Genome analysis of Getah virus (GETV) isolated from and contaminated in a live swine vaccine: The commercial live vaccines might be a potential route for GETV transmission

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Abstract

GETV cause fetus death and reproductive disorders in pigs. In this study, a GETV strain, named GETV-V1, was isolated from a commercial modified live vaccine (MLV) against porcine reproductive and respiratory syndrome virus (PRRSV), which is widely used on pigs in China. Further results showed that nine batches of MLV vaccine (three batches per year) from the same manufacturer between 2015 and 2017 were all positive for GETV. We then further characterized the GETV-V1 strain and performed phylogenetic analysis, revealing that the GETV strains circulating in China are genetically diverse and providing a potential platform for evolution. Therefore, this research firstly reported the contamination of GETV in live attenuated PRRS vaccines in China, implying that monitoring of exogenous virus in live vaccines for pigs needs to be improved.

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Running Head: Getah virus (GETV) isolated from a vaccine

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To the Editor,

Getah virus (GETV) belongs to the genus *Alphavirus* within the family *Togaviridae* and is a mosquito-transmitted arbovirus(Berge et al., 1975) that can infect many vertebrates and arthropods(Doherty et al., 1966). The first GETV strain was isolated from Culex in Malaysia in 1955(Go et al., 2014). The prototype virus strain was MM2021(Elisberg et al., 1963). Serological survey of GETV showed that GETV antibodies have been identified in humans, pigs, cattle, horses, goats, rabbits, kangaroos, chickens, foxes and some wild birds in many countries within Europe, Asia and Oceania. GETV can cause reproduction disorders and fetal death in pigs, as well as body rash, leg edema and fever in horses. The potential risk to animal health posed by GETV must not be overlooked(Li et al., 1992; Kurogi et al., 1975; Doherty et al., 1972; Tajima et al., 2014; Yago et al., 1987; Fukunaga et al., 2000)

The first GETV strain in China was isolated from mosquitoes in Hainan province in 1964. Since then, GETVs have been isolated from mosquitoes in more than 10 provinces in China, including Hainan, Shanghai, Sichuan, Yunnan, Hubei and Gansu(Yang et al., 1984; Li et al., 2017; Li et al., 2017; Li et al., 1992).

Porcine reproductive and respiratory syndrome virus (PRRSV) and GETV are different but common viruses associated with reproduction disorders in sows, and both are capable of growing in Marc-145 cells. PRRSV modified live vaccine (MLV) is widely used in China. In October 2017, in the titer test of a PRRSV MLV sample from a pig farm where abortion in a pregnant sow occurred, we found that the caused cytopathic effect (CPE) by the supernatant of vaccine in Marc-145 cells was significantly different from that of the previously vaccinated PRRSV strain. Similarly, unusual CPE was also observed in other cell lines, including Vero, PK-15, BHK-21 and human hepatocellular carcinoma HepG-2 cells. Therefore, exogenous virus was detected by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers, demonstrating positivity for GETV. Because the pig farm had also been vaccinated against classical swine fever virus, Japanese encephalitis virus, porcine parvovirus and PRRSV. We suspected that GETV might be a contaminant in these commercial vaccines. As expected, a GETV strain was isolated from the commercial PRRS MLV vaccine following isolation, passage, purification by plaque assay and observation by transmission electron microscopy. This virus was designated as GETV-V1. In this study, we characterized the isolated GETV strain and the genome data of this newly identified GETV strain.

Further RT-PCR results showed that the commercial PRRS MLV vaccine was positive for GETV, including nine batches of vaccine (three batches per year) from the same manufacturer between 2015 and 2017. Immunofluorescence assay (Figure 1) showed that Marc-145 cells infected with the GETV isolate reacted specifically with GETV-specific monoclonal antibody (produced by our laboratory). The fairly uniform morphological appearance of GETV indicated that the diameter of the virus was 60–80 nm and close to spherical in shape. The fiber protein was clearly visible on the membrane (Figure 2). These data demonstrated that GETV-V1 was isolated from a contaminated live swine vaccine.

