7/19) for pigs. Rates in 324 total serum samples were 7.8% (15/193) for dairy cattle, 5.9% (3/51) for buffalo, and 33.8% (27/80) for goats. The influenza D virus–positive rate was also high (28.9%, 13/45) in swine lung samples. In contrast, we found no or low prevalence ( $\leq 2\%$ ) in asymptomatic animals tested (Table). Moreover, all of the archived serum samples were found to be influenza D virus negative. Interestingly, 1 of 8 rectal swabs of goats with severe diarrhea tested positive (Table). Samples from animals with reproductive problems had a positive rate of 4.3% (5/116) (Table).

Sequence alignment analysis showed that the nucleotide sequences of influenza D viruses found in this study shared high similarity (99%–100%) with previously described sequences from China (7) and low similarity (93.8%–98.8%) with sequences originating from the United States, France, Italy, Mexico, and Japan (1,8–12). Similarly, phylogenetic analysis revealed that all influenza D virus sequences in this study clustered together with previous sequences from China and belonged to the D/OK lineage (Figure 2).

## Conclusions

When first discovered, influenza D virus was reported in diseased pigs in the United States (1). Later, it was

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**Table.** Animal species, location, sample data, and detection rate of influenza D virus, Guangdong Province, China\*

Animal species and farm	Farm type†	Farm location	No. animals	Age range of animals	Sample type	No. positive/no. samples	Detection rate, %
<b>Holstein dairy cattle</b>							
A	Not all-in-all-out	Guangzhou: Tianhe	2,000	3–5 y	Nasal swab	14/86‡	16.3
A	Not all-in-all-out	Guangzhou: Tianhe	2,000	3–5 y	Serum	10/94‡	10.6
B	Not all-in-all-out	Guangzhou: Luogang	800	3–6 y	Nasal swab	6/70‡	8.57
B	Not all-in-all-out	Guangzhou: Luogang	800	3–6 y	Serum	5/99‡	5.05
C	Not all-in-all-out	Guangzhou: Tianhe	175	2–5 y	Nasal swab	1/50§	2
<b>American Landrace pig</b>							
D	Not all-in-all-out	Guangzhou: Huadu	200	10–15 wks	Lung	4/10‡	40
E	All-in-all-out	Heyuan: Yuancheng	1,000	5–5 wks	Nasal swab	4/10‡	40
E	All-in-all-out	Heyuan: Yuancheng	1,000	3–5 wks	Lung	1/8‡	12.5
F	All-in-all-out	Jiangmen: Kaiping	800	8–20 wks	Nasal swab	3/9‡	30
F	All-in-all-out	Jiangmen: Kaiping	800	8–20 wks	Lung	8/27‡	29.6
G	All-in-all-out	Heyuan: Dongyuan	600	9–15 wks	Nasal swab	1/50§	2
<b>Native hybrid white goat</b>							
H	Not all-in-all-out	Guangzhou: Zengcheng	200	0.5–5 y	Serum	7/25‡	28
I	Not all-in-all-out	Guangzhou: Luogang	300	2–4 y	Serum	20/55¶	36.4
<b>Native hybrid black goat</b>							
J	Not all-in-all-out	Qingyuan: Jiangkou	150	1–3 y	Rectal swab	1/8#	12.5
K	Not all-in-all-out	Jiangmen: Enping	500	1–4 y	Nasal swab	0/50§	0
<b>Asian buffalo</b>							
L	Not all-in-all-out	Guangzhou: Nansha	150	3–5 y	Serum	2/26¶	7.7
M	Not all-in-all-out	Guangzhou: Panyu	180	3–6 y	Serum	1/25¶	4
N	Not all-in-all-out	Qingyuan: Yingde	400	1–4 y	Nasal swab	0/50§	0
<b>Native yellow cattle</b>							
O	Not all-in-all-out	Qingyuan: Qingxin	200	2–5 y	Nasal swab	4/55‡	7.3
P	Not all-in-all-out	Qingyuan: Fogang	230	1–3 y	Nasal swab	0/50§	0

\*Feeding type of farms A–G was in captivity (poor biosecurity and high density). Feeding type of farms H–K and N–P was free grazing on the hills in the daytime and in captivity (poor biosecurity and high density) in the nighttime. Feeding type of farms L and M was free grazing in wetland in the daytime and in captivity (poor biosecurity and high density) in the nighttime.

†All-in-all-out is a strategy for the control of infectious disease. The barn is emptied of all animals and the accommodation is cleaned and disinfected and then refilled, all on 1 day.

‡These animals had severe respiratory diseases with a 10%–30% mortality rate, mainly characterized by expiratory dyspnea and abdominal respiration.

§These animals were asymptomatic.

¶These animals had severe reproductive disorders with a 60%–70% abortion rate.

#These animals had severe diarrheal disease, characterized by watery diarrhea, limb weakness, and nearly dying.

identified in cattle and swine herds in several other countries, with or without clinical manifestation (7–11). Moreover, antibodies to influenza D virus were detected in goats, sheep, and humans (5–6). Under experimental conditions, influenza D virus replicated and transmitted among ferrets and guinea pigs (13). We confirmed that influenza D virus is widely present in cattle species (dairy cattle, yellow cattle, and buffalo). We also found influenza D virus at a high prevalence (>30%) in pigs and goats (Table), which is in contrast to the low prevalence found in previous investigations (1,5,10). The high prevalence may be caused by poor biosecurity measures and high-density feeding mode practices in China's animal industry as well as possible cross-species transmission (13). Taken together, our findings expand the host range of influenza D virus and further emphasize the health concern this virus poses to multiple animal species.

