Development of Tissue Culture Living Hog Cholera Vaccine

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At the FAO conference on "pig disease and production in developing countries" held in Singapore in December, 1963 and FAO/OIE meeting on hog cholera and African swine fever held in Rome in May[3], 1965, authors presented reports on the modification of the virulence of hog cholera (HC) virus.

The highly virulent ALD strain of HC virus was attenuated by 142 serial passages in swine testicle cells, and then by 36 passages in bovine testicle cell. Resulting virus was designated as the B-line strain. Continuing the attenuatuion of B-line virus, 4 sublines were developed. (1) The C30 line was evolved through 14 serial passages in swine testicle cells at 30°C. Better growth of the virus at 30°C indicated the adaptation to low temperature condition. (2) The P-line virus was isolated from the PK2a cell persistently infected with the B-line virus for 72 culture passages (600 days). (3) The GPK-line was a continuation of B-line but grown in guinea pig kidney (GPK) cells. Two variant viruses were separated from the 32nd passage in GPK culture using a technique of terminal dilution passage. The growth of both variants in GPK was much better than that of the original B-line virus. One of them interfered with Newcastle disease virus (NDV) and western equine encephalitis (WEE) virus in the swine testicle cell culture, while the other one enhanced the NDV (END phenomenon) the same as virulent and other lines of attenuated viruses do. (4) The interfering and enhancing characters were designated as E^- (END negative) and E^+(END positive). The $GPKE^-$ virus could be detected and measured in a swine testicle cell culture, and its result was

Table 1. Comparative pathogenicity of attenuated hog cholera viruses

Viruses	Adult pig of 5 month old			Baby pig of half day old*		
	Fever	Leukopenia	Duration of viremia in day	Fever	Leskopenia	Duration of viremia in day
B	No or transient	Distinct	1~4			
C30	No	Slight	0~2	No	Slight	2~8
P	No	Slight or none	0~1	No	Slight	2~5
$GPKE^+$	No	Slight or none	0~2	No	Slight	2~0
$GPKE^-$	No	Almost none	0	No	Slight	0

* Farrowed and nursed by susceptible sow. Given viruses in 10~12 hours after birth. Grew normally. Manifested no clinical changes after inoculation with viruses.

* PU: One protective unit (PU) is expressed in terms of the highest dilution of the vaccine that can protect pigs against the challenge of 100 MLD of the virulent ALD strain made with in 10~21 days after vaccination.

read by interference with WEE virus. The pathogenicity of B-line and its 4 subline viruses was repraised basing upon the grade of fever, leukopenia and viremia in adult and halfday-old baby pigs. Comparative pathogenicity of 5 line viruses was summarized in Table 1. The original B-line virus caused fever in only 30% of the adult pigs, and then was mild leukopenia and viremia lasting from 1 to 4 days. Three subline viruses other than GPKE$^-$ caused no clinical change other than mild or slight leukopenia. They manifested only a transient viremia lasting 2 days or in less adult pigs, but longer viremia lasting from 2 to 8 days in baby pigs. A definitive drop of pathogenicity was shown by GPKE$^-$ line which manifested no clinical change, almost no leukopenia and no detectable viremia even in baby pigs.

Vaccination with the B and these 4 subline viruses gave satsisfactory immunity to almost all experimental pigs. The neutralizing antibody rose to the highest level ranging from 1 : 36 to 1 : 512 in titer and lasted for more than one year without a significant drop of titer. The pigs vaccinated with GPKE$^+$ and GPKE$^-$ vaccines, when passive antibody still remained at as high level as 1 : 64 in titer, developed a considerable amount of antibody and resisted to challenges of virulent HC virus, even after one year from vaccination.

As was introduced at the conference in Rome, Sato et al.[4] developed a modified antigenic strain of HC virus by a unique method called the CCVP method (LOM strain). To give support to this research, preliminary field trials of this LOM strain were instituted to determine the safety and efficacy of this vaccine strain in Taiwan. A total of 2,177 pigs, including 1,452 weighing less than 15 kg, were inoculated subcutaneously with 5,000 PU* of virus during the week beginning February 1, 1964. Of them, 2,159 survived in good condition, 13 survived after showing anorexia, conjunctivitis, and diarrhea, and 4 died. This presents a mortality rate of only 0.18 percent.

