ゲタウイルスによる新生子豚の死亡例

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A Fatal Case in Newborn Piglets with Getah Virus Infection: Pathogenicity of the Isolate

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ABSTRACT. The Kanagawa strain of Getah virus isolated from newborn piglets which died of peracute disease was examined for pathogenicity in gnotobiotic piglets. Its pathogenicity depended on the route of inoculation. Piglets inoculated intramuscularly on 5 to 18 day old exhibited anorexia, depression, tremor of whole body, red discoloration of the skin, trembling of the tongue, and incoordination of pelvic limbs 20 hrs and died or became moribund 2 to 3 days after inoculation. The virus was recovered at high titer from various organs and serum of dead and moribund piglets.—KEY WORDS: Getah virus, gnotobiotic piglet.

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The pathogenicity of Getah virus (GETV) for animals is well known in horses with natural and experimental infection [1, 6] and in suckling mouse with experimental infection [7]. It is well known that pigs in Japan are infected widely with GETV but relationship between GETV and disease in pigs is unknown. In the previous paper [9], it has been described that GETV was isolated from newborn piglets which died from peracute disease. In this paper we attempted to confirm pathogenicity of the isolated GETV in gnotobiotic piglets which have not antibody against GETV.

MATERIALS AND METHODS

Virus: The Kanagawa strain described in the previous paper [9] was used.

Gnotobiotic piglet: Gnotobiotic piglets were produced and reared by the method in the previous paper [2].

Experimental design: Five 5-day-old and one 18-day-old piglets were inoculated intramuscularly with 1 m*l* of virus material containing $10^{5.5}$ PFU/m*l* of the virus. Three 5-day-old piglets were inoculated oronasally with 1 m*l* of the virus material. They were observed daily clinical signs and rectal and oral swabs were collected daily for 5 days after inoculation. The swabs were immersed with 2 m*l* of Eagle's minimum essential medium (MEM) and stored at −80°C until used for virus recovery. Dead and moribund piglets were necropsied and 2 piglets inoculated intramuscularly (piglet No. 5) and oronasally (piglet No. 6) were killed 5 days after inoculation. The cerebrum, cerebellum, mandibular salivary glands, tonsil, lungs, heart, liver, spleen, pancreas, kidneys, adrenal glands, small intestine, rectum, and inguinal lymphnodes were harvested for virus recovery and for histopathological examination.

Virus titration: Virus titration was performed by the plaque method as mentioned in the previous paper [9]. Ten percent emulsions of the organs harvested were made in MEM and stored at −80°C until used for virus titration. Isolated virus was identified as GETV by the fluorescent antibody technique or plaque size.

Table 1. Clinical signs in gnotobiotic piglets inoculated with the Kanagawa strain

Piglet Number	Route of inoculation[a)]	Age in days	Days after inoculation				
			1	2	3	4	5
1	Oronasally	5	−[b)]	−	−	−	−
2	Oronasally	5	−	−	−	−	−
3	Intramuscularly	5	++[c)]	++	K		
4	Intramuscularly	5	++	+++[d)]	D		
5	Intramuscularly	5	++	−	−	−	K[e)]
6	Oronasally	5	−	+	−	−	K
7	Intramuscularly	5	++	+++	D[f)]		
8	Intramuscularly	5	+++	K			
9	Intramuscularly	18	+++	K			

a) $10^{5.5}$ PFU per head
b) Negative
c) Moderate
d) Severe
e) Killed
f) Died

RESULTS

Clinical signs: Clinical signs in gnotobiotic piglets inoculated with the Kanagawa strain are presented in Table 1. All of 6 piglets inoculated intramuscularly exhibited anorexia, depression, tremor of whole body, red discoloration of the skin, trembling of the tongue, and incoordination of pelvic limbs 20 hrs after inoculation. Three piglets (piglet Nos. 3, 8, and 9) became moribund 2 to 3 days after inoculation. Two piglets (piglet Nos. 4 and 7) died between 60 to 70 hrs after inoculation. One (piglet No. 5) of 6 piglets inoculated intramuscularly recovered from clinical signs 2 days after inoculation. On the other hand, one (piglet No. 6) of the 3 piglets inoculated oronasally presented slight clinical signs 2 days after inoculation and recovered next day.

Pathological changes: At necropsy, no gross and histopathological lesions were observed in all of the organs of dead, moribund, and killed piglets.