Previous studies have shown that influenza D viruses are mainly found in respiratory tract samples (1–4,7,9–12) and that they have played an etiologic role in bovine respiratory diseases (2–4). In this study, we found that influenza D virus RNA was present in cattle and goat serum samples; it was also present in goat rectal swabs, accompanied by peste des petits ruminants virus and caprine kobovirus (data not shown). The distribution of influenza D virus in our study is not the same as that described under experimental conditions (3).

Influenza viremia, an indicator of disease severity (14), has been detected in 20.9% of severe cases during the acute phase of infection or before host death. Our detection of influenza D virus genome in serum samples from severely diseased animals (Table) implies that the virus could enter transiently into the animal's circulatory system through capillaries lining the respiratory tract, which



**Figure 1.** Farm locations for study of influenza D viruses in cattle, goats, buffalo, and pigs, Guangdong Province, China.

further contributes to the possibility of detecting virus in other organs. Similar to previous studies (2,4), we also found that the reverse transcription PCR positive rate was significantly higher (4%–40%) in diseased animals than the rate ( $\leq 2\%$ ) observed in asymptomatic animals ( $p < 0.05$ ), which suggests a potential correlation between the disease severity and presence of influenza D virus. For influenza D virus found in rectal swabs, it might be that animals have swallowed the virus. Another possibility is that, similar to influenza A and B viruses, influenza D virus can replicate within the intestinal tract (15).

We detected influenza D virus in cattle with reproductive disorders. However, we could not determine whether influenza D virus is associated with reproductive problems. Future studies can be designed to investigate these scientific issues.

To date, 2 lineages of influenza D virus (D/OK and D/660) co-circulate in North America and Europe (8–10,12). However, only the D/OK lineage has been found in China, and a potential third lineage was found in Japan (7,11). Our study confirms and further extends the previous observation that D/OK lineage circulates in East Asia. The viral, host, and ecologic factors that shape the observed contrasting phylodynamics of influenza D viruses among different geographic regions warrant further investigation.

In addition, we found different minor genetic variants circulating on the same farm (Figure 2), indicating the ongoing evolution of influenza D viruses in their hosts

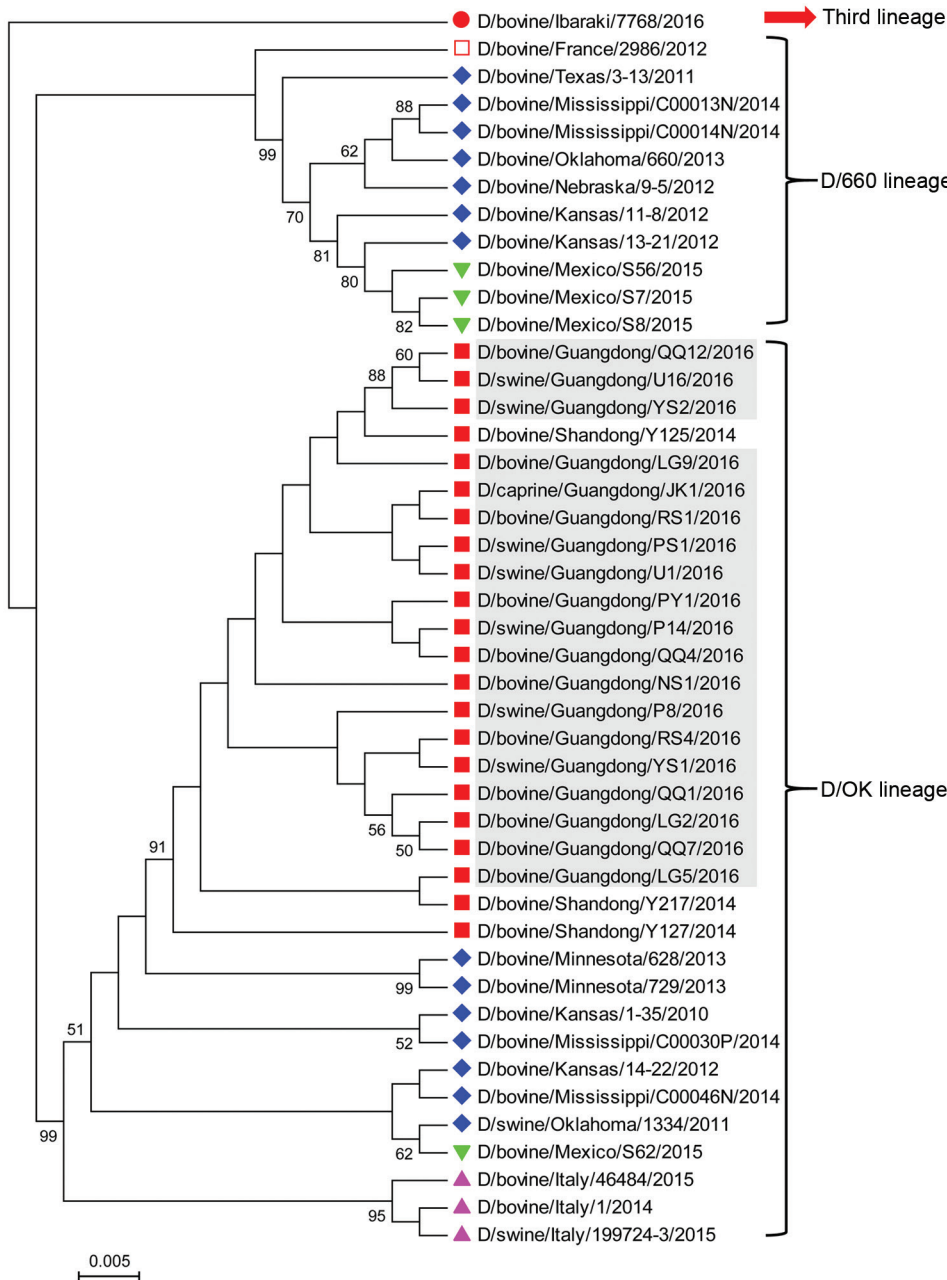
(7,8,11). In comparing our sequences to the reference sequences from different animal species, we found 4 frequent nucleotide mutations (at positions 136, 231, 263, and 486) (online Technical Appendix Figure 1), which caused 2 amino acid mutations at positions 77 and 88 (online Technical Appendix Figure 2). Interestingly, among 4 nucleotide mutations, 1 unique nucleotide (T at position 486) was originally from the D/660 lineage. Moreover, we found several consistent sequences co-circulating in multiple animal species (online Technical Appendix Figure 1). Our speculation is that homologous recombination among different influenza D viruses and potential cross-species transmission under field conditions are possible, but further study is needed.

