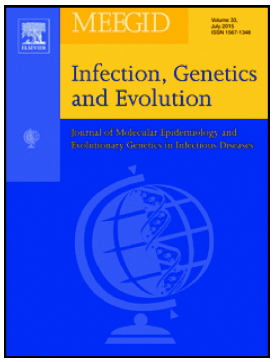

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Epidemiological investigation and genomic characterization of Caprine herpesvirus 1 from goats in China

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Abstract

Caprine herpesvirus 1 (CpHV-1) is a member of the alpha subfamily of herpersviruses, and is responsible for genital lesions and latent infections in goat population worldwide. Here, we describe goats suffered severe respiratory diseases caused by alphaherpesvirus during 2013 to 2014 in Jiangsu province of China. CpHV-1 was detected out by PCR with a prevalence of 21.1% (40/190), among which three novel CpHV-1 strains were firstly identified and isolated in China. Phylogenetic analysis of glycoprotein B (gB) gene revealed that these new viruses were closely clustered with CpHV-1 strain E/CH. The isolate JSHA1405 was further studied by transmission electron microscopy, and displayed typical herpesvirus morphology. Then, for the first time, complete viral genome of JSHA1405 was sequenced by Illumina Hiseq and third-generation sequencing technology. The viral genome is 134,617 bp in length and the genome characteristics were deeply analyzed. 69 open reading frames were predicted and annotated, which was less than that of BoHV-1.

[#] These two authors contribute equally to this work.

Phylogenetic analysis of the complete genome revealed that JSHA1405 was classified into the same branch with previous CpHV-1 strains as well. Moreover, the pathogenicity test is further evidence that JSHA1405 strain induced obvious symptoms of high fever and nasal discharge in infected goats, consistent with clinical manifestations. This is the first report about isolation and identification of CpHV-1 in China and the first characterization of CpHV-1 genome structure. The research also provides a basis for understanding the characteristics, viral genome and pathogenicity of the virus.

Key words: Caprine herpesvirus 1; epidemiology; goats; genome; pathogenicity.

1. Introduction

Herpesviruses are important pathogens to human and animals. They pose severe impact on human health under a number of conditions and cause economic losses to different animal industries. Caprine herpesvirus 1 (CpHV-1) is a member of the order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*, which is genetically and antigenically related to bovine alphaherpesvirus 1 (BoHV-1) (Davison, 2010; Thiry et al., 2006). The virus was firstly isolated from goats in 1974 and being further characterized in 1975 (Berrios et al., 1975; Gonzalez et al., 2017). The symptoms of which was similar to other members of the alpha subfamily of herpersviruses that induced genital lesions and latent infections (Benavides et al., 2015; Camero et al., 2017), however, the pathogenesis of the infection is not yet completely understood. The virus is believed to infect goats through the respiratory or reproductive route, displaying high tropism to the genital tract (Tempesta et al., 2001; Tempesta et al., 2004). Evidences proved that CpHV-1 causes systemic disease in young kids with high morbidity and mortality, while in infected adult goats causes vulvovaginitis, balanoposthitis, respiratory disease and occasionally abortions (Gonzalez et al., 2017; Grewal and Wells, 1986; Horner et al., 1982; Suavet et al., 2016; Tarigan et al., 1987). Emerging evidence showed that CpHV-1 could infect varied human cell lines and provide a potential candidate for oncolytic virotherapy (Montagnaro et al., 2019). Partial genome sequences had been deposited in GenBank, but the whole viral genome sequence and the genome structure were not identified and analyzed. CpHV-1 was reported in goats globally, including USA, Australia, New Zealand, Canada, Brazil and Mediterranean countries (such as Greece, Italy, Spain, France, etc.) (Camero et al., 2017; Chenier et al., 2004; Gonzalez et al., 2017; Suavet et al., 2016). However, no CpHV-1 infection had been reported in China until now.

From 2013 to 2014, goats in Jiangsu of China suffered severe respiratory diseases with high morbidity and varied mortality. Pathogens detection and isolation was conducted to clarify the causative agents. Three different CpHV-1 strains were

identified from the clinical samples from 13 farms, and in our study, for the first time, the full genome sequence of JSHA1405 strain was characterized, and the pathogenicity of the CpHV-1 JSHA1405 in goats was evaluated.