Distribution of the virus in organs and serum: The results are shown in Table 2. The virus was recovered from all of the organs and/or serum of dead (piglet Nos. 4 and 7) and moribund (piglet Nos. 3 and 8) piglets inoculated intramuscularly at 5-day-old. The infective titer of the virus in organs and sera ranged from $10^{2.0}$ to $10^{5.6}$ PFU/m*l*. The virus titer was more than $10^{3.0}$ PFU/m*l* in the spleen, tonsil, adrenal glands, small intestine, liver, inguinal lymphnodes, and serum. The virus was recovered from the organs exclusive of the heart, pancreas, and small intestine of moribund piglet (piglet No. 9) inoculated intramuscularly at 18 days of age. The virus titer was lower than those of 5-day-old ones, ranging from $10^{0.7}$ to $10^{3.4}$ PFU/m*l*. The virus was recovered from the tonsil and rectum of piglet No. 5 and from the tonsil and inguinal lymphnodes of piglet No. 6 killed 5 days after inoculation.

Recovery of the virus from rectal and oral swabs: The virus was recovered from rectal swabs of piglets, inoculated intramuscularly at 5 days of age, 1 to 3 days after inoculation and from oral swabs of 2 piglets 2 days after inoculation. The virus titer ranged from $10^{0.7}$ to $10^{3.4}$ PFU/m*l*. On the contrary, the

Table 2. Distribution of the virus in organs and serum of gnotobiotic piglets inoculated with the Kanagawa strain

Specimen	Piglet number[a]						
	3	4	5	6	7	8	9
Cerebrum	3.0[b]	3.0	–[c]	–	3.3	3.1	1.8
Cerebellum	3.4	3.0	–	–	3.2	3.2	1.8
Mandibular Salivary glands	3.2	2.0	–	–	2.2	2.5	1.0
Tonsil	4.1	4.4	1.7	2.2	3.7	4.0	1.2
Lungs	2.7	3.3	–	–	4.0	3.2	1.5
Heart	2.9	3.0	–	–	3.2	3.4	–
Liver	3.3	3.8	–	–	4.2	3.9	2.0
Spleen	4.5	5.4	–	–	5.6	5.2	3.0
Pancreas	2.7	2.5	–	–	3.1	3.9	–
Kidneys	3.5	3.4	–	–	3.0	3.5	1.4
Adrenal Glands	4.3	4.9	–	–	4.4	3.9	2.3
Small intestine	4.3	3.3	–	–	3.7	4.9	–
Rectum	3.0	2.7	2.7	–	3.2	4.5	0.7
Inguinal lymphnodes	3.1	3.2	–	2.2	4.3	3.7	3.0
Serum	4.5	NT[d]	–	–	NT	5.0	3.4

a) Same as in Table 1.
b) Log-PFU/m*l*.
c) Negative in 10% suspensions of the organ.
d) Not tested.

Table 3. Recovery of the virus from rectal and oral swabs in gnotobiotic piglets inoculated with the Kanagawa strain at 5 days of age

Piglet Number[a]	Swab	Route of inoculation	Day after inoculation				
			1	2	3	4	5
1	Rectal	Oronasally	–[b]	–	–	–	–
	Oral		–	–	–	–	–
2	Rectal	Oronasally	–	–	–	–	–
	Oral		0.7[c]	–	–	–	–
3	Rectal	Intramuscularly	–	1.7	0.7		
	Oral		–	1.7	–		
4	Rectal	Intramuscularly	–	2.2	–		
	Oral		–	–	–		
5	Rectal	Intramuscularly	–	1.2	–	–	–
	Oral		–	–	–	–	–
6	Rectal	Oronasally	–	–	–	–	–
	Oral		0.7	–	–	–	–
7	Rectal	Intramuscularly	1.0	2.0			
	Oral		–	2.0			
8	Rectal	Intramuscularly	2.9	3.4			
	Oral		–	–			

a) Same as in Table 1.
b) Negative in original suspension.
c) Log-PFU/m*l*.

virus was only demonstrated in oral swabs of 2 piglets (piglet Nos. 2 and 6), inoculated oronasally, 1 day after inoculation. The virus titer was $10^{0.7}$ PFU/m*l*, respectively (Table 3).

DISCUSSION

This study demonstrated that the same peracute fatal disease as natural infection was reproduced in gnotobiotic piglets when the Kanagawa strain of GETV was inoculated by the intramuscular route. The present and previous [9] papers are the first report of disease due to natural and experimental GETV infection in pigs.