In summary, our study investigating the infection status of influenza D virus in different farmed animal species in Guangdong Province provides novel insights into the epidemiology and evolution of this virus. In particular, we document the molecular evidence for influenza D virus infection in goats and buffalo.

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**Figure 2.** Phylogenetic analysis of viruses from study of influenza D viruses in cattle, goats, buffalo, and pigs in Guangdong Province, China, compared with reference viruses. Partial hemagglutinin-esterase-fusion gene sequences (496 bp) were aligned by using ClustalW implemented in DNASTar software (DNASTar, Madison, WI, USA), and the phylogenetic tree was obtained using neighbor-joining method within MEGA 5.1 software (<http://www.megasoftware.net>). Numbers at nodes are percentages of bootstrap values obtained by repeated analyses (1,000 times) to generate majority consensus tree. Only bootstrap scores of at least 50 were retained. Scale bar indicates 0.5% nucleotide divergence. Gray shading indicates viruses from this study; reference viruses obtained from the United States are marked with ◆; from China, ■; from Italy, ▲; from Mexico, ▼; from France, □; and from Japan ●. Note that D/swine/Guangdong/YS1/2016 and D/swine/Guangdong/YS2/2016 are from the same farm; D/swine/Guangdong/P8/2016 and D/swine/Guangdong/P14/2016 are from the same farm; D/swine/Guangdong/U1/2016 and D/swine/Guangdong/U16/2016 are from the same farm; D/bovine/Guangdong/LG2/2016, D/bovine/Guangdong/LG5/2016 and D/bovine/Guangdong/LG9/2016 are from the same farm; D/bovine/Guangdong/QQ1/2016, D/bovine/Guangdong/QQ4/2016, D/bovine/Guangdong/QQ7/2016 and D/bovine/Guangdong/QQ12/2016 are from the same farm; D/bovine/Guangdong/RS1/2016 and D/bovine/Guangdong/RS4/2016 are from the same farm.

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## Technical Appendix

### RT-PCR method and subcloning protocol used in this study

A classic reverse transcription PCR (RT-PCR) method was developed using a pair of primers (HEF-F: 5'-AAC CRC ATC TTC TTG TTC TTC A-3' and HEF-R: 5'-TGC TTC TTC WGT GGC ATT ATC T-3') targeting at the partial hemagglutinin-esterase-fusion (HEF) gene (at positions 582–1077) to test the presence of IDVs and further define IDV genetic lineages in those samples. For RT-PCR, the primers were diluted to 10  $\mu$ M with ddH<sub>2</sub>O.

A 25  $\mu$ L RT-PCR system was constructed using a one-step RT-PCR kit (Takara Bio Inc.), which contained 0.5  $\mu$ L of each primer, 0.5  $\mu$ L of enzyme mixture (including PrimeScript RTase, DNA polymerase, RNase inhibitor), 12.5  $\mu$ L of 2  $\times$  buffer, 8.5  $\mu$ L of ddH<sub>2</sub>O, and 2.5  $\mu$ L of viral RNA. RT-PCR was performed as follows: reverse transcription (RT) at 50°C for 30 min, 95°C for 5 min, followed by 35 PCR cycles (95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec), and a final extension at 72°C for 10 min. The positive PCR fragments ( $\approx$ 500 bp) were purified and cloned into the pGEM-T vector (TIANGEN, Inc., Beijing). Positive recombinant plasmids were purified according to the manufacturer's instructions (TIANGEN, Inc., Beijing) and sequenced using the Sanger sequencing method (Sangon Biotech Co., Ltd., Shanghai). RT-PCR detection data were analyzed by chi-square test (Ziyue software), and significance was set at  $p < 0.05$ .