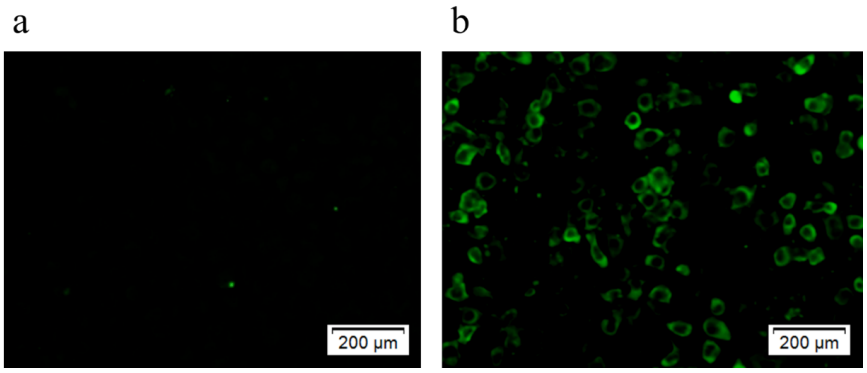

Figure 1 Cells infected with the isolate showed a strong reaction to GETV C protein-specific polyclonal antibodies by indirect immunofluorescence. Marc-145 cells were infected with (b) or without (a) GETV isolate, then IFA were performed.

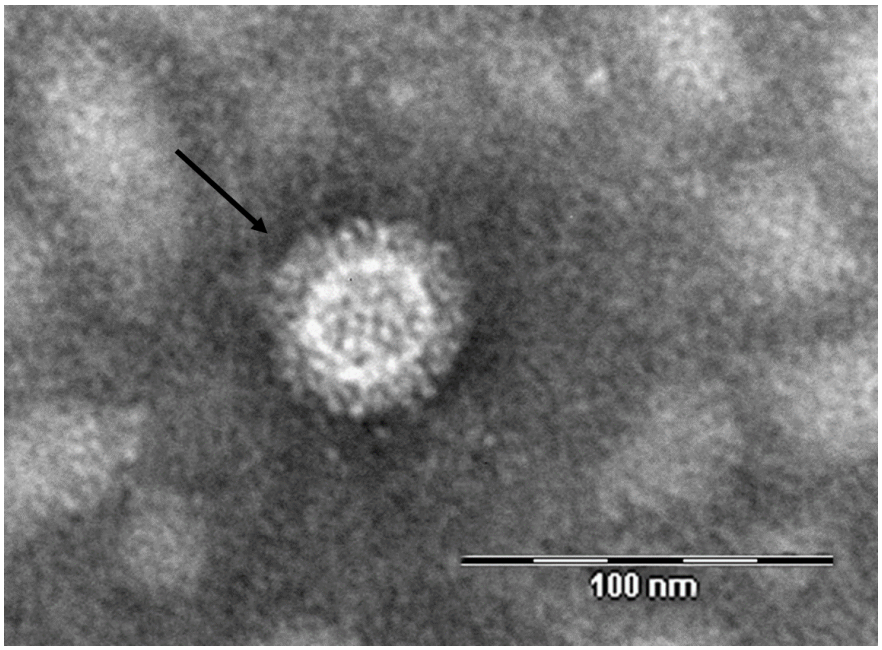

Figure 2 GETV-V1 particle (black arrow) revealed by transmission electron microscopy (scale bar = 100 nm)

Excluding the poly(A) tail, the complete genome sequence of GETV-V1 was 11,689 nucleotides (nt) in length with a 5'-UTR of 78 nt and 3'-UTR of 402 nt (Table 1). The genome was similar to that of previously reported GETV strains. Sequence alignment of GETV-V1 with all GETV sequences available in GenBank revealed that GETV-V1 shared the highest identity (98.9%) with 16-I-674 (isolated from a horse in Japan in 2016), 98.1% identity with Chinese strains JL1707, JL1708, 12IH26 and Japanese strain 14-I-605-C2. Phylogenetic analysis showed that GETV-V1, together with HB0234, JL1707, JL1708, HNJZ-S1, HNJZ-S2 strains, was clustered into the Japanese GETV branch (Fig.3). Interestingly, although the Chinese reference strains were close to GETV-V1 geographically and temporally, they were phylogenetically much closer to each other, compared with GETV-V1. These results revealed that the GETV strains circulating in China are genetically diverse, thus providing a potential platform for evolution.