2. Materials and Methods

2.1 Samples

From Oct 2013 to Nov 2014, goats (3-6 months) of many farms in Jiangsu of China suffered severe respiratory diseases, showed respiratory syndromes in different degree, including coughing, nasal discharges, dyspnea, and anorexia emaciation, and displaying high morbidity and varied mortality. 190 nasal swab (n=99) and serum (n=91) samples were collected from 13 goat farms in Jiangsu province. All samples were divided into two parts, one for virus isolation, and the other for PCR or RT-PCR detection.

2.2 Virus isolation

Virus isolation was performed on Madin Darby bovine kidney (MDBK) cells as the procedure previously described (Li et al., 2014). Briefly, sera/nasal swab samples were centrifuged at 12,000 rpm for 20 min, the supernatants were filtered through 0.22 mm filter (Millipore) and inoculated onto MDBK cell monolayers cultured in 24-well cell culture plates. Subsequently, the cell cultures were observed for cytopathic effect (CPE) for 3–5 days, and then harvested and passaged on MDBK cells blindly for 3 more times. The cell cultures were store at -80℃ for the future analysis.

2.4 PCR and RT-PCR

Total RNA and DNA was extracted from seral and nasal swabs by viral RNA/DNA purification kit (Axygen, China) according to the manufacturer's instructions. To identify the virus from clinical samples and cell cultures, RT-PCR was employed to amplify the target gene of pestivirus (BVDV, BDV), PPRV, BTV, CPIV3 and RSV as previously described (Li et al., 2014; 2016; Mao et al., 2015;2016; 2017). PCR was performed with primers specific for the glycoprotein B gene of the ruminant alphaherpesviruses and the expected size was 444 bp (Ros and Belak, 1999). The

amplicons were recovered from the agarose using the Axygen gel extraction kit (Axygen, China). Then, the purified products were directly sequenced by Genscript (Nanjing) Co., Ltd. Sequences were further compared to existing sequences in databases using the BLAST analysis.

2.5 Transmission electron microscopy (TEM)

To observe the morphology characteristics of the virus, the virus particles in cell cultures were isolated by differential centrifugation. In brief, 200 mL infected cell culture were harvested at 48 hours post infection (hpi) and centrifuged at 10,000 × *g* for 30 min to remove the cells and debris, the supernatants were centrifugated at 100,000 × *g* for 2 h (70Ti rotor, Beckman) to harvest the virions, followed by one wash with 20 mL phosphate-buffered saline (PBS) and resuspended in 1 mL PBS. The virions were subsequently spotted onto formvar-carbon-coated grids (200 meshes) and fixed with 2% (vol/vol) paraformaldehyde for 5 min at room temperature. Then, the viruses were directly negatively stained using phosphotungstic acid (PTA) and the grids were observed by electron microscope (H-7650, HITACHI) at 80 kV. In addition, the CpHV-1 infected MDBK cells were fixed, sliced into ultraslices to observe the viral morphology and locations in the cells through TEM study.

2.6 The complete genome sequencing

The complete viral genome was sequenced by Illumina Hiseq combining with the third-generation sequencing technology. Sequencing data was analyzed and corrected with SOAPdenovo (v2.04) and blasR alignment. The genome scaffold was constructed based on the overlapping sequencing data and further assembled by Celera Assembler 8.0. To understand the functions of genome, we used *ab initio* prediction method to get gene models for strain CpHV-1 JSHA1405. Gene models were identified using GeneMark. Then all gene models were blastp against non-redundant (NR in NCBI) database, SwissProt (http://uniprot.org), KEGG (http://www.genome.jp/kegg/), and COG (http://www.ncbi.nlm.nih.gov/COG) to do functional annotation by blastp module. In addition, tRNA were identified using the tRNAscan-SE (v1.23, http://lowelab.ucsc.edu/tRNAscan-SE) and rRNA were determined using the RNAmmer (v1.2, http://www.cbs.dtu.dk/services/RNAmmer/),

respectively.

2.7 Phylogenetic analysis

The nucleotide regions of glycoprotein B (gB) gene and complete genome were aligned with CLUSTAL W program. Molecular Evolutionary Genetics Analysis version 6 (MAGE6) was used for phylogeny inference according to the neighbor-joining criterion. The robustness of the hypothesis was test with 1000 nonparametric bootstrap analyses.

