



Communication

A Novel Enzootic Nasal Tumor Virus Circulating in Goats from Southern China

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Abstract: Enzootic nasal tumor virus (ENTV) has two types, ENTV-1 in sheep and ENTV-2 in goats, respectively. In China, the incidence of ENTV-2 related diseases has increased year by year. In this study, we reported an outbreak of ENTV-2 in a commercial goat farm in Qingyuan city, Guangdong province, southern China. A full-length genome of ENTV-2 (designated GDQY2017), with 7479 base pairs, was sequenced. Although GDQY2017 shared the highest nucleotide identity with a Chinese ENTV-2 isolate (ENTV-2CHN4, GenBank accession number KU258873), it possesses distinct genome characteristics undescribed, including a non-continuous 21-nucleotide insertion in the *gag* gene and a non-continuous 12-nucleotide deletion in the *env* gene. Notably, most of these indel nucleotide sequences were originated from a Chinese jaagsiekte sheep retrovirus (JSRV) isolate (GenBank accession number DQ838494). In the *gag* and *env* genes, GDQY2017 was phylogenetically related to those Chinese ENTV-2 isolates and a Chinese JSRV isolate (DQ838494). For GDQY2017-like viruses, more surveillance work should be made to explain their pathogenicity in goat herds. To our knowledge, this study represents the first to demonstrate the circulating pattern of ENTV-2 in Guangdong province, China, which will help to better understand the epidemiology and genetic diversity of ENTV-2.

Keywords: enzootic nasal tumor virus; ENTV-2; novel variation; genetic diversity; goats; Guangdong province

1. Introduction

Enzootic nasal tumor virus (ENTV) is a single positive-stranded RNA virus that belongs to the genus *Betaretrovirus* in the family *Retroviridae*. The genome of ENTV is about 7.5 kilo-base pairs and

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contains four open reading frames (ORFs), which encode *gag* (group antigen) protein, *pro* protease protein, *pol* protein, and *env* protein, respectively. Genetically, ENTV is close to jaagsiekte sheep retrovirus (JSRV, another ovine betaretrovirus), which is the causative agent of ovine pulmonary adenocarcinoma (OPA) [1]. ENTV has two types, ENTV-1 in sheep and ENTV-2 in goats [2,3], which mainly cause enzootic nasal adenocarcinoma (ENA), including copious serous nasal discharge, dyspnea, and even death due to respiratory failure [4,5].

Previous studies showed that ENTV-2 was mainly found in Inner Mongolia, Hunan, and Sichuan [6–9], while in past five years, ENTV-2 has spread to Anhui, Shaanxi, Chongqing, Hunan, and Fujian, suggesting a potential outbreak of ENTV-2 in China [10–14]. In this study, we reported the first case of ENTV-2 infection with distinct genome characteristics in Guangdong, China.

2. Materials and Methods

2.1. Case Description

In September 2017, a suspected case of ENA occurred on a goat farm (total livestock: 82) in Qingyuan city, a city of Guangdong province in southern China. The eight affected goats aged from three-month to one-year. The affected goats presented initial symptoms as nasal polyps (Figure 1a), followed by copious serous nasal discharge, dyspnea emerged, and even death (Figure 1b). To define potential causes of ENA, one affected goat (goat 3) was sent to the Animal Disease Diagnostic Center, Institute of Animal Health, Guangdong Academy of Agricultural Sciences. After euthanasia, the autopsy report showed that the main pathological changes (tumor-like tissue hyperplasia) were observed within the goat nose (Figure 1c). These clinical manifestations suggested that ENTV-2 was considered the potential cause. Serum, tumor-like tissue, and nasal discharge of goat 3 were collected and stored at -80 °C for further use. Tumor-like tissues were stained by hematoxylin-eosin (HE). Sample collection was approved by the ethical and ethics commission (Institute of Animal Health, Guangdong Academy of Agricultural Sciences, China). The license number was SYXK(Yue) 2011–0116. Moreover, all methods in this study were performed in accordance with national and local laws and guidelines.



Figure 1. Clinical manifestations and pathological changes of goats affected by enzootic nasal tumor virus (ENTV)-2. (a) Bloody polyps appeared around the nostrils of a goat's nose (Goat 1, about 3-month-old). (b) A lot of nasal discharge appeared around the nose (Goat 2, about 10-month-old). (c) The nasal cavity (Goat 3, about one-year-old) filled with tumor-like tissues.

2.2. Detection and Complete Genome Amplification of ENTV-2

One-step RT-PCR kit (Takara Inc., Dalian, Liaoning, China) was performed to detect ENTV-2 in collected samples, using a pair of previously reported primers (3F: 5'-CACTCCTAATTTGTGCCCACG-3' and 3R: 5'-GGCCACTGATCGACCCATAC-3') targeting gag-pro-pol fusion gene (Table 1), according to the manufacturer's instructions. Four additional pairs of primers (Table 1) were synthesized and used to amplify the genome of ENTV-2, according to

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one previous literature [13]. One step RT-PCRs were performed with a final reaction volume of 50 μ L, containing 25 μ L 2 × RT-PCR buffer (Takara, Inc., Dalian, Liaoning, China), 1 μ L Enzyme Mix (including reverse transcriptase and DNA polymerase), 5 μ L viral RNA, 1 μ L each of the primers (10 pmol), and 17 μ L RNase-free water. Cycling conditions were as follows: 50 °C for 30 min, 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 70 s. A final extension of 5 min at 72 °C concluded the program. The PCR products were purified and cloned into the pGEM-T vector (Tiangen Biotech Co., Ltd. Beijing, China). In addition, the positive recombinant plasmids were sequenced by the Sanger sequencing (Sangon Biotech Co., Ltd. Shanghai, China).

Primer	Primer Sequence(5'-3')	Location	Product Length
1F	ACAAGGCATCAGCCATTTTGGTCTGATCCTCTCAACCCCA	1-40	
1R	AGGAGGAGCATCATAACCAGGCTCTGGGTCAGGAATA	627-588	627 bp
2F	GTTTTCCTCGCCACTACTCTTG	151-172	•
2R	TACCCAATAAGCGTCGGATGAT	2399-2378	2249 bp
3F	CACTCCTAATTTGTGCCCACG	1848-1869	•
3R	GGCCACTGATCGACCCATAC	2934-2915	1087 bp
4F	GAAGAGGTTTGGGGTGTTTTCCCTAGGGACCTCTGATTCTCCTGTGAC	2830-2878	_
4R	GTTTAAGACGTTGATGAGCTCGTTCTACAATCCCTTGTCCCTGTGGGT	5087-5040	2258 bp
5F	AGAACGAGCTCATCAACGTCTTAAACATCAACT	5062-5094	•
5R	CTTGTTGTTTTATTGTGTCATAGTATATAT	7440-7411	2379 bp

Table 1. Primers used in this study.