Table 1 Composition of genome and the encoded proteins of GETV-V1 strain

Nucleotide	Nucleotide	Protein	Location	Length/nt	Length/aa
5'UTR	/	/	1-78	78	/
Non-structural protein orf	Non-structural protein orf	NSP1234	79-7482	7404	2468
Junction	Junction	/	7483-7526	44	/
structural protein orf	structural protein orf	C-E3-E2-6K-E1	7527-11288	3762	1254
3'UTR	3'UTR	/	11289-11690	402	/

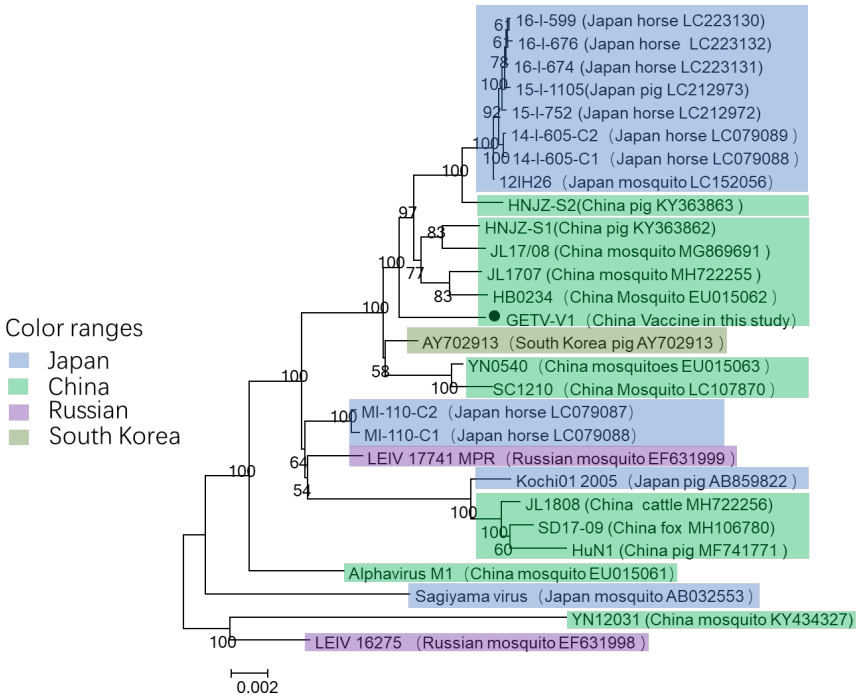

Figure 3 Phylogenetic analysis based on the nucleotide sequences corresponding to the complete genome

Note: GETV isolated in the present study is indicated by *; Bootstrap values (based on 1000 replicates) for each node are given if >60%.

Analysis using MegAlign software showed that GETV-V1 had 36 unique nucleotide mutations in its genome, including 35 nucleotide substitutions and 1 nucleotide insertion (Table 2). Interestingly, only 15 nucleotide mutations resulted in amino acid substitutions. These mutations with the nucleotide insertion in the 3' UTR of the genome could be a unique hallmark of GETV-V1.

Table 2 Comparison of genomic sequences of GETV-V1 strain with reference strains

Gene	position	Mutation (nt/aa)	Gene	position	Mutation (nt/aa)
Nsp1	821	C-T/T-I	Nsp4	6450	T-C/-
	975	A-G/-		6687	C-T/-
	1200	C-T/-		7230	G-A/-
Nsp2	2096	A-T/K-M	C	7715	T-C/M-T
	2100	A-G/-		8081	C-T/T-I
	3078	G-A/-	E3	8876	A-G/Q-R
	3168	A-G/-	E2	9050	A-G/Q-R
	3171	T-A/D-E		9302	G-A/-
	3332	G-A/R-K		9368	G-A/-
Nsp3	4272	C-T/-		9665	G-A/C-Y
	4456	T-C/-	E1	10265	T-A/L-Q
	5415	T-C/-		10277	G-A/S-N
	5517	G-A/-		10289	A-G/H-R
	5586	C-T/-		10292	C-T/T-M
	5613	C-T/-		10838	C-T/A-V
Nsp4	5976	G-A/-		10931	C-T/S-L

Gene	position	Mutation (nt/aa)	Gene	position	Mutation (nt/aa)
	6072	C-T/-	3'UTR	11371	C-T
	6327	C-T/-		11536	A*

Note "-" represents no variation, and "*" represents insertion.