**Technical Appendix Table.** Reference influenza D virus isolates with partial hemagglutinin-esterase-fusion sequences used in this study

Strain or isolate	Specimen origin of isolate	Country	Year	GenBank
				accession no.
D/bovine/Nebraska/9–5/2012	Bovine (nasal swab)	USA	2012	KM392471
D/bovine/Oklahoma/660/2013	Bovine (nasal swab)	USA	2013	KF425662
D/bovine/Mississippi/C00014N/2014	Bovine (nasal swab)	USA	2014	KT581417
D/bovine/Mississippi/C00013N/2014	Bovine (nasal swab)	USA	2014	KT581416
D/bovine/Kansas/11–8/2012	Bovine (nasal swab)	USA	2012	KM392506
D/bovine/Kansas/13–21/2012	Bovine (nasal swab)	USA	2012	KM392492
D/bovine/Texas/3–13/2011	Bovine (nasal swab)	USA	2011	KM392485
D/bovine/Mexico/S56/2015	Bovine (nasal swab)	Mexico	2015	KU171128
D/bovine/Mexico/S8/2015	Bovine (nasal swab)	Mexico	2015	KU171127
D/bovine/Mexico/S7/2015	Bovine (nasal swab)	Mexico	2015	KU171126
D/bovine/France/2986/2012	Bovine (nasal swab)	France	2012	LN559126
D/bovine/Kansas/1–35/2010	Bovine (nasal swab)	USA	2010	KM392478
D/bovine/Mexico/S62/2015	Bovine (nasal swab)	Mexico	2015	KU171129
D/swine/Oklahoma/1334/2011	Swine (nasal swab)	USA	2011	JQ922308
D/bovine/Mississippi/C00030P/2014	Bovine (nasopharyngeal swab)	USA	2014	KT581418
D/bovine/Mississippi/C00046N/2014	Bovine (nasal swab)	USA	2014	KT581412
D/bovine/Minnesota/729/2013	Bovine (nasal swab)	USA	2013	KF425669
D/bovine/Minnesota/628/2013	Bovine (nasal swab)	USA	2013	KF425655
D/bovine/Kansas/14–22/2012	Bovine (nasal swab)	USA	2012	KM392499
D/bovine/Shandong/Y125/2014	Bovine (nasal swab)*	China	2014	KM015494
D/bovine/Shandong/Y127/2014	Bovine (nasal swab)*	China	2014	KM015501
D/bovine/Italy/46484/2015	Bovine (nasal swab)	Italy	2015	KT592526
D/bovine/Italy/1/2014	Bovine (nasal swab)	Italy	2014	KT592522
D/bovine/Shandong/Y217/2014	Bovine (nasal swab)*	China	2014	KM015508
D/swine/Italy/199724–3/2015	Swine (nasal swab)	Italy	2015	KT592533
D/bovine/Ibaraki/7768/2016	Bovine (nasal swab)	Japan	2016	LC128433
D/swine/Guangdong/YS1/2016	Swine (lung)	China	2016	KY441104
D/swine/Guangdong/YS2/2016	Swine (lung)*	China	2016	KY441105
D/swine/Guangdong/P8/2016	Swine (lung)	China	2016	KY441106
D/swine/Guangdong/P14/2016	Swine (nasal swab)	China	2016	KY441107
D/swine/Guangdong/PS1/2016	Swine (nasal swab)*	China	2016	KY441108
D/swine/Guangdong/U1/2016	Swine (serum)	China	2016	KY441109
D/swine/Guangdong/U16/2016	Swine (nasal swab)*	China	2016	KY441110
D/bovine/Guangdong/LG2/2016	Bovine: dairy cow (serum)	China	2016	KY441111
D/bovine/Guangdong/LG5/2016	Bovine: dairy cow (nasal swab)	China	2016	KY441112

Strain or isolate	Specimen origin of isolate	Country	Year	GenBank
				accession no.
D/bovine/Guangdong/LG9/2016	Bovine: dairy cow (nasal swab)*	China	2016	KY441113
D/bovine/Guangdong/QQ1/2016	Bovine: dairy cow (nasal swab)	China	2016	KY441114
D/bovine/Guangdong/QQ4/2016	Bovine: dairy cow (serum)	China	2016	KY441115
D/bovine/Guangdong/QQ7/2016	Bovine: dairy cow (serum)	China	2016	KY441116
D/bovine/Guangdong/QQ12/2016	Bovine: dairy cow (nasal swab)*	China	2016	KY441117
D/bovine/Guangdong/NS1/2016	Bovine: buffalo (serum)	China	2016	KY441118
D/bovine/Guangdong/RS1/2016	Bovine: yellow cattle (nasal swab)	China	2016	KY441119
D/bovine/Guangdong/RS4/2016	Bovine: yellow cattle (nasal swab)*	China	2016	KY441120
D/bovine/Guangdong/PY1/2016	Bovine: buffalo (serum)	China	2016	KY441121
D/caprine/Guangdong/JK1/2016	Caprine: goat (rectal swab)	China	2016	KY441122

\*Healthy.