Kawakubo et al.[1] also reported on the modification of the virulence of lapinized SFA strain in a persistently infected culture of PK2a cells (NIBS strain).

After making sure that the vaccinated pigs showed no clinical symptoms, slight leukopenia, and almost no viremia in pigs over 4 month of age, field trials of these LOM, GP KE$^+$ and NIBS strains were carried out to determine the safety and efficacy to pigs in Japan during a period from December, 1964 to November 1965.

As is shown in Table 2, 14 of 1,665 pigs inoculated with GPKE$^+$, 5 of 1,562 pigs with LOM, and 3 of 1,517 pigs with NIBS died during from 10 to 26 days after vaccination. The deaths occured mainly in young pigs under poor conditions. Six of them had pneumonia associated with *Pasteurella multocida*, hemolytic streptococcus, lung-worm or swine enzootic pneumonia. Five dead pigs showed septicemia of *Escherichia coli*, D type of staphylococcus and *Erysipelothrix insidiosa*, and 3 died with enteritis, 1 with peritonitis, 1 with

Table 2. Results of field trials of different vaccines

	Pigs vaccinated				Pigs dead			
Vaccines	Exp. 1	Exp. 2*	Exp. 3	Total	Exp. 1	Exp. 2	Exp. 3	Total
GPKE+	170	111	1384	1665	4	3	7	14
LOM	168		1394	1562	3		2	5
NIBS	169		1348	1517	0		3	3
Total	507	111	4126	4744	7	3	12	22(0.46%)
CVV	169	100	1347	1516	4	2	0	6(0.33%)

* Pigs of 29 litters were divided into 2 groups. One group was vaccinated with GPKE+ and another with CVV.

Japanese B encephalitis, and 1 with tetanus. The remaining 5 could not be diagnosed because the carcasses had been scrapped. Consequently, the deaths are considered to have been caused by factors other than vaccination. Vaccination with these 3 vaccine viruses gave satisfactory immunity to the most vaccinated pig. The neutralizing antibody rose to high level, ranging from 1 : 32 to 1 : 521 in titer. On the other hand, vaccination with crystal-violet vaccine gave neutralizing antibody titers in the lower level ranging from 1 : 2 to 1 : 16.

The field trials reported herein indicated that the living hog cholera vaccines of tissue culture origin developed in Japan gave successful results with regard to their safety and immunogenicity.

References

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3) Sasahara, J. and Kumagai, T.: Live virus hog cholera vaccine in Japan. Working paper No. 21. FAO/OIE international meeting on hog cholera and African swine fever. (1965)
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As was already reported in newspapers or on waves, the Conference on Agricultural Development in Southeast Asia was held in Tokyo last December.

The Conference especially placed a stress on and reaffirmed the importance of improvement of agricultural techniques. For that reason, the editors intended to introduce STATEMENT read by His Excellency Mr. Tadao Kuraishi, Minister of Agriculture and Forestry of Japan and the full text of JOINT COMMUNIQUE hereunder.

Comments on the concept of technical improvement of agriculture in Southeast Asia will be warmly welcom by the editors.

STATEMENT BY HIS EXCELLENCY MR. TADAO KURAISHI, MINISTER OF AGRICULTURE AND FORESTRY OF JAPAN

It gives me great pleasure to witness the opening of the Conference on Agricultural Development in Southeast Asia, with the participation of prominent officials responsible for agricultural development policy from our friendly neighbors of Southeast Asia, as well as representatives from FAO, ECAFE and Asian Development Bank.

It was at last April's Ministerial Conference for Economic Development of Southeast Asia that the important role of agriculture in economic development was emphasized, and out of that Conference has been born the present meeting. This very fact, I believe, is a proof that there now exists a common recognition among us of the importance of agricultural development. It hardly needs to be restated that the majority of peoples of