2.8 The pathogenicity experiments

To analyze the pathogenicity of the novel virus, ten goats (3-4 months old) were randomly divided into two groups of five each and housed separately. Group CC was intramuscularly inoculated with JSHA1405 (3 mL/goat, $10^{7.5}$ $TCID_{50}$/mL). Group NC was inoculated with PBS and used as negative control. After challenge, all animals were monitored for 35 days. Rectal temperatures and clinical signs (respiratory signs) of all animals were examined and recorded daily. The serum samples and swabs (both nasal and rectal swabs) were collected at 0, 1, 3, 5, 7, 9, 12, 14, 16, 19, 21, 28 and 35 days post infection (dpi), all samples were subjected to TaqMan-based qRT-PCR (Elia et al., 2008). Serum samples of 0, 7, 14, 21, 28 and 35 dpi were performed to determine neutralizing antibodies (NAs) level against CpHV-1 by virus neutralization test (VNT).

2.9 Virus neutralization test

MDBK cells were seeded in 96-well plates with the presence of 200 $TCID_{50}$ CpHV-1 and 2-fold dilution series of sera. The cytopathic effect was observed at 48 hours post seeding. The neutralization titer was determined as the reciprocal of the highest dilution that inhibited formation of cytopathic effect. This assay was repeated 3 times.

2.10 Ethics approval and consent to participate

The owners consented to the use and disclosure of questionnaire data for the current study. All experimental procedures were approved by the Ethical and Animal Welfare Committee of the Jiangsu Academy of Agricultural Sciences (Reference 189).

3. Results and Discussion

In order to explore the cause of the severe respiratory disease in fattened goat herds in Jiangsu province during 2013 to 2014, virus isolation was done firstly. MDBK cells were inoculated with the 190 nasal swab (n=99) /serum (n=91) samples. Caprine parainfluenza virus 3 (CPIV3) was detected from 26.3% (50/190, Table 1) samples, and two CPIV3 stains designated as JS2013 and JSHA2014 with CPE on MDBK cells were previously isolated from nasal swab samples (Li et al., 2014; Yang et al., 2016). Eight other unknown viruses showing CPE were isolated from nasal swabs originated from eight farms.

The unknown viruses were identified by RT-PCR and the results showed it is negative for pestivirus (BVDV, BDV), PPRV, BTV, CPIV3 and RSV. Interestingly, the amplification of a 444 bp product was shown by using the alphaherpesviruses glycoprotein B (gB) universal primers. Further sequencing and BLAST analysis revealed that these unknown viruses performed the highest homology with CpHV-1. Based on this result, the 190 samples from 13 farms collected in 2013-2014 were re-detected by alphaherpesviruses-specific PCR. As shown in Table 1, 21.1% (40/190) of the samples and 61.5% (8/13) of the farms were positive for CpHV-1. According to the sequence information and BLAST analysis, the eight isolates were designated as three stains (named as JSHA1401, JSHA1405 and JSNJ1401). Two of them were isolated from Nantong and one from Nanjing (Table 1).

Phylogenetic analysis of the glycoprotein B genes with other ruminant representative alphaherpesviruses was conduct using the Neighbor-Joining method with MEGA6. The glycoprotein B (gB) gene of JSHA1401, JSHA1405 and JSNJ1401 shared the highest nucleotide identity (99.2-99.3%) with CpHV-1 E/CH strain, and shared 84.77%-85.13% identity with BoHV-1. The phylogenetic tree showed that the isolates closely clustered with CpHV-1 strain E/CH (Figure 1A). Because of the closed relationship between CpHV-1 and BoHV-1, the pathogenicity of CpHV-1 was reported very similar to BoHV-1 infection in calves (Engels and Ackermann, 1996). Previous reports indicated that CpHV-1 infection was observed worldwide, e.g., in the USA, Canada, New Zealand and Mediterranean countries such as Italy, Spain, France