Majority	AACCGCATCTTCTGTCTTCAAGCTGGATGAAAAGCCCGTTGTGGTATGCAGAACTTCTGTTAATCCTGGAGCTAAACCTCAAGTTTGTGGGACTGAG										
	10	20	30	40	50	60	70	80	90	100	
JK1.seq	.	.	.	.	.	.	.	.	.	.	100
LG2.seq	.	.	.	.	.	.	.	.	.	.	100
LG5.seq	.	.	.	.	.	.	.	.	.	.	100
LG9.seq	.	.	.	.	.	.	.	.	.	.	100
NS1.seq	.	.	.	.	.	.	.	.	.	.	100
P14.seq	.	.	.	.	.	.	.	.	.	.	100
P8.seq	.	.	.	.	.	.	.	.	.	.	100
PS1.seq	.	.	.	.	.	.	.	.	.	.	100
PY1.seq	.	.	.	.	.	.	.	.	.	.	100
QQ1.seq	.	.	.	.	.	.	.	.	.	.	100
QQ12.seq	.	.	.	.	.	.	.	.	.	.	100
QQ4.seq	.	.	.	.	.	.	.	.	.	.	100
QQ7.seq	.	.	.	.	.	.	.	.	.	.	100
RS1.seq	.	.	.	.	.	.	.	.	.	.	100
RS4.seq	.	.	.	.	.	.	.	.	.	.	100
U1.seq	.	.	.	.	.	.	.	.	.	.	100
U16.seq	.	.	.	.	.	.	.	.	.	.	100
YS1.seq	.	.	.	.	.	.	.	.	.	.	100
YS2.seq	.	.	.	.	.	.	.	.	.	.	100
Y125.seq	.	.	.	.	.	.	.	.	.	.	100
Y127.seq	.	.	.	.	.	.	.	.	.	.	100
Y217.seq	.	.	.	.	.	.	.	.	.	.	100
628.seq	.	.	.	.	.	.	.	.	.	.	100
729.seq	.	.	.	.	.	.	.	.	.	.	100
1-35.seq	.	.	.	.	.	.	.	.	.	.	100
C00030P.seq	.	.	.	.	.	.	.	.	.	.	100
14-22.seq	.	.	.	.	.	.	.	.	.	.	100
C00046N.seq	.	.	.	.	.	.	.	.	.	.	100
1334.seq	.	.	.	.	.	.	.	.	.	.	100
S62.seq	.	.	.	.	.	.	.	.	.	.	100
46484.seq	.	.	.	.	.	.	.	.	.	.	100
1.seq	.	.	.	.	.	.	.	.	.	.	100
199724-3.seq	.	.	.	.	.	.	.	.	.	.	100
S8.seq	.	.	.	.	.	.	.	.	.	.	100
S7.seq	.	.	.	.	.	.	.	.	.	.	100
S56.seq	.	.	.	.	.	.	.	.	.	.	100
13-21.seq	.	.	.	.	.	.	.	.	.	.	100
11-8.seq	.	.	.	.	.	.	.	.	.	.	100
9-5.seq	.	.	.	.	.	.	.	.	.	.	100
660.seq	.	.	.	.	.	.	.	.	.	.	100
C00014N.seq	.	.	.	.	.	.	.	.	.	.	100
C00013N.seq	.	.	.	.	.	.	.	.	.	.	100
3-13.seq	.	.	.	.	.	.	.	.	.	.	100
2986.seq	.	.	.	.	.	.	.	.	.	.	100
7768.seq	.	.	.	.	.	.	.	.	.	.	100