In summary, we isolated and characterized one GETV strain from a commercial live pig vaccine produced in China. Complete genome analysis showed that GETV-V1 share high identity with GETV isolates HNJZ-S1, 14-I-605-C1 and 16-I-599, and these strains are pathogenic to pigs or horses(Nemoto et al., 2016). This study reported for the first time contamination with GETV in live attenuated PRRS vaccines in China, implying that monitoring of exogenous virus in live vaccines for pigs needs to be improved. Furthermore, we should also pay more attention to the potential public health effect of vaccines contaminated with this virus.

Acknowledgements

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Conflict of interest statement

The authors declare no conflict of interest.

Ethics Statements

All the sample collections were performed after obtaining verbal approval of farm owner. This study was approved by the Ethics Review Committee of the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention. All the animal studies were conducted in accordance with the principles of Henan agricultural university Animal Care and Use Committee (IACUC).

Data Availability Statement

All data generated or analyzed during this study are included in the article.

References

Berge, T. O. (1975). International catalogue of arboviruses, including certain other viruses of vertebrates.

Go, Y. Y., Balasuriya, U. B., & Lee, C. K. (2014). Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. Clinical and experimental vaccine research, 3(1), 58-77.

Elisberg, B. L., & Buescher, E. L. (1963). Arbovirus catalogue card No. 47. Arbovirus Information Exchange.

Doherty, R. L., Gorman, B. M., Whitehead, R. H., & Carley, J. G. (1966). Studies of Arthropod-borne Virus Infections in Queensland: V. Survey of Antibodies to Group A Arboviruses in Man and other Animals. Australian Journal of Experimental Biology and Medical Science, 44(4), 365-378.

Li, X. D., Qiu, F. X., Yang, H., Rao, Y. N., & Calisher, C. H. (1992). Isolation of Getah virus from mosquitos collected on Hainan Island, China, and results of a serosurvey. The Southeast Asian journal of tropical medicine and public health, 23(4), 730-734.

Kurogi, H., Inaba, Y., Goto, Y., Miura, Y., Takahashi, H., Sato, K., ... & Matumoto, M. (1975). Serologic evidence for etiologic role of Akabane virus in epizootic abortion-arthrogryposis-hydranencephaly in cattle in Japan, 1972–1974. Archives of virology, 47(1), 71-83.

Doherty, R. L. (1972). Arboviruses of Australia. Australian veterinary journal, 48(4), 172-180.

Tajima, S., Kotaki, A., Yagasaki, K., Taniwaki, T., Moi, M. L., Nakayama, E., ... & Takasaki, T. (2014). Identification and amplification of Japanese encephalitis virus and Getah virus propagated from a single porcine serum sample: a case of coinfection. Archives of virology, 159(11), 2969-2975.

Yago, K., Hagiwara, S., Kawamura, H., Narita, M.(1987). A fatal case in newborn piglets with Getah virus infection: isolation of the virus. Nihon Juigaku Zasshi, 49:989-94.

Fukunaga, Y., Kumanomido, T., & Kamada, M. (2000). Getah virus as an equine pathogen. *Veterinary Clinics of North America: Equine Practice* , *16* (3), 605-617.

Yang, H., Rao, YN., Chen, RG., Hu.(1984). A strain of alphavirus togavviridae isolated in Hainan island-isolation, identification and aero-epidemic investigation. Chinese Journal of Microbiology and Immunology ,4(2):107.

Li, Y. Y., Fu, S. H., Guo, X. F., Lei, W. W., Li, X. L., Song, J. D., ... & Wang, H. Y. (2017). Identification of a newly isolated Getah Virus in the China-Laos border, China. Biomedical and Environmental Sciences, 30(3), 210-214.

Li, L., Guo, X., Zhao, Q., Tong, Y., Fan, H., Sun, Q., ... & Zhang, J. (2017). Investigation on mosquito-borne viruses at Lancang river and Nu river watersheds in Southwestern China. Vector-Borne and Zoonotic Diseases, 17(12), 804-812.

Li, X. D., Qiu, F. X., Yang, H., Rao, Y. N., & Calisher, C. H. (1992). Isolation of Getah virus from mosquitos collected on Hainan Island, China, and results of a serosurvey. The Southeast Asian journal of tropical medicine and public health, 23(4), 730-734.

Nemoto, M., Bannai, H., Tsujimura, K., Yamanaka, T., & Kondo, T. (2016). Genomic, pathogenic, and antigenic comparisons of Getah virus strains isolated in 1978 and 2014 in Japan. Archives of virology, 161(6), 1691-1695.