NOTE

The Propagation of a Strain of Swine Vesicular Disease Virus in One-Day-Old Mice

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A characteristic of swine vesicular disease virus (SVDV) may be the pathogenicity in suckling mice [3, 7, 8], since close antigenical relationship was demonstrated between SVDV and Coxsackie B5 virus [1, 5]. Suckling mice were used to compare the virulence of SVDV strains isolated in different countries [2] after Nardelli *et al.* [8] indicated their susceptibility in their first report on SVDV.

The present experiment was conducted to study the virus propagation of a strain of SVDV, Pd 894, isolated in Italy in 1-day-old mice. A foot epithelial sample collected from a swine showing typical clinical signs of SVD in Veneto, Italy, in March, 1973, was kindly supplied by Dr. R. Zoletto of this institute.

In vitro virus assay was carried out with an established cell line, IB-RS-2 [4]. The cell culture media, the way of virus assay in microplates and the incubator system with CO₂-gas flow were basically the same as mentioned previously [6]. The agar overlay medium consisted of serum-free cell maintenance medium (MM) supplemented with 0.125% bovine albumin (BDH, Fraction V) and 0.8% Bact agar (Difco.). The virus isolation from the original epithelial extract was per-

formed directly by the plaque method as usual. The original epithelial material contained 4×10^5 PFU virus per gram. After three plaque clonings, the cloned virus was propagated in bottle cultures of IB-RS-2 cells. When more than 75% of the cells showed CPE, the cultures were freeze-thawed. The clarified virus containing $10^{7.5}$ TCID₅₀ per ml was stored at -30°C until required. An aliquot of the stock virus was sent to the SVD diagnosis center in Italy, Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia, Brescia, and identified as SVDV antigenically related to the strain Italy/66. Two litters, enumerated 16 in total of 1-day-old mice, NMRI strain, were each inoculated intraperitoneally with 0.01 ml of the stock virus. When found moribund or dead after illness, the mice were kept in plastic sacks separately and stored at -30°C . Four days after the last mouse was put in the freezer, all frozen mice brought on to a bench and thawed. In each mouse, four groups of the organs, e.g. the brain, the mixture of the liver and the spleen, the intestines and the skeletal muscles including the bones were separated. A 10% suspension (w/v) was made in a glass mortar by adding an adjusted amount of MM. All mice infect-

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Table 1. Virus recovery from the organs of 1-day-old mice infected with SVDV strain Pd 894

Mouse No.	The day showing illness and or death	Intestines	Mixture of Liver and spleen	Brain	Skeletal muscles with bones
1	2	3.25*	5.00	4.00	7.00
2	2	4.25	4.75	5.50	7.50
3	2	3.50	4.00	5.50	7.00
4	2	4.25	5.50	5.00	7.50
5	2	5.00	5.25	5.50	7.50
6	2	4.00	5.00	4.75	6.75
7	2	5.00	4.50	5.00	6.50
6	2	3.75	4.25	6.75	6.75
9	2	4.25	5.50	5.75	7.25
10	2	4.50	5.00	5.75	6.50
11	2	4.75	5.75	6.25	7.25
12	4	4.75	6.00	4.75	7.25
13	5	5.25	6.00	5.00	7.25
14	5	5.00	5.00	4.75	6.75
15	6	NT	NT	NT	NT
16	6	3.75	3.75	7.50	6.75
$\bar{x}$	3	4.35	5.02	5.45	7.03

*: Virus infective titer in TCID₅₀ per gram of organ (neg. log₁₀).

NT: Not tested.

ed showed severe clinical symptoms characterized by tremor and ascending paralysis starting from the hind legs, finally leading to death in 2 to 6 days after infection.

The mean virus infective titers was $10^{7.03}$ in the skeletal muscles with the bones, $10^{5.45}$ in the brain, $10^{5.02}$ in the mixture of the liver and spleen and $10^{4.35}$ in the intestines as shown in Table 1.

The present results, in the point of the highest virus recovery in the skeletal muscles with the bones, was contrary to that of Burrows *et al.* [2] who reported in 5-day-old mice infected with SVDS England/72. They found a greater amount of virus in the brain than in the whole carcass. At the moment, the difference in these results is obscure since no comparative study has been made between them. The strain PD 894 seems to be less virulent; however, the develop-

ment of immune response in such a premature stage is assumed to be a factor involved in this phenomenon. The relationship among mortality, virus propagation in each individual organ and the age of the mouse has remained to be studied.

REFERENCES

1. Brown, F., Talbot, P., and Burrows, R. (1973). *Nature* **245**, 315–316.
2. Burrows, R., Mann, J. A., and Goodridge, D. (1974). *J. Hyg.* **73**, 109–117.
3. Dawe, P. S., Forman, A. J., and Samale, C. J. (1973). *Nature* **241**, 540–542.
4. De Castro, M. P. (1964). *Arq. Inst. Biol. S. Paulo* **31**, 63–78.
5. Graves, J. H. (1973). *Nature* **245**, 314–315.
6. Kadoi, K. (1983). *Jpn. J. Vet. Sci.* **45**, 131–133.
7. Mowat, G. N., Darbyshire, J. H., and Huntley, J. F. (1972). *Vet. Rec.* **90**, 618–621.
8. Nardelli, L., Lodetti, E., Gualandi, G. F., Burrows, R., Goodridge, D., Brown, F., and Cartwright, B. (1968). *Nature* **219**, 1275–1276.

要 約

豚水疱病ウイルス Pd894 株の 1 日齢マウスにおける増殖（短報）：門井克幸（日本大学農獣医学部獣医伝染病学研究室）——豚水疱病ウイルス（Pd894 株）を 1 日齢マウスの腹腔内に接種し，発症・病死時に凍結保存した各個体の 4 群の臓器からウイルスの回収を試みた。臓器別の平均ウイルス感染価は，躯幹筋（骨を含む）が 7.03 ($\log_{10}$ TCID₅₀/g) で最高値を示し，ついで，脳が 5.45，肝と脾の混和物で 5.02，腸管で 4.35 あった。