2.3. Sequence and Phylogenetic Analysis

Nucleotide sequence editing, full-length genome assembling, amino acid sequence prediction, sequence alignment, and analysis were performed by using Lasergene v7.1 sequence analysis software package (DNASTAR Inc., Madison, WI, USA). Phylogenetic analysis was performed by the maximum likelihood method with 1000 bootstrap replicates using MEGA 7 software (https://www.megasoftware.net/). One thousand bootstrap replicates were used. Recombination analysis was performed using a recombination detection program (RDP) 4.95 (http://web.cbio.uct.ac.za/~{}darren/rdp.html).

3. Results and Discussion

According to the results of HE staining (Figure 2), the tumor appeared in the goat was considered to be nasal adenoma, which was in compliance with the feature of ENA.

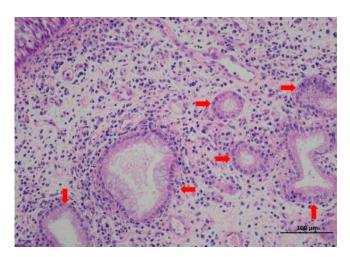


Figure 2. Microscopic feature of tumor tissues in goat 3. There were some different sizes of secretory glandular structures and a lot of interstitial epithelioid cells (labeled by the red arrows) in the figure (Bar = $100 \mu m$).

Serum, tumor-like tissue, and nasal discharge of goat 3 were positive for ENTV-2 by RT-PCR detection. A complete genome sequence (designated GDQY2017) of ENTV-2 with 7479 base pairs was

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obtained from the goat sera and deposited in the GenBank database (accession number: MK164396). GDQY2017 has four main encoding gene regions, *gag* gene (position 266–2125), *pro* gene (position 2017–2886), *pol* gene (position 3132–4259), *env* gene (position 5351–7207). Complete genome alignment results showed that GDQY2017 had the highest nucleotide similarity (96.6%) with a Chinese ENTV-2 strain (ENTV-2CHN4, GenBank accession number KU258873), and the lowest nucleotide similarity (88.2%) with a Spanish ENTV-2 strain (GenBank accession number AY197548) isolated in the 1990s (Table 2). Compared with ENTV-1, JSRV, endogenous JSRV (enJSRV), and other ENTV-2 sequences, the *gag* gene and *env* gene of GDQY2017 had higher diversities (85.7–96.8% vs. 85.8–95%) than the *pro* gene and *pol* gene (90.9–99.4% vs. 88–99.2%).

Table 2. Similarity (%) of GDQY2017 with other reference retroviruses in goats and sheep.

Isolation (GenBank No.)	Complete Genome	gag Gene	gag Protein	env Gene	env Protein	
JSRV (M80216)	88.2%	86.6%	88.9%	85.9%	89.4%	
JSRV21 (AF105220)	88.2%	86.1%	89.4%	86.2%	89.7%	
sheep C1 China 2013 G1 (KP691837)	87.6%	85.7%	89.1%	85.8%	89.2%	
JSRV (DQ838494)	92.2%	93.6%	87.7%	93.6%	92.2%	
sheep TNO28 (Y16627)	88.4%	87.7%	89.7%	87.7%	88.2%	
ENTV-1NA1 (GU292317)	88.2%	87.5%	89.4%	87.8%	87.9%	
ENTV-1NA2 (GU292315)	88.2%	87.9%	89.4%	87.7%	88.2%	
ENTV-1NA3 (GU292318)	88.2%	87.7%	89.3%	87.6%	88.2%	
ENTV-1NA4 (FJ744146)	88.2%	87.8%	89.3%	87.6%	88.1%	
ENTV-1NA5 (GU292316)	88.2%	87.8%	89.3%	87.7%	88.2%	
ENTV-1NA6 (FJ744149)	88.4%	87.9%	89.4%	87.8%	88.1%	
ENTV-1NA7 (FJ744150)	88.4%	87.9%	89.4%	87.8%	88.1%	
ENTV-1NA8 (FJ744147)	88.3%	87.8%	89.6%	87.8%	88.1%	
ENTV-1NA9 (FJ744148)	88.3%	87.8%	89.6%	87.7%	88.2%	
ENTV-1NA10 (GU292314)	88.2%	88%	89.6%	87.5%	88.1%	
ENTV-1OVC (KC189895)	88.2%	87.7%	89.4%	87.7%	88.2%	
ENTV2 (AY197548)	88.2%	87.2%	88.6%	91.2%	89.8%	
Shaanxi (KU179192)	91.9%	86.7%	88.7%	89.1%	91%	
Shaanxi2 (KU980910)	92%	86.6%	88.7%	89.1%	91%	
Shaanxi3 (KU980911)	92.2%	87.2%	88.9%	89.2%	91.1%	
Shaanxi4 (KU980912)	91.8%	86.7%	88.7%	89.1%	91%	
CQ1 (MK164400)	92.7%	86.6%	88.3%	90.8%	90.5%	
ENTV-SC (HM104174)	90.9%	86.5%	88.3%	89%	90.8%	
ENTV-2CHN1 (KU258870)	95.7%	96.3%	94.9%	93.3%	92.4%	
ENTV-2CHN2 (KU258871)	96%	95.8%	93.3%	93.3%	91.6%	
ENTV-2CHN3 (KU258872)	95.8%	95.9%	94.1%	93.5%	92.2%	
ENTV-2CHN4 (KU258873)	96.6%	96.8%	94.9%	94.8%	93.7%	
ENTV-2CHN5 (KU258874)	95.2%	96%	93.5%	91.7%	91.6%	
ENTV-2CHN6 (KU258875)	94.7%	96.8%	95.1%	92.1%	93.1%	
ENTV-2CHN7 (KU258876)	94.4%	95.5%	95.1%	90.3%	93.9%	
ENTV-2CHN8 (KU258877)	95%	96.6%	94.9%	90.4%	93.5%	
ENTV-2CHN9 (KU258878)	92.6%	95.9%	93.3%	90.3%	93.4%	
ENTV-2CHN10 (KU258879)	93.3%	96.1%	93.6%	90.1%	93.2%	
ENTV-2CHN11 (KU258880)	92.2%	95.6%	95.1%	90.3%	93.4%	
enJSRV-1 (EF680311)	94.6%	95.1%	94.5%	_a	_a	
enJSRV-16 (EF680300)	93.3%	91.6%	91.4%	94.6%	94.3%	
HamJ1 (MF175067)	93.6%	91.8%	91.7%	94.9%	94.4%	
KarM (MF175071)	93.6%	91.8%	91.7%	95%	94.6%	

Note: ^a It means no comparability between enJSRV-1 and GDQY2017 because the *env* gene of enJSRV-1 was incomplete or uncertain. ENTV, enzootic nasal tumor virus; JSRV, jaagsiekte sheep retrovirus; enJSRV, endogenous JSRV.