and Greece. Up to now, CpHV-1 has not been recorded in Asian countries. BoHV-1 is able to replicate and establish latent infection in goats; and it has also been reported that CpHV-1 replicated efficiently in experimentally infected calves during acute infection and established latent infection, which support the ability of these viruses to cross-infect the respective heterologous hosts (Camero et al., 2019; Engels et al., 1992; Martins et al., 2019; Six et al., 2001). Epidemiology investigation and meta-analysis showed that BoHV-1 was highly prevalent in Chinese cattle herds (Chen et al., 2018; Yan et al., 2008). But the existence of CpHV-1 in Chinese cattle remains uncertain and need further investigation. In addition, viruses like BVDV and Hobi-like virus had been frequently detected in commercial calf serum and caused incidence of contamination of cell cultures and vaccines, which may result in the infection of inoculated animals (Falcone et al., 2003; Giammarioli et al., 2015; Silveira et al., 2017; Uryvaev et al., 2012). Considering that the potential contact of goats and cattle under clinical conditions and the widely usage of calf serum in biological products in China, the cross-species infection of the goats by virus infected cattle or virus contaminated biological products might be the potential source of CpHV-1 infections in Chinese goat herds.

Of the 190 samples, 91 serum samples were further tested with VNT and 35 samples were positive (NA titers >4, Table 1), showing a higher positive rate (35/91) than PCR detection in nasal swab and serum samples (24/99 and 16/91, Table 1). Respiratory diseases are multifactorial and there are multiple etiological agents responsible for the respiratory disease complex, viral infection plus stress conditions and other secondary or co-infections usually resulted in severe clinical observations. In the 13 farms, 46.2% (6/13) were co-infected by CPIV3 and CpHV-1 (Table 1). The results of our study implied the high prevalence in goat farms and the potential role of CpHV-1 in clinical disease.

To identify the virion morphology, ultracentrifugation purified CpHV-1 JSHA1405 strain was examined by TEM and representative herpesvirus morphology was observed. The viral particles are circular and composed of two parts, the core and the envelope. The inner part of the virion consists of a core containing the viral

genome, protected by an icosahedral nucleocapsid of 100 nm (Figure 2A, 2B, 2C). A number of virions were gathered and arranged orderly in JSHA1405 infected MDBK cells (Figure 2D, red circle). Circular or oval capsids with an annular protein structure, empty capsids, and DNA-containing nucleocapsids aggregated and arrayed as pseudocrystals were present in intranuclear (Figure 2D, red circle). In addition, mature virions and immature virions were found simultaneously in the same infected cell. In contrast to immature virions (Figure 2-E, blue arrows), mature virions (Figure 2-E, red arrows) contain multilayer structure, including nucleocapsid, tegument and envelope. After cytolysis, the released virus could be found in the infected cells (Figure 2-F, red circle). The complete viral genome of JSHA1405 was obtained as 134,617 bp in length, with an average G+C content of 74.16%, and deposited in GenBank under accession number MG939243. The virus was deposited in China center for type culture collection (CCTCC) under accession number V201745. Phylogenetic tree based on the complete genome sequences of twelve alphaherpesvirus strains indicated the JSHA1405 was differ from BoHV-1 strains and formed a distinct branch (Figure 1B). The potential genes encoded by the viral genome were predicted with Glimmer 3.02 software (http://www.cbcb.umd.edu/software/glimmer/) and the genome map was drawn by Geneious (v11.1.4). As shown in Figure 3A, it consists of an arrangement of a 95,156 bp of long unique unit (UL) and a 9,087 bp of short unique unit (US) flanked by two inverted repeat sequences, named internal repeat (IR) and terminal repeat (TR). As shown in Table S1, there are 69 predicted genes (open reading frames, ORFs) in CpHV-1 genome, of which 38 are positive strand and 31 are negative strand. A few of the genes are overlapped with each other. Compared to BoHV-1, three ORFs (UL26.5、UL0.7、US1.67) were missing in CpHV-1 genome. Similar to other alphaherpesvirus, the UL region of the CpHV-1 genome encoded 57 ORFs and the US region encoded seven putative genes (US2 to US4 and US6 to US9) (Table S1). Both of IR and TR regions contain two regulatory genes, US1 (also named ICP22) and ICP4. The circle graph of the CpHV1-JSHA1405 genome was drew by Circos v0.64 based on the sequencing results. As shown in Figure 3B, the predicted CDS of the

positive and negative chains were annotated as the different functional classification of cluster of orthologous groups (COGs), mainly including virion, virion part, binding, catalytic activity, cellular component organization, structural molecular activity. No rRNA or tRNA was found to be encoded by the genome as analyzed by RNAmmer-1.2 and tRNAscan-SE v1.3.1 software. In three regions, the G+C content was obviously higher than that in the whole genome (average level).