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Majority	CAATCGGCAACTTTTACTTTGCCGACAAGCTTCGGAAATTTACA AATGCAACAAGCATGTAGTGCAGCTTTGTTACTTTGTGTACGAAAACAAAACACAT										
	110	120	130	140	150	160	170	180	190	200	
JK1.seq	.	.	.	.	.	.	.	.	.	G	200
LG2.seq	.	.	.	.	.	.	.	.	.	.	200
LG5.seq	.	.	.	.	.	.	.	.	.	.	200
LG9.seq	.	.	.	.	.	.	.	.	.	.	200
NS1.seq	.	.	.	.	.	.	.	.	.	.	200
P14.seq	.	.	.	.	.	.	.	.	.	.	200
P8.seq	.	.	.	.	.	.	.	.	.	.	200
PS1.seq	.	.	.	.	.	.	.	.	.	.	200
PY1.seq	.	.	.	.	.	.	.	.	.	.	200
QQ1.seq	.	.	.	.	.	.	.	.	.	.	200
QQ12.seq	.	.	.	G	.	.	.	.	.	.	200
QQ4.seq	.	.	.	.	.	.	.	.	.	.	200
QQ7.seq	.	.	.	.	.	.	.	.	.	.	200
RS1.seq	.	.	.	.	.	.	.	.	.	.	200
RS4.seq	.	.	.	.	.	.	.	.	.	.	200
U1.seq	.	.	.	.	.	.	.	.	.	.	200
U16.seq	.	.	.	G	.	.	G	.	.	.	200
YS1.seq	.	.	.	.	.	.	.	.	.	.	200
YS2.seq	.	.	.	G	.	.	.	.	.	.	200
Y125.seq	.	.	.	.	.	.	.	.	.	.	200
Y127.seq	.	.	.	.	.	.	.	.	.	.	200
Y217.seq	.	.	.	.	.	.	.	.	.	.	200
628.seq	.	.	.	.	C	.	.	.	.	.	200
729.seq	.	.	.	C	.	.	.	.	.	.	200
1-35.seq	.	.	T	.	.	.	.	.	.	.	200
C00030P.seq	.	.	.	.	.	.	.	.	.	.	200
14-22.seq	.	.	.	.	.	.	.	A	.	.	200
C00046N.seq	.	.	.	A	TAC	.	.	.	.	.	200
1334.seq	.	.	.	.	.	.	.	.	G	A	200
S62.seq	.	.	.	.	.	.	.	.	G	A	200
46484.seq	.	.	.	.	.	.	.	.	G	.	200
1.seq	.	.	C	.	.	.	.	.	G	.	200
199724-3.seq	.	.	.	.	.	.	.	.	G	.	200
S8.seq	A	.	.	T	.	.	A	.	T	G	200
S7.seq	A	.	.	T	.	.	A	.	T	G	200
S56.seq	A	.	.	T	.	.	A	.	T	G	200
13-21.seq	A	.	.	T	.	.	A	.	T	G	200
11-8.seq	A	.	.	.	.	.	A	.	T	G	200
9-5.seq	A	.	T	.	.	.	A	.	T	G	200
660.seq	A	.	T	.	.	.	A	.	T	GM	200
C00014N.seq	A	.	T	.	.	.	A	.	T	G	200
C00013N.seq	A	.	T	.	.	.	A	.	T	G	200
3-13.seq	A	.	.	.	.	.	A	.	T	G	200
2986.seq	A	T	.	.	.	.	.	T	G	G	200
7768.seq	G	.	.	.	T	.	.	.	G	G	200

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Majority	TTAACACTTTTGGCTGTGGAGATTATTACCAAAATTACTATGATGGGAATGGAAACCTGATACGGGGAATGGATAACAGAGTGGCAGCATAACAGAGGAAT									
	210	220	230	240	250	260	270	280	290	300
JK1.seq	.	.	.G.	.	.	.C.	.	.	.	300
LG2.seq	.	.	.G.	.	.C.	.	A.	.	.	300
LG5.seq	.	.	.G.	.	.C.	.	.	.G.	.	300
LG9.seq	.	.	.G.	.	.C.	.	.	.	.	300
NS1.seq	.	.	.G.	.	.C.	.	.	.	.	300
P14.seq	.	.	.G.	.	.C.	.	.	.	.	300
P8.seq	.	.	.G.	.	.C.	.	.	.	.	300
PS1.seq	.	.	.GG.	.	.C.	.	.	.	.	300
PY1.seq	.	.	.G.	.	.C.	.	.	.	.	300
QQ1.seq	.	.	.G.	.	.C.	.	A.	.	.	300
QQ12.seq	.	.	.G.G.	.	.C.	.	.	.	.	300
QQ4.seq	.	.A.	.G.	.	.C.	.	.	.	.	300
QQ7.seq	.	.	.G.	.	.C.	.	A.	.	.	300
RS1.seq	.	.	.G.	.	.C.	.	.	.	.	300
RS4.seq	.	.	.G.	.	.C.	.	A.	.	.	300
U1.seq	.	.	.G.	.	.C.	.	.	.	.	300
U16.seq	.	.	.G.G.	.	.C.	.	.	.	.	300
YS1.seq	.	.	.G.G.	.	.C.	.	A.	.	.	300
YS2.seq	.	.	.G.G.	.	.C.	.	.G.	.	.	300
Y125.seq	.	.	.G.	.	.C.	.	.	.	.	300
Y127.seq	.	.	.	.	.C.	.	.	.	.	300
Y217.seq	.	.	.	.	.C.	.	.	.T.	.	300
628.seq	.	.G.	.T.	.	.	.	.	.	.GC	300
729.seq	.	.G.	.T.	.	.	.	.	.	.GC	300
1-35.seq	.	.	.	.	.A.	.	.C.	.	.	300
C00030P.seq	.	.	.	.	.	.	.	.	.	300
14-22.seq	.	.	.	.	.	.	.	.	.	300
C00046N.seq	.	.	.	.	.	.	.	.	.	300
1334.seq	.	.	.	.	.	.	.	.	.	300
S62.seq	.	.	.	.	.	.	.	.	.	300
46484.seq	.	.	.	.	.	.	.	.	.	300
1.seq	.	.	.	.	.	.	.	.	.	300
199724-3.seq	.	.C.	.	.	.	.	.A.	.	.	300
88.seq	.C.	.	.	.	.C.	.G.	.	.C.	.G.	300
87.seq	.C.	.	.	.C.	.	.G.	.	.C.	.G.	300
S56.seq	.C.	.T.	.	.C.	.	.G.	.	.C.	.	300
13-21.seq	.C.	.	.	.C.	.	.G.	.	.C.	.	300
11-8.seq	.C.	.A.	.	.C.	.	.G.	.	.	.	300
9-5.seq	.C.	.R.	.	.C.	.	.G.	.R.	.	.	300
660.seq	.C.	.	.	.C.	.	.G.	.	.	.	300
C00014N.seq	.C.	.	.	.C.	.	.G.	.A.	.	.	300
C00013N.seq	.C.	.	.	.C.	.	.G.	.A.	.	.	300
3-13.seq	.C.	.	.C.	.	.G.	.	.	.	.	300
2986.seq	.	.	.	.	.	.	.G.	.	.	300
7768.seq	.G.	.	.	.	.	.	.	.	.	300