In addition to nucleotide substitution, the *gag* gene of GDQY2017 had a distinctive non-continuous 21-nucleotide insertion (Figure 3). Another difference from most of the ENTV-2 sequences is a non-continuous 12-nucleotide deletion in the *env* gene (Figure 4). Interestingly, most of these indel nucleotide sequences were originated from a JSRV isolate (DQ838494), and some nucleotide sequences were identical to one endogenous JSRV isolate (enJSRV-1, GenBank accession number DQ838494) (Figures 3 and 4).

At the amino acid level, the gag protein sequences of GDQY2017 showed the highest similarity (95.1%) with ENTV-2CHN6 (KU258875), ENTV-2CHN7 (KU258876), and ENTV-2CHN11 (KU258880),

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and shared the lowest similarity (88.3%) with CQ1 (MK164400) and ENTV-SC (HM104174). Compared to previous strains, there were seven non-continuous amino acid insertion sites in the gag protein of GDQY2017 (Figure 5). While, the env protein sequences of GDQY2017 had the highest similarity (94.6%) with one endogenous JSRV isolate (KarM, MF175071), and shared the lowest similarity with a Spanish ENTV-2 isolate (AY197548). Compared to previous ENTV-2 strains, four continuous amino acid deletion sites were found in the env protein of GDQY2017 (Figure 6), which is identical to ENTV-2CHN1 (KU258870).

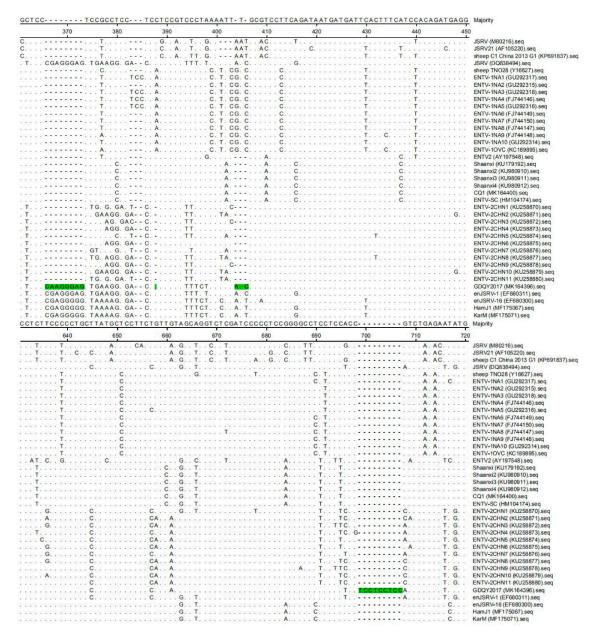


Figure 3. Multiple sequence alignment results of the *gag* gene of ENTV-2 with ENTV-1, jaagsiekte sheep retrovirus (JSRV), and endogenous JSRV (enJSRV). The nucleotide insertion sites in the *gag* gene of GDQY2017 were marked with green color.

In the phylogenetic tree based on the *gag* and *env* genes, GDQY2017 was clustered with ENTV-2 branch and phylogenetically related to a Chinese JSRV isolate (DQ838494) or an endogenous JSRV isolate (enJSRV-1, EF680311) (Figures 7 and 8).

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Moreover, no possible recombination points among ENTV-2 and other retroviruses were found by RDP.