The pathogenicity test in goats indicated that JSHA1405 induced high fever (peaked at 41.8℃) and lasted for about one week. Other symptoms including depression and nasal discharge were observed simultaneously (Table 2). Virus shedding could be detected from nasal and rectal swabs during 3-12 dpi and 3-9 dpi, respectively (Table 2). Similarly, bubaline alphaherpesvirus 1 (BuHV-1) is able to infected goats via intranasal route, a slight transient increase in temperature and virus shedding was observed in half of the infected animals (Camero et al., 2017). Neutralizing antibodies appeared from 7 dpi (1:16-1:32) and peaked at 28 dpi (1:512-1:1024). These results reflected the pathogenicity of CpHV-1 in infected goats, which might contribute to the clinical diseases. Generally, the pathogenesis of acute CpHV-1 infection in goats is similar to that of BoHV-1 in calves (Engels and Ackermann, 1996). Natural CpHV-1 infections were associated with systemic disease and high mortality in young kids (Roperto et al., 2000), while induced reproductive disorders, abortions or subclinical infection in adult goats (Chenier et al., 2004; Gonzalez et al., 2017; Piper et al., 2008; Tempesta et al., 2004). However, in our study, no clinical sign of genital pathology was observed. Furthermore, the three CpHV-1 strains were identified and isolated from goats with respiratory disease. It is interesting to found that in the study of Suavet et al.(Suavet et al., 2016), nasal swabs instead of vaginal swabs were positive by PCR detection. Since the goats in clinical diseases and experimental infection were less than 6 months, short-term breeding might limit the appearance of reproductive disorders and abortions. The results present in this study might broaden the tissue tropism and pathogenesis of CpHV-1. To our knowledge, this is the first description of respiratory disease caused by CpHV-1 worldwide. CpHV-1 should be considered as the novel infectious factor

responsible for respiratory disease in goats, and serological and etiological examination would be necessary to know the prevalence state of the infection, especially in the regions with a large goat population.

4. Conclusion

In conclusion, this is the first report about isolation, identification, pathogenicity and epidemiology study of CpHV-1 in Chinese goat herds. This finding suggests the pathogen complexity of respiratory disease in fattened goat herds during 2013 to 2014 and CpHV-1 might be a new potential threat for goat populations. And for the first time, the complete viral genome was sequenced by powerful sequencing technology. The genome structure and genes/proteins were predicted based on the sequencing results. These results will facilitate the epidemiology, pathogenesis and protein functional study of CpHV-1 in the future.

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Conflict of Interest Statement

None.

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Figure legend

Figure 1. Phylogenetic analysis based on the glycoprotein B (gB) regions (A) and complete genome sequences (B) by the neighbor-joining (NJ) method with the sequences available in GenBank. The numbers at the phylogenetic branches indicated the bootstrap values (1000 replicates) in percentage supporting each group. The bar represents the genetic distance.

Figure 2. Virion morphology was observed by transmission electron microscopy using ultracentrifugation purified virions. A, B, C: The morphology of viral particles; D: The red circle showed the DNA-containing nucleocapsids aggregated and arrayed as pseudocrystals presented in intranuclear; E: The virions in the infected cells, the red and blue arrows represented the mature and immature virions, respectively; F: The released virions in the extracellular space, the red circle showed the orderly gathered and arranged released virions.

Figure 3. The genome characteristics of CpHV-1. A) Map of the features of the CpHV1-JSHA1405 (MG989243) genome. The map was drawn by Geneious (v11.1.4). B) The circle graph of the CpHV1-JSHA1405 genome drawn by Circos v0.64. From outside to inside, the outermost circle indicates the genome size, the scale is 0.5Mb. The second and third circles are respectively the CDS of the positive and negative chains, and the different colors indicate the different functional classification of COGs. The fourth circle shows GC content. Thereinto, the outward red part indicates that the GC content in this region is higher than that in the whole genome. The higher the peak value, the greater the difference from the average GC content is. Likewise, the inward blue peak indicates the GC content which is lower than the whole genome average GC content. The higher the peak value indicates the greater the difference from the average GC content. The innermost circle indicates the GC skew value. The specific algorithm is G-C/G+C. When the value is positive, the positive chain is more inclined to transcribe CDS, and when it

is negative, the negative chain is more inclined to be transcribed.