It is considered that high infective titer of the virus in the organs of dead and moribund piglets might be derived from viremia, since fluorescent antigens and virus particles in the spleen, which had highest titer of the virus, could not be observed and, the virus has been isolated from blood samples of pigs [3, 5].

GETV was originally isolated from mosquitoes and it is assumed that infection in mammals and birds is transmitted by these insects. It has been demonstrated that GETV grows well in *Aedes vexans* [4, 8] and *Culex tritaeniorhynchus* [8] mosquitoes after feeding of blood meal containing high titer of the virus and is detected at high titer from 7 to 21 days after feeding. In this experiment, piglets showed severe disease by intramuscular inoculation but not by oronasal inoculation and a small amount of the virus was recovered from rectal and oral swabs. From the facts mentioned above, it is strongly suggested that natural infection in newborn piglets [9] might occur by biting mosquitoes infected with the virus.

Our unpublished data showed that 4-month-old pigs without antibody to GETV exhibited no clinical sign when they were inoculated intramuscularly with the same dose of the Kanagawa strain. Five-month-old pigs without antibody to GETV exhibited also no clinical sign after subcutaneous inoculation with the Haruna, 2078 (Kubota *et al.* at the 85th Meeting of the Japanese Society of Veterinary Science in 1978) and Sakai strains of GETV (Izumida *et al.* at the 88th Meeting of the Japanese Society of Veterinary Science in 1979). These facts indicate that the pathogenicity of GETV for pigs is influenced by age.

REFERENCES

1. Kamada, M., Ando, Y., Fukunaga, T., Kumanomido, T., Imagawa, H., Wada, R., and Akiyama, Y. 1980. Equine Getah virus infection: Isolation of the virus from racehorses during an enzootic in Japan. *Am. J. Trop. Med. Hyg.* 29: 984–988.
2. Kasiwazaki, M., Namioka, S., Umoto, K., Shibata, S. and Akaike, Y. 1967. Studies on the rearing of germ-free pigs. I. A germ-free rearing isolator apparatus and procurement of baby pigs. *Bull. Exp. Anim.* 16: 85–92 (in Japanese with English summary).
3. Kumanomido, T., Fukunaga, Y., Ando, Y., Kamada, M., Imagawa, H., Wada, R., Akiyama, Y., and Tanaka, Y. 1982. Ecological survey on Getah virus among swine in Japan. *Bull. Equine Res. Inst.* No. 19: 89–92.
4. Kumanomido, T., Fukunaga, Y., Kamada, M., and Wada, R. 1982. Experimental transmission studies of Getah virus in *Aedes vexans*. *Bull. Equine Res. Inst.* No. 19: 93–96.
5. Matsuyama, T., Nakamura, T., Isahai, K., Oya, A., and Kobayashi, M. 1967. Haruna virus, a group A arbovirus isolated from swine in Japan. *Gumma J. Med. Sci.* 16: 131–134.
6. Sentsui, H., and Kono, Y. 1980. An epidemic of Getah virus infection among racehorses: Isolation of the virus. *Res. Vet. Sci.* 29: 157–161.
7. Sentsui, H., and Kono, Y. 1981. Pathogenicity of Getah virus for mice. *Natl. Inst. Anim. Health Q. (Jpn)* 21: 7–13.
8. Takashima, I., Hashimoto, N., Arikawa, J., and Matsumoto, K. 1983. Getah virus in *Aedes Vexans nipponii* and *Culex triaeniorhynchus*: Vector susceptibility and ability to transmit. *Arch. Virol.* 76: 299–305.
9. Yago, K., Hagiwara, S., Kawamura, H., and Narita, M. A fatal case in newborn piglets with Getah virus infection: Isolation of the virus. *Jap. J. Vet. Sci.* 49: 989–994.

要　　約

ゲタウイルスによる新生子豚の死亡例：分離ウイルスの病原性試験：川村　齊・矢後啓司[1)]・成田　實・今田忠男・西森知子・播谷　亮（農林水産省家畜衛生試験場，[1)]神奈川県家畜病性鑑定所）――甚急性経過で死亡した新生子豚から分離したゲタウイルス Kanagawa 株を無菌子豚の筋肉内に接種すると20時間後に食欲不振，元気消失，全身のふるえ，舌のふるえ及び後肢の不調整，皮膚の赤色化などの症状を現わし，接種後２～３日でほとんどが死亡または瀕死状態となった．これらの子豚の臓器と血清から高い値のウイルスが回収された．経口・経鼻接種の場合は症状は軽く，死亡例はなかった．