	1750	1760	1770	1780	1790	1800)
C. C	. CGT	A. G.	A	C		CT	JSRV (M80216).seq
	. CGC		A				JSRV21 (AF105220).seq
G	. CGC			C. G		. T	sheep C1 China 2013 G1 (KP691837).s
T	A.		G TT. A		C A		JSRV (DQ838494).seq
T A.		. CC A.		. A			sheep TNO28 (Y16627).seq
T A.			A				ENTV-1NA1 (GU292317).seq
T A.					T		ENTV-1NA2 (GU292315).seq
T A.				. A	T		ENTV-1NA3 (GU292318).seq
T A.		. CC A.		. A	T		ENTV-1NA4 (FJ744146).seq
T. A.							ENTV-1NA5 (GU292316).seq
T . A							ENTV-1NA6 (FJ744149).seq
T A.						710 MI 0	ENTV-1NA7 (FJ744150), seq
T. A.							ENTV-1NA8 (FJ744147).seq
T A.							ENTV-1NA9 (FJ744148).seq
T A.		CC A		Α	T		ENTV-1NA10 (GU292314).seq
				. A	T		ENTV-10VC (KC189895).seq
			C			. T	ENTV2 (AY197548).seq
				T C			Shaanxi (KU179192).seq
т.			C				Shaanxi2 (KU980910).seq
T.							Shaanxi3 (KU980911).seq
T .		C.					Shaanxi4 (KU980912).seq
T .		C.		T C			CQ1 (MK164400).seq
T			C				ENTV-SC (HM104174).seq
T .							ENTV-3C (HM104174).seq ENTV-2CHN1 (KU258870).seq
1			C. TC			. T	
T							ENTV-2CHN2 (KU258871).seq
	A.		C. TC			. T	ENTV-2CHN3 (KU258872).seq
	A.				C C		ENTV-2CHN4 (KU258873).seq
	A.				CC		ENTV-2CHN5 (KU258874).seq
	A.		C		CC		ENTV-2CHN6 (KU258875).seq
	A.		C				ENTV-2CHN7 (KU258876).seq
	A.		C		C		ENTV-2CHN8 (KU258877).seq
1	A.		C				ENTV-2CHN9 (KU258878).seq
I	A.		C		C C		ENTV-2CHN10 (KU258879).seq
T	A.		C		CC		ENTV-2CHN11 (KU258880).seq
TA.	A.		T		A		GDQY2017 (MK164396).seq
T	A.	C			C A		enJSRV-16 (EF680300).seq
	A.		TT. A		C A		HamJ1 (MF175067).seq
T	A .		TT. A		C A		HamJ1 (MF175067).seq KarM (MF175071).seq
TT GC					C A		
TT GC A	AATACCGAC	CATCTTATGG	AGCTTTTAAAAA	ATAAAGAG	C A C A AGGGGAGCT	cgggg	KarM (MF175071).seq Majority
	1810	1820	AGCTTTTAAAAA 1830	ATAA AGAG 1840	C A C A AGGGGAGCT G		KarM (MF175071).seq Majority
A	1810 . C	1820	1830	ATAA AGAG 1840	C A C A AGGGGAGCT G	cgggg	KarM (MF175071).seq Majority) JSRV (M80216).seq
A	1810 . C GC A	1820		ATAA AGAG 1840	C A	cgggg	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq
A	1810 . C	1820 A	AGCTTTTAAAAA 1830	ATAA AGAG 1840	C A	1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s
A	1810 . C	1820 A	TT. A AGCTTTTAAAAA 1830 G.	ATAA AGAG 1840	C A	1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq
A	1810 . C	1820 A	1830 G	ATAA AGAG 1840	- C . A	1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF10520).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq
A	1810 . C	1820 A	1830 G. TT. G. G.	1840	C. A. C. A. AGGGGAGCT G 1850 A. A.	1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq
A	1810 . C	1820 A	1830	1840 1840	C. A. C. A. AGGGGAGCTG 1850	1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq
A	1810 . C	1820		ATAA- AGAG 1840 	C. A C. A. AGGGGAGCTG 1850	186C	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (D0838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq
A	1810 . C	1820 A	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G. T. G.	1840 1840 	C. A. C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq
A	1810 C. GC. A. GC. A.	1820 A	TT. A AGCTTTTAAAAA 1830 G	1840 1840 	- C. A. - C. A. AGGGGAGCT G 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (GJ44146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA5 (GU292316).seq
A	1810 C	1820	1830 1830 G. C. TT. G. G. T. G.		C. A C. A. AGGGGAGCT 6 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA1 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744149).seq
A	1810 C	1820 1820 A A A A A A A A.	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G.	1840 1840 	- C. A. - C. A. AGGGGAGCT G 1850	3CGGGG 1866	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (GU744149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ7441450).seq
A	1810 C	1820 A	TT. A AGCTTTTAAAAA 1830 G	1840 1840 	- C. A. - C. A. AGGGGAGCT 0 1850	3CGGGG	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA8 (FJ744147).seq
A	1810 C	1820 1820 A A A A A A A A.	TT. A AGCTTTTAAAAA 1830	1840 1840 	C. A C. A. AGGGGAGCT G 1850	186C	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TMO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ74417).seq ENTV-1NA6 (FJ74417).seq ENTV-1NA6 (FJ74417).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq
. A	1810 C	1820 A	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A. C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292318).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA5 (GU292316).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (GU292314).seq
. A	1810 C	1820 A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. TT. G. G. TT. G. G. TT. G.	1840 1840 	C. A C. A. AGGGGACTO 1850	1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TMO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ74417).seq ENTV-1NA6 (FJ74417).seq ENTV-1NA6 (FJ74417).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq
A. A	1810 C	1820	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G.	ATAA AGAG 1840 G G G G G G G G G G	C. A C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292318).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA5 (GU292316).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (GU292314).seq
A. A	1810 C	1820	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A. C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292316).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ74419).seq
A. A	1810 C	1820 1820A AAAAAAAA.	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A C. A. AGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744148).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ74418).seq ENTV-1NA9 (FJ74418).seq ENTV-1NA9 (FJ74418).seq ENTV-1NA9 (FJ74418).seq ENTV-1NA9 (FJ74418).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KC189895).seq
A. A	1810 C	1820 A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A C. A. AGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292316).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (GU292314).seq ENTV-1NA9 (GU292314).seq ENTV-1NA9 (GU292314).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ749159).seq ENTV-10VC (KC189895).seq ENTV2 (AV197548).seq Shaanxi (KU179192).seq Shaanxi (KU179192).seq
A	1810 C	1820 A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA1 (GU292316).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ74418).seq ENTV-1NA9 (FJ74418).seq ENTV-1NA9 (FJ74419).seq Shaanxi (KU1980910).seq Shaanxi3 (KU980911).seq
A. A	1810 C	1820 1820A AAAAAAAA.	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A. C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep T028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (GJ292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744170).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA0 (GU292316).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (GU292314).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KC189895).seq ENTV2 (AY197548).seq Shaanxi (KU1979192).seq Shaanxi (KU1980911).seq Shaanxi (KU980911).seq Shaanxi (KU980911).seq Shaanxi (KU980911).seq
A. A	1810 C	1820 A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. T. G.	1840 1840 	C. A C. A. AGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ74418).seq ENTV-1OVC (KC189895).seq ENTV-10VC (KC189895).seq ENTV-10VC (KU1990910).seq Shaanxi (KU980910).seq Shaanxi (KU980911).seq Shaanxi (KU980911).seq Shaanxi (KU980912).seq CQ1 (MK164400).seq
A. A	1810 C	1820AAAAAAAAA	TT. A AGCTTTTAAAAA 1830 G C G.	ATAA- AGAG 1840	C. A C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA0 (GU292316).seq ENTV-1NA0 (GU292316).seq ENTV-1NA9 (FJ74419).seq ENTV-1NA9 (GU292314).seq ENTV-1NA9 (GU292314).seq ENTV-1NA9 (FJ74619).seq Shaanxi (KU980911).seq Shaanxi (KU980911).seq CQ1 (MK164400).seq ENTV-5C (HM104174).seq
A. A	1810 C	1820 1820 . A	TT. A AGCTTTTAAAAA 1830 G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. G. T.	ATAA- AGAG 1840	- C. A. - C. A. AGGGGAGCT G 1850	3C GGGG 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep T028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA6 (GY244148).seq ENTV-1NA6 (GY244149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744175).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA10 (GU292316).seq ENTV-1NA10 (GU292316).seq ENTV-1NA10 (GU292314).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KV189891).seq Shaanxi2 (KU980911).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980912).seq CQ1 (MK164400).seq ENTV-SC (HM104174).seq ENTV-SC (HM104174).seq ENTV-SC (HM104174).seq