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Majority	AGCAAACGCTGGAGTTAAABATTGAATGTCCTTCCAAAATCTTGAACCCCTGGGACTTACAGCATTAGATCAACACCAAGATTCCCTTCTAGTACCAAAAAGG									
	310	320	330	340	350	360	370	380	390	400
JK1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
LG2.seq	.A.....	.....	.....	.....	.....	.....	.....	.....	.....	400
LG5.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
LG9.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
NS1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
P14.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
P8.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
PS1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
PY1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
QQ1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
QQ12.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
QQ4.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
QQ7.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
RS1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
RS4.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
U1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
U16.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
YS1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
YS2.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
Y125.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
Y127.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
Y217.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
628.seq	.....	.....	.....	.....	.....	.....	.....	.....G.....	.....	400
729.seq	.....	.....	.....	.....	.....	.....	.....	.....G.....	.....	400
1-35.seq	.....A.....	.....	.....	.....	.....	.....	.....	.....	.....	400
C00030P.seq	.....A.....	.....	.....	.....	.....	.....	.....	.....	.....	400
14-22.seq	.....	.....	.....	.....	.....	.....	.....	.....C.....	.....	400
C00046N.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	400
1334.seq	.....	.....	.....	.....	.....	.....A.....	.....	.....	.....	400
S62.seq	.....	.....	.....	.....	.....	.....A.....	.....	.....	.....	400
46484.seq	.....T.....	.....	.....	.....	.....	.....	.....	.....	.....	400
1.seq	.....T.....	.....	.....	.....	.....	.....	.....	.....	.....	400
199724-3.seq	.....T.....	.....	.....	.....T.....	.....	.....	.....	.....	.....	400
S8.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....	.....	...C.A.....	.G.....	400
S7.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....	.....	...C.A.....	.G.....	400
S56.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....C.....	.....	...C.A.....	.G.....	400
13-21.seq	...GG.T.....	.G.....	.....A.....	.....	.....C.....	.....	.....	...A.....	.G.....	400
11-8.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....	.....	...A.....	.G.....	400
9-5.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....R.....	.....	...A.....	.G.....	400
660.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....	.....	...A.....	.G.....	400
C00014N.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....	.....	...A.....	.G.....	400
C00013N.seq	...GG.T.....	.G.....	.....A.....	.....	.....	.....	.....	...A.....	.G.....	400
3-13.seq	...GG.T...R.....	.G.....	.....A.....	.....	.....	.....	.....	...A.....	.G.G.G.....	400
2986.seq	...GGT...C.....	.G.....	.....T.....	.....	.....A.....	.....	.....	...G.A.....	.G.....	400
7768.seq	...G...A.....	.G.....	.....	.....	.....	.....	.....	...G.A.....	.G.....	400

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Majority	TCATACTGCTTCGACACTGATGGAGGGTACCCCTATACAAGTAGTTCAATCTGAGTGGTCAGCTTCACGAAGATCAGATAATGCCA	T	AAAGAAGCA							
	410	420	430	440	450	460	470	480	490	
JK1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
LG2.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
LG5.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
LG9.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
NS1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
P14.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
P8.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
PS1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
PY1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
QQ1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
QQ12.seq	.....C.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
QQ4.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
QQ7.seq	.....G.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
RS1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
RS4.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
U1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
U16.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
YS1.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
YS2.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
Y125.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
Y127.seq	.....G.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
Y217.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
628.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
729.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
1-35.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
C00030P.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
14-22.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
C00046N.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
1334.seq	.....G.....	.....	.....	.....	.....	.....	.....	.....	.....	496
S62.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
46484.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
1.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
199724-3.seq	.....	.....	.....	.....	.....	.....	.....	.....	.....	496
S8.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
S7.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
S56.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
13-21.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
11-8.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
9-5.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
660.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
C00014N.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
C00013N.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
3-13.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496
2986.seq	.....T.....	.....A.....	.....	.....	.....	.....	.....	.....	.....	496
7768.seq	.....T.....	.....G.....	.....	.....	.....	.....	.....	.....	.....	496

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**Technical Appendix Figure 1.** Multiple sequence alignment results of the current IDV nucleotide sequences (496 bp) and corresponding published reference sequences.