Author Contributions

FH and LM performed sampling, virus isolation, purification, identification, sequencing analysis and animal experiments. WLL designed and participated in sampling, virus identification, sequencing and animal experiments. JZL, LLY and WWZ participated in animal experiments and PCR detection. FH, LM, WLL, MS and XX analyzed the data. FH, LM and WLL wrote and revised the manuscript. MJL and JYJ help the experiment design and draft the manuscript . All authors read and approved the final manuscript.

Conflict of Interest Statement

None.

Table 1 Summary of the sampling and detection results.

Farm	Location	Collection date	No. positive of CPIV3 by RT-PCR / total		No. positive of CpHV-1 by PCR / total		CpHV-1 VNT/ total	CpHV-1 strain
			Nasal swabs	Sera	Nasal swabs	Sera	Sera	
A	Nantong	Oct-2013	0	1/5	0	0/5	0/5	/
B	Nantong	Oct-2013	0	6/15	0	3/15	10/15	JSHA1401
C	Nantong	Oct-2013	0	1/5	0	0/5	0/5	/
D	Nantong	Nov-2013	2/9	0	0/9	0	0	/
E	Nantong	Nov-2013	4/5	0	1/5	0	0	JSHA1401
F	Nantong	Jan-2014	13/13	/	5/13	0	0	JSHA1401
G	Nanjing	Feb-2014	4/6	4/6	3/6	2/6	4/6	JSNJ1401
H	Nanjing	Mar-2014	1/	1/1	0/2	0/1	0/1	/
I	Nanjing	Apr-2014	0/11	0/11	5/11	3/11	7/11	JSNJ1401
J	Nantong	May-2014	6/11	3/11	3/11	2/11	6/11	JSHA1405
K	Nantong	May-2014	0/9	0/9	0/9	0/9	0/9	/
L	Nantong	Sep-2014	4/10	0/10	6/10	5/10	8/10	JSHA1405
M	Nantong	Nov-2014	0/18	0/18	1/18	1/18	0/18	JSHA1405
Total			34/99	16/91	24/99	16/91	35/91	

Table 2 Clinical signs, virus shedding and neutralizing antibody detection at different time points post-infection.

Group/No.		nasal discharges (dpi) [a]							Virus shedding (nasal/rectal swabs) (dpi)							neutralizing antibody(log_2) (dpi)					
		0	3	5	7	9	12	14	0	3	5	7	9	12	14	0	7	14	21	28	35
NC	11	-	-	-	-	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	0	0	0	0	0	0
	12	-	-	-	-	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	0	0	0	0	0	0
	13	-	-	-	-	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	0	0	0	0	0	0
	14	-	-	-	-	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	0	0	0	0	0	0
	15	-	-	-	-	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	0	0	0	0	0	0
CC	21	-	+	++	++	++	+	-	-/-	29.32[b]/30.62	28.3/33.6	31.12/34.69	32.32/-	-/-	-/-	0	5	8	10	10	10
	22	-	-	+	++	++	++	-	-/-	31.55/-	29.67/34.3	32.56/-	34.69/-	-/-	-/-	0	4	6	9	9	9
	23	-	-	++	++	+	+	-	-/-	25.03/31.55	18.56/23.99	23.99/28.12	29.51/33.21	33.8/-	-/-	0	5	8	9	9	9
	24	-	+	++	++	++	+	-	-/-	25.62/-	20.14/26.66	24.96/30.98	30.52/33.26	33.97/-	-/-	0	5	8	10	10	10
	25	-	+	+	++	++	+	-	-/-	28.89/33.03	23.12/28.7	29.63/32.9	31.31/34.62	-/-	-/-	0	4	7	9	10	10

[a] The clinical signs were evaluated as −, + and ++.
[b] Ct values determined by qRT-PCR.

Highlights

Epidemiological investigation of caprine herpesvirus 1 in goats of Jiangsu province was carried out, and this is the first clinical report in China.

The genomic characteristics of caprine herpesvirus 1 was deeply analyzed.

Pathogenicity test showed caprine herpesvirus 1 was one of the pathogens causing respiratory diseases.