A	1810 C	1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 C. TT. G. G. T. G. G. T. G. G	ATAA- AGAG 1840	C. A. C. A. AGGGGAGCTG 1850	3C GGGG	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (GJ744149).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (KU292314).seq ENTV-10VC (KC189895).seq ENTV-2 (AY197548).seq Shaanxi (KU980910).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980911).seq CQ1 (MK164400).seq ENTV-SC (HM104174).seq
A	1810 C	1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. G. T. G. G	ATAA AGAG 1840	C. A C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TN028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA10 (GU292316).seq ENTV-1NA10 (GU292316).seq ENTV-1NA9 (FJ74419).seq ENTV-1NA9 (FJ74419).seq ENTV-1NA9 (FJ74419).seq ENTV-1NA9 (FJ7481).seq ENTV-1NA9 (FJ7481).seq ENTV-1NA10 (GU292314).seq ENTV-2 (KU1980911).seq Shaanxia (KU980911).seq Shaanxia (KU980911).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN1 (KU258871).seq
A. A	1810 C	1820 1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. G. C. T. G. G. T. G. G	ATAA- AGAG 1840	C. A C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep T028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (GJ292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (GU292314).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KC189895).seq ENTV-2 (AY197548).seq Shaanxi2 (KU980910).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980912).seq CQ1 (MK164400).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN2 (KU258871).seq ENTV-2CHN3 (KU258871).seq
A. A	1810 C	1820 . A	TT. A AGCTTTTAAAAA 1830 G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. T. G. G. T. G. G	ATAA- AGAG 1840	C. A. C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA7 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (GU292314).seq ENTV-10VC (KC189895).seq ENTV-10VC (KC189895).seq ENTV-10VC (KU1898910).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980911).seq Shaanxi5 (KU980911).seq ENTV-SC (HM104174).seq ENTV-SC (HM104174).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN2 (KU258871).seq ENTV-2CHN3 (KU258871).seq ENTV-2CHN3 (KU258873).seq ENTV-2CHN5 (KU258873).seq
A. A	1810 C	1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. T. G. G. T. G. G	ATAA- AGAG 1840	C. A C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TN028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA2 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA9 (FJ744149).seq ENTV-1NA10 (GU292314).seq ENTV-2 (KU1980910).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980911).seq Shaanxi4 (KU980912).seq ENTV-2CHN10 (KU258870).seq ENTV-2CHN10 (KU258871).seq ENTV-2CHN3 (KU258872).seq ENTV-2CHN4 (KU258873).seq ENTV-2CHN5 (KU258873).seq ENTV-2CHN5 (KU258873).seq
A. A	1810 C	1820	TT. A AGCTTTTAAAAA 1830 G. G. C. T. G. G	ATAA- AGAG 1840	C. A C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA6 (GJ2414149).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (GU292314).seq ENTV-1OVC (KC189895).seq ENTV-1OVC (KC189895).seq ENTV-2 (AY197548).seq Shaanxi2 (KU980910).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980912).seq CQ1 (MK164400).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN3 (KU258873).seq ENTV-2CHN5 (KU258873).seq ENTV-2CHN5 (KU258873).seq ENTV-2CHN5 (KU258873).seq
A. A	1810 C	1820 . A	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G. G. T. G. G	ATAA- AGAG 1840	C. A. C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TNO28 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292316).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (GU292314).seq ENTV-1OVC (KC188895).seq ENTV-10VC (KC188895).seq ENTV-10VC (KC188895).seq ENTV-2 (AY197548).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980912).seq CQ1 (MK164400).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN2 (KU258871).seq ENTV-2CHN3 (KU258871).seq ENTV-2CHN4 (KU258873).seq ENTV-2CHN5 (KU258874).seq ENTV-2CHN6 (KU258875).seq ENTV-2CHN6 (KU258876).seq
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A. A	1810 C	1820 . A	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G.	ATAA- AGAG 1840	C. A. C. A. AGGGGAGCTG 1850	3C GGGG 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep EN 2028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (GU292316).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA6 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744147).seq ENTV-1OVC (KC189895).seq ENTV-1NA9 (KU29314).seq Shaanxi2 (KU980911).seq Shaanxi2 (KU980911).seq Shaanxi2 (KU980911).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN1 (KU258873).seq ENTV-2CHN6 (KU258873).seq ENTV-2CHN6 (KU258875).seq ENTV-2CHN9 (KU258877).seq ENTV-2CHN9 (KU258879).seq ENTV-2CHN10 (KU258879).seq ENTV-2CHN10 (KU258879).seq ENTV-2CHN11 (KU2588879).seq ENTV-2CHN11 (KU2588879).seq
A	1810 C	1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G. G. T. G. G	ATAA- AGAG 1840	C. A. C. A. AGGGGAGCTG 1850	1860 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep EN 2028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292318).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (GJ292316).seq ENTV-1NA6 (FJ744149).seq ENTV-1NA7 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (GU292314).seq ENTV-1NA10 (KU179192).seq Shaanxi (KU179192).seq Shaanxi (KU1880911).seq Shaanxi (KU980911).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN3 (KU258871).seq ENTV-2CHN1 (KU258873).seq ENTV-2CHN5 (KU258873).seq ENTV-2CHN6 (KU258873).seq ENTV-2CHN6 (KU258873).seq ENTV-2CHN7 (KU258873).seq ENTV-2CHN7 (KU258875).seq ENTV-2CHN7 (KU258877).seq ENTV-2CHN8 (KU258877).seq
A. A	1810 C	1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. C. T. G. G	ATAA- AGAG 1840	- C. A. - C. A. AGGGGAGCT G 1850	1860 1860	KarM (MF175071).seq Majority JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep EN 2028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (FJ744146).seq ENTV-1NA6 (GU292316).seq ENTV-1NA6 (FJ744147).seq ENTV-1NA6 (FJ744150).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744147).seq ENTV-1NA9 (FJ744148).seq ENTV-1NA9 (FJ744147).seq ENTV-1OVC (KC189895).seq ENTV-1NA9 (KU29314).seq Shaanxi2 (KU980911).seq Shaanxi2 (KU980911).seq Shaanxi2 (KU980911).seq ENTV-2CHN1 (KU258870).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN1 (KU258873).seq ENTV-2CHN6 (KU258873).seq ENTV-2CHN6 (KU258875).seq ENTV-2CHN9 (KU258877).seq ENTV-2CHN9 (KU258879).seq ENTV-2CHN10 (KU258879).seq ENTV-2CHN10 (KU258879).seq ENTV-2CHN11 (KU2588879).seq ENTV-2CHN11 (KU2588879).seq
A. A	1810 C	1820 A A A A A A A A A A A A A	TT. A AGCTTTTAAAAA 1830 G. C. TT. G. G. T. G. G. T. G. G	ATAA- AGAG 1840	- C. A. - C. A. AGGGGAGCT G 1850	1860 1860	KarM (MF175071).seq Majority) JSRV (M80216).seq JSRV21 (AF105220).seq sheep C1 China 2013 G1 (KP691837).s JSRV (DQ838494).seq sheep TN028 (Y16627).seq ENTV-1NA1 (GU292317).seq ENTV-1NA3 (GU292315).seq ENTV-1NA4 (F1744146).seq ENTV-1NA5 (GU292316).seq ENTV-1NA6 (G1744149).seq ENTV-1NA6 (F1744145).seq ENTV-1NA7 (F1744150).seq ENTV-1NA9 (F1744145).seq ENTV-1NA9 (F1744147).seq ENTV-1NA9 (G1744147).seq ENTV-1NA9 (G1744147).seq ENTV-1NA9 (F1744147).seq ENTV-10VC (KC189895).seq ENTV-2 (AV197548).seq Shaanxi3 (KU980911).seq Shaanxi4 (KU980911).seq Shaanxi4 (KU980911).seq ENTV-SC (HM104174).seq ENTV-SC (HM104174).seq ENTV-SC (HM104174).seq ENTV-2CHN1 (KU258871).seq ENTV-2CHN3 (KU258871).seq ENTV-2CHN3 (KU258871).seq ENTV-2CHN6 (KU258875).seq ENTV-2CHN9 (KU258878).seq ENTV-2CHN9 (KU258878).seq ENTV-2CHN11 (KU258887).seq ENTV-2CHN10 (KU258887).seq ENTV-2CHN11 (KU258887).seq ENTV-2CHN11 (KU258887).seq ENTV-2CHN11 (KU258887).seq ENTV-2CHN11 (KU258887).seq ENTV-2CHN11 (KU258887).seq ENTV-2CHN11 (KU258887).seq