Majority	T	A	S	C	S	S	S	W	M	K	S	P	L	W	A	E	S	S	V	N	P	G	A	K	P	Q	V	C	G	T	E	Q	S	A	T	F	T	L	P	T	S	F	G	I	Y	K	C	N	K	H	V	V	Q	L	C	Y	F	V	Y	E	N	K	T	F	N	T	F	G	C	G	D	Y	V	N	Y	D	G	N	G	N	L	I	S	M	D	N	R	V	A	A	R	G	I
	10	20	30	40	50	60	70	80	90	100																																																																																			
JK1.pro	.....																										D	.....																			100																																														
LG2.pro	.....																										D	.....																			100																																														
LG5.pro	.....																										D	.....																			100																																														
LG9.pro	.....																										D	.....																			100																																														
NS1.pro	.....																										D	.....																			100																																														
F14.pro	.....																										D	.....																			100																																														
P8.pro	.....																										D	.....																			100																																														
PS1.pro	.....																										D	.....																			100																																														
PY1.pro	.....																										D	.....																			100																																														
QQ1.pro	.....																										D	.....																			100																																														
QQ12.pro	.....																										FD	.....																			100																																														
QQ4.pro	.....P.....																										N	.....																			100																																														
QQ7.pro	.....																										D	.....																			100																																														
RS1.pro	.....																										D	.....																			100																																														
RS4.pro	.....																										D	.....																			100																																														
U1.pro	.....																										D	.....																			100																																														
U16.pro	.....																										FD	.....																			100																																														
YS1.pro	.....																										D	.....																			100																																														
YS2.pro	.....																										FD	.....																			100																																														
Y125.pro	.....																										D	.....																			100																																														
Y127.pro	.....																											.....																			100																																														
Y217.pro	.....																											.....																			100																																														
628.pro	.....																											.....																			A 100																																														
729.pro	.....																											.....																			A 100																																														
1-35.pro	.....																											.....																			100																																														
C00030P.pro	.....																											.....																			100																																														
14-22.pro	.....																											.....																			100																																														
C00046N.pro	.....																										NLQ	.....																			100																																														
1334.pro	.....																											.....AK.....																			100																																														
862.pro	.....																											.....AK.....																			100																																														
46484.pro	.....																											.....A.....																			100																																														
1.pro	.....																											.....A.....																			100																																														
199724-3.pro	.....																										T	.....A.....																			I..... 100																																														
S8.pro	.T.....																										R	.A.....																			A..L.....V..... 100																																														
S7.pro	.T.....																										R	.A.....																			A..L.....V..... 100																																														
S56.pro	.T.....																										R	.A.....																			A..L.....V..... 100																																														
13-21.pro	.T.....																										R	.A.....																			A..L.....V..... 100																																														
11-8.pro	.T.....																										R	.A.....																			A..L.....V..... 100																																														
9-5.pro	.T.....																										R	.A.....																			A..L.....V..X..... 100																																														
660.pro	.T.....																										R	.A.....																			A..L.....V..... 100																																														
C00014N.pro	.T.....																										R	.A.....																			A..L.....V..I..... 100																																														
C00013N.pro	.T.....																										R	.A.....																			A..L.....V..I..... 100																																														
3-13.pro	.T.....																											.A.....																			A..L.....V..... 100																																														
2986.pro	.T.....																										S	.....I.....																			S..A..... 100																																														
7768.pro	.T.....																										S	.L.....																			S.AA..A..... 100																																														

This study

D/OK

D/660

Majority	ANAGVKIECPKILNPGTYSIRSTPRFLLVPKRSYCFDITDGGYPIQVVQSEWSASRRSDNATEEA	
	110      120      130      140      150      160	
JK1.pro	.....	165
LG2.pro	T.....	165
LG5.pro	.....	165
LG9.pro	.....	165
NS1.pro	.....	165
P14.pro	.....	165
P8.pro	.....	165
PS1.pro	.....	165
PY1.pro	.....	165
QQ1.pro	.....	165
QQ12.pro	.....H.....	165
QQ4.pro	.....	165
QQ7.pro	.....R.....	165
RS1.pro	.....	165
RS4.pro	.....	165
U1.pro	.....	165
U16.pro	.....	165
YS1.pro	.....	165
YS2.pro	.....	165
Y125.pro	.....	165
Y127.pro	.....G.....	165
Y217.pro	.....	165
628.pro	.....	165
729.pro	.....	165
1-35.pro	.T.....	165
C00030P.pro	.T.....	165
14-22.pro	.....	165
C00046N.pro	.....	165
1334.pro	.....K.....	165
862.pro	.....K.....	165
46484.pro	.V.....	165
1.pro	.V.....	165
199724-3.pro	.V.....	165
S8.pro	.GS..R.....K.....	165
S7.pro	.GS..R.....K.....	165
S56.pro	.GS.....K.....	165
13-21.pro	.GS..R.....R.....K.....	165
11-8.pro	.GS.....K.....I.....	165
9-5.pro	.GS.....X.....K.....	165
660.pro	.GS.....K.....	165
C00014N.pro	.GS.....K.....	165
C00013N.pro	.GS.....K.....	165
3-13.pro	.GSX.....K.....	165
2986.pro	.G..A.....	165
7768.pro	.S.E.....S.....K.....	165

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**Technical Appendix Figure 2.** Multiple sequence alignment results of the current IDV amino acid sequences (165 aa) and corresponding published reference sequences.