Figure 4. Multiple sequence alignment results of the *env* gene of ENTV-2 with ENTV-1, JSRV, and enJSRV. The nucleotide deletion sites in the *env* gene of GDQY2017 were marked with green color. Moreover, compared to ENTV-2CHN1, GDQY2017 had an additional three-nucleotide deletion (marked with green color) in position 1839–1841 of the *env* gene.

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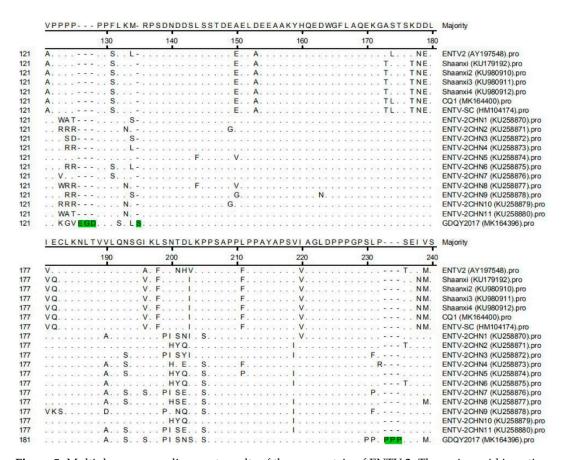


Figure 5. Multiple sequence alignment results of the gag protein of ENTV-2. The amino acid insertion sites (EGD-S-PPP) in the gag protein of GDQY2017 were marked with green color.

	570	580	590	600	610	620	
							JSRV (M80216).pro
L. F.	1 LIF.	V. GM. R	K M	Q.		. D D	JSRV21 (AF105220).pro
							sheep C1 China 2013 G1 (KP691837).pr
. A. L. F	. L I I A.	E	LI	HK Q.	N	D	JSRV (DQ838494).pro
A. I. F.	L	T. GLI	Q I I	Y YQF	DFV. K RE	SC. GQ. AEG.	sheep TNO28 (Y16627).pro
						SC. GQ. AEG.	ENTV-1NA1 (GU292317).pro
A. I. F.	L	T. DLI	Q I	Y YQH	D. V. K GE	SC. GQ. AEG.	ENTV-1NA2 (GU292315).pro
A. I. F.		T. DL1	0	. Y YOF	D. V. K GE	SC. GQ. AEG.	ENTV-1NA3 (GU292318).pro
						SC. GQ. AEG.	ENTV-1NA4 (FJ744146).pro
						SC. GQ. AEG.	ENTV-1NA5 (GU292316).pro
						SC. GQ. AEG.	ENTV-1NA6 (FJ744149),pro
						SC. GQ. AEG.	ENTV-1NA7 (FJ744150).pro
						SC. GQ. AEG.	ENTV-1NA8 (FJ744147).pro
						SC. GQ. AEG.	ENTV-1NA9 (FJ744148).pro
						SC. GQ. AEG.	ENTV-1NA10 (GU292314).pro
						SC. GQ. AEG.	ENTV-10VC (KC189895).pro
					G		ENTV2 (AY197548).pro
					2 G		Shaanxi (KU179192).pro
					2 G		
							Shaanxi2 (KU980910).pro
					G		Shaanxi3 (KU980911).pro
) G		Shaanxi4 (KU980912).pro
) G		CQ1 (MK164400).pro
) G		ENTV-SC (HM104174).pro
					N. K.		ENTV-2CHN1 (KU258870).pro
							ENTV-2CHN2 (KU258871).pro
							ENTV-2CHN3 (KU258872).pro
							ENTV-2CHN4 (KU258873).pro
					.		ENTV-2CHN5 (KU258874).pro
							ENTV-2CHN6 (KU258875).pro
							ENTV-2CHN7 (KU258876).pro
. A. L. V	. LIL A.	E	A. S. Y				ENTV-2CHN8 (KU258877).pro
. A. L. V	. LIL A.	E	A. S. Y				ENTV-2CHN9 (KU258878).pro
. A. L. V	. LIL A.	E	A. S. Y	H	K.		ENTV-2CHN10 (KU258879).pro
. A. L. V	. LIL A.	E	A. S. Y		K.		ENTV-2CHN11 (KU258880).pro
. T. L. V	. L.I.I T.	I . E	Q LI	HK		0.0000000000000000000000000000000000000	GDQY2017 (MK164396),pro
							enJSRV-16 (EF680300).seq
							HamJ1 (MF175067).seq
							KarM (MF175071).seq

Figure 6. Multiple sequence alignment results of the env protein of ENTV-2 with ENTV-1, JSRV, and enJSRV. The amino acid deletion sites in the env protein of GDQY2017 were marked with green color.

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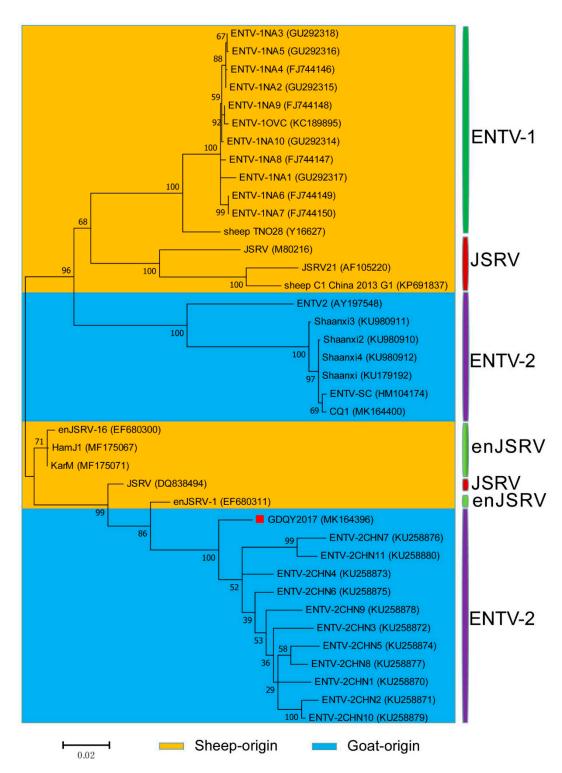


Figure 7. Phylogenetic analysis of the *gag* gene of ENTV-2 with ENTV-1, JSRV, and enJSRV. GDQY2017 described in this study was marked with the red square.

In China, reports of OPA and ENA could be traced back to 1982 in Inner Mongolia of north China [6]. Although cases of OPA and ENA were restricted in northern China previously, increasing cases have been reported in recent years [6,7]. ENTV spread from northern to southern gradually, including Sichuan province, Chongqing city, Guizhou province, Fujian province, Anhui province, and Hunan province [8–14]. Here, we reported the first ENTV-2 case in Guangdong province, southern China. The possible reasons for this outbreak are as follows: 1) the rapid development of the logistics industry

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and the policy guidance for southern herbivore animal husbandry that made that live goats move around more and more frequently; 2) the lack of ENTV-2 pathogen quarantine when farms introduce or export goats. Therefore, effective animal disease surveillance agencies in China still need improvement.

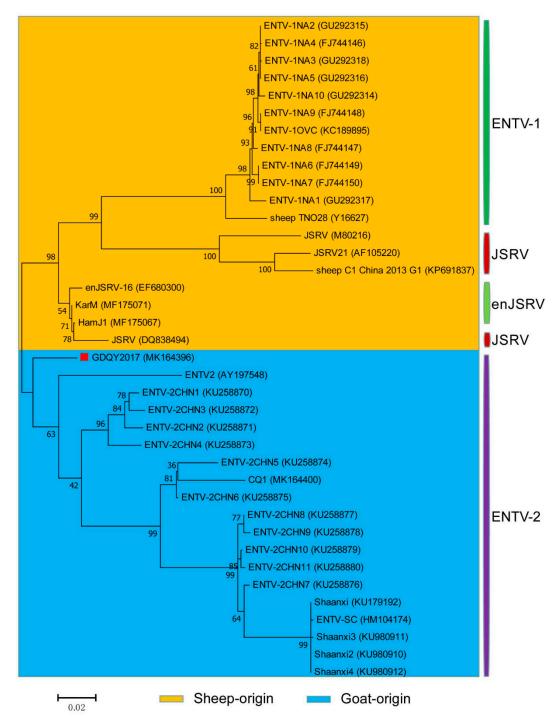


Figure 8. Phylogenetic analysis based on the *env* gene of ENTV-2 with ENTV-1 and JSRV. GDQY2017 described in this study was marked with the red square.

One previous study showed that an unusually high degree of genetic stability (>96%) existed in the genome of ENTV-1 isolated from North America and Europe [15]. However, JSRV isolates had lower genetic similarity (89.9–95.7%) than ENTV-1 isolates, showing three main variable regions (VRs) (VR1 and VR2 in *gag* genes, and VR3 in *env* gene) [16–18]. In this study, the genome similarity of ENTV-2 varied from 87.1% to 99.8%, indicating that ENTV-2 had higher genetic diversity than

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ENTV-1 and JSRV. Notably, we identified a novel ENTV-2 (GDQY2017) with gene insertion and deletion among the genome sequence that is significantly different from previously reported ENTV-2 strains. Although GDQY2017 is similar to the ENTV-2CHN1 and the Chinese JSRV sequence (DQ838494) and enJSRV sequence, it had the same six-amino acid deletion in the *env* gene, and ENTV-2CHN1 had an additional amino acid (N) insertion in the *env* gene (Figure 6). Other than previous ENTV-2 sequences, GDQY2017 had a higher identity (92.2%) to a Chinese JSRV sequence (DQ838494) and an endogenous JSRV sequence (enJSRV-1, EF680311) than other JSRV and ENTV-1 sequences (87.6–88.4%). Interestingly, some sequences of GDQY2017 were similar or identical to certain sequences of the Chinese JSRV sequence (DQ838494) and enJSRV-1 (EF680311) (Figures 3 and 4). Surprisingly, most of the indel points in the GDQY2017 genome located in VR regions of the JSRV genome [16–18]. All the genome features above indicated that GDQY2017 was a novel ENTV-2 isolate with unique features among JSRV and enJSRV.

In this study, five pairs of overlapping primers were used to amplify the genome of ENTV-2 and derived five PCR products were cloned and sequenced. Due to high sequence similarity between both exogenous and endogenous viruses, it is still possible that some of determined sequences may come from endogenous viral sequences. Further experiment is needed towards amplifying and sequencing the full genome-size clone or large PCR products so any possible components of the endogenous viruses would be excluded.

Generally, ENTV-1 and JSRV only infected sheep, while ENTV-2 only infected goats. But, one previous study reported that the experimental goats could be infected with JSRV and developed lung tumors [19], which improve our understanding of retrovirus in sheep and goats, and revealed that JSRV could break across the species barriers. In China, goats carrying ENTV-2 and sheep carrying JSRV were raised together on some farms. The feeding mode might potentially promote the emergence of the chimeric virus. In this study, GDQY2017 had certain identical or similar gene sequences with the Chinese JSRV sequence (DQ838494), which further supports our speculation. As far as we checked, any clinical lesions in the lungs of the affected goats were not observed (data not shown). In subsequent studies, relevant animal experiments should be made to verify this hypothesis.

Usually, ENTV-2 infections occur in goats older than six-month-old [6,20,21]. However, in this study, we found that younger goats (about 3-month old) could be also infected with ENTV-2 and showed tumor-like lesions, suggesting that the ENTV-2 strain could cause acute infection in goats, which is similar to the acute infection in lambs caused by ENTV-1 [5]. Generally, tumor formation and growth require a certain amount of time. The occurrence of tumors in low-age lambs or goats revealed a possibility of vertical transmission. As the age rose, the number and size of tumors increased in goats (Figure 1). Due to the lack of in vitro cell lines, it is necessary to construct an infectious clone according to the infectious clone strategy of ENTV-1, which may help identify the pathogenicity of the novel ENTV-2 isolate in goats [22].

4. Conclusions

In summary, we identified the first case of ENTV-2 on a commercial goat farm in Guangdong province of southern China. Our data could help understand the epidemiology and genetic diversity of ENTV-2. The epidemic significance and potential pathogenicity of ENTV-2 in the goat population need to be further investigated.

Author Contributions: Conceptualization, S.-L.Z. and H.Z.; methodology, S.-L.Z. and H.Z.; software, S.-L.Z.; writing—original draft preparation, S.-L.Z.; writing—review and editing, D.-H.L., Z.-H.X., J.-S.Y., X.-H.W., C.-L.J., X.-R.Z., Q.Z., and P.C.Y.W.; supervision, S.K.P.L., F.L., D.W., and W.-K.W.; project administration, S.-L.Z. and O.-L.C.

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Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Palmarini, M.; Sharp, J.; de las Heras, M.; Fan, H. Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *J. Virol.* **1999**, *73*, 6964–6972. [PubMed]
- 2. Cousens, C.; Minguijon, E.; Dalziel, R.G.; Ortin, A.; Garcia, M.; Park, J.; Gonzalez, L.; Sharp, J.M.; de las Heras, M. Complete sequence of enzootic nasal tumor virus, a retrovirus associated with transmissible intranasal tumors of sheep. *J. Virol.* 1999, 73, 3986–3993. [PubMed]
- 3. Ortín, A.; Cousens, C.; Minguijón, E.; Pascual, Z.; Villarreal, M.P.; Sharp, J.M.; Heras Mde, L. Characterization of enzootic nasal tumour virus of goats: Complete sequence and tissue distribution. *J. Gen. Virol.* 2003, 84, 2245–2252. [CrossRef]
- 4. De las Heras, M.; García de Jalón, J.A.; Minguijón, E.; Gray, E.W.; Dewar, P.; Sharp, J.M. Experimental transmission of enzootic intranasal tumors of goats. *Vet. Pathol.* 1995, 32, 19–23. [CrossRef] [PubMed]
- 5. Walsh, S.R.; Linnerth-Petrik, N.M.; Yu, D.L.; Foster, R.A.; Menzies, P.I.; Diaz-Méndez, A.; Chalmers, H.J.; Wootton, S.K. Experimental transmission of enzootic nasal adenocarcinoma in sheep. *Vet. Res.* **2013**, *44*, 66. [CrossRef]
- 6. Liu, F.X.; Lin, X.; Huang, F.; Hao, X.P.; Zhao, Z.H.; Yu, W.S.; Wang, H.Z. An investigation and pathologic morphological study on tumors of domestic animals in Inner Mongolia. *Vet. Ori.* **1984**, *3*, 15–25. (in Chinese).
- 7. Lei, H.Y.; Su, J.M.; Ning, L.Z.; Kang, Y.; Chen, K.Y.; Zeng, D.N. Investigation and diagnosis of an enzootic nasal tumor of goat. *Prog. Vet. Med.* **2006**, 27, 112–114. (in Chinese).
- 8. Yi, G.; Kaiyu, W.; Qigui, Y.; Zhongqiong, Y.; Yingdong, Y.; Defang, C.; Jinlu, H. Descriptive study of enzootic nasal adenocarcinoma in goats in southwestern China. *Transbound. Emerg. Dis.* **2010**, *57*, 197–200. [CrossRef]
- 9. Liu, F.; Feng, Y.C.; Yan, Q.G.; Han, G.Q. Diagnosis of an enzootic nasal tumor of goat in Sichuan. *Anim. Husb. Vet. Med.* **2011**, *43*, 83–86. (in Chinese).
- 10. Yu, Y.D.; Wei, L.F.; Huang, X.J.; Zhang, B.; Yu, X.H.; Tang, C. Diagnosis of four cases of enzootic nasal adenocarcinoma of goats. *Prog. Vet. Med.* **2014**, *35*, 129–131. (in Chinese).
- 11. Jiang, J.X.; Lin, Y.S.; Jiang, B.; Mao, K.M.; You, W.; Zhang, J.P.; Hu, Q.L. Molecular epidemiology of enzootic nasal tumor virus on goats in Fujian *J. Agr. Sci.* **2017**, *32*, 837–841. (in Chinese).
- 12. Hou, H.; Zhu, D.; Zhang, D.; Hu, X.; Zhao, R.; Dai, Y. Cloning and Analysis of gag Gene of Enzootic Nasal Tumor Virus in Goats. *Chin. Herb. Sci.* **2018**, *38*, 53–55. (in Chinese).
- 13. He, Y.; Zhang, Q.; Wang, J.; Zhou, M.; Fu, M.; Xu, X. Full-length genome sequence analysis of enzootic nasal tumor virus isolated from goats in China. *Virol. J.* **2017**, *14*, 141. [CrossRef] [PubMed]
- 14. Ye, C.; Huang, Q.; Chen, T.; Jiang, J.; Hou, F.; Xu, D.; Peng, Y.; Fang, R.; Chen, J. First detection and genotypic analysis of goat enzootic nasal tumor virus 2 in Chongqing, China. *Arch. Virol.* **2019**, *164*, 1647–1650. [CrossRef] [PubMed]
- 15. Walsh, S.R.; Linnerth-Petrik, N.M.; Laporte, A.N.; Menzies, P.I.; Foster, R.A.; Wootton, S.K. Full-length genome sequence analysis of enzootic nasal tumor virus reveals an unusually high degree of genetic stability. *Virus Res.* **2010**, *151*, 74–87. [CrossRef] [PubMed]
- 16. Hallwirth, C.; Maeda, N.; York, D.; Fan, H. Variable regions 1 and 2 (VR1 and VR2) in JSRV gag are not responsible for the endogenous JSRV particle release defect. *Virus Genes.* **2005**, *30*, 59–68. [CrossRef]
- 17. Mura, M.; Murcia, P.; Caporale, M.; Spencer, T.E.; Nagashima, K.; Rein, A.; Palmarini, M. Late viral interference induced by transdominant Gag of an endogenous retrovirus. *Proc. Natl. Acad. Sci. USA* **2004**, 101, 11117–11122. [CrossRef]
- 18. Palmarini, M.; Maeda, N.; Murgia, C.; De-Fraja, C.; Hofacre, A.; Fan, H. A phosphatidylinositol 3-kinase docking site in the cytoplasmic tail of the Jaagsiekte sheep retrovirus transmembrane protein is essential for envelope-induced transformation of NIH 3T3 cells. *J. Virol.* **2001**, *75*, 11002–11009. [CrossRef]

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19. Caporale, M.; Martineau, H.; De las Heras, M.; Murgia, C.; Huang, R.; Centorame, P.; Di Francesco, G.; Di Gialleonardo, L.; Spencer, T.E.; Griffiths, D.J.; et al. Host species barriers to Jaagsiekte sheep retrovirus replication and carcinogenesis. *J. Virol.* **2013**, *87*, 10752–10762. [CrossRef]

- 20. De las Heras, M.; Garcia de Jalon, J.A.; Sharp, J.M. Pathology of enzootic intranasal tumor in thirty-eight goats. *Vet. Pathol.* **1991**, *28*, 474–481. [CrossRef]
- 21. Vitellozzi, G.; Mughetti, L.; Palmarini, M.; Mandara, M.T.; Mechelli, L.; Sharp, J.M.; Manocchio, I. Enzootic intranasal tumour of goats in Italy. *J. Vet. Med. Ser. B* **1993**, *40*, 459–468. [CrossRef] [PubMed]
- 22. Walsh, S.R.; Gerpe, M.C.; Wootton, S.K. Construction of a molecular clone of ovine enzootic nasal tumor virus. *Virol. J.* **2016**, *13*, 209. [CrossRef] [PubMed]



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