GP VACCINE FOR CONTROL OF HOG CHOLERA IN JAPAN

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GP vaccine is a hog cholera vaccine derived from modified live virus adapted to guinea pig kidney cell cultures. Since the vaccine was put into practical use in May 1969 in Japan, outbreaks of hog cholera have decreased sharply. Outbreaks of hog cholera have disappeared since the latest occurrence in 1975.

The following report summarizes the development, production, and current use of the GP vaccine in Japan.

History of hog cholera vaccine in Japan

In Japan, phenol-glycerine vaccine was practically applied to hog cholera control over the period extending from 1922 to 1928. Thereafter formal vaccine was used for control programs from 1929 to 1951 while crystal violet vaccine was put in practical use from 1951 to 1968. However, more than 5,000 cases were diagnosed as hog cholera every year. Under these circumstances it was considered that the crystal violet vaccine was not effective enough to control hog cholera in Japan. Several disadvantages of this vaccine were pointed out by laboratory experiments. 1) Slow establishment of immunity 2) Duration of immunity was short-lived 3) Antibody response varied in each pig 4) Efficacy of vaccine was greatly influenced by maternal antibody titer. To overcome these shortcomings, studies were started on development of live virus vaccine.

In the United States, Koprowski and his co-workers developed an attenuated strain of hog cholera virus by passage through rabbits. The strain has been widely used as good and practical vaccine for active immunization of pigs against hog cholera in the United States and some other countries. Since the strain was introduced into Japan in 1952, evaluation has been conducted on its safety and efficacy. This vaccine had so solid immunogenicity that immunity was conferred to pigs 3 days after inoculation but the degree of attenuation was not sufficient. The results of field experiments indicated that the Koprowski's strain was not safe enough for pigs in Japan. Studies were made to produce more attenuated hog cholera virus and a caprinized virus could be obtained by conducting serial passages of the lapinized strain. This lapinized-caprinized virus gave satisfactory results in a laboratory pig inoculation test, but a considerable number of pigs manifested moderate and severe symptoms and some of them died in the field experiments. Therefore, it was estimated that the lapinized-caprinized virus could not be used practically for a vaccination program.

Establishment of vaccinal strain

Studies were carried out actively to produce an attenuated strain by tissue culture passages. The virulent ALD strain of hog cholera virus was subjected to 142 passages in swine testicle cells (S strain). Then a clone was purified by the limiting dilution method and subjected to 36 passages in bovine testicle cells. A clone was again obtained by the limiting dilution method (B strain). After that, the strains were made to adapt to guinea-pig kidney cells by 41 passages in these cells. As a result, they were separated into two groups of clones, a clone showing no END (exaltation of Newcastle disease virus) effect and a clone showing this effect, which were designated as GPE^- and GPE^+ virus respectively. GPE^- virus multiplied better at 30°C than at 40°C. Wild or virulent virus multiplied well both at 40°C and at 30°C. In the case of vaccine virus, however, the ability to multiply was very low at 40°C (T marker). While vaccine virus multiplied well in guinea-pig kidney

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cells, wild or virulent virus hardly multiplied in these cells (G marker).

The importance of establishing a criterion for the development of a low virulent strain which could be used as vaccine virus was emphasized. In 1964, the Research Committee on Live Virus Vaccine, Japanese Society of Veterinary Science, passed a resolution to approve the following items as minimum requirements for hog cholera live virus vaccine. 1) When pigs are inoculated with vaccine virus, they shall not manifest such clinical symptoms as anorexia or pyrexia. 2) In pigs inoculated with vaccine virus, leukopenia which is one of the symptoms characteristic of hog cholera, shall be very mild or shall hardly be recognized. 3) In pigs inoculated with vaccine virus, viremia induced by the vaccine virus shall not be observed, or it shall not last long even it is recognized. 4) No vaccine virus shall be discharged in excreta, so that pigs other than the inoculated ones may be prevented from contact infection. Three strains, LOM, NIBS and GPE^+, which met these conditions, were tested in field experiments. The LOM strain was produced by Sato and his co-workers, who had employed the continuous cell-virus propagation (CCVP) method. The strain was subjected to a field experiment in Taiwan in 1964, and its safety was confirmed. The NIBS strain was produced by Kawakubo and his co-workers from the lapinized SFA strain, which was attenuated by persistent virus infection of established pig kidney cell lines.

In the field experiments of these 3 strains, a total of 4,126 pigs were inoculated with one of the three types of vaccine. Concurrently, 1,347 pigs were inoculated with crystal violet vaccine as controls. The neutralizing antibody titer increased up to 1:32 to 1:512 in 242 out of 261 (92.7%) pigs. The results of all the field experiments indicated that the tissue culture modified-live virus vaccine was quite safe. After all these experiments on 3 strains, GPE^- virus was isolated in our institute. In an experiment with newborn piglets, it was assumed from the results obtained that GPE^- virus might be lower in pathogenicity, as judged from the severity of viremia, than any other vaccine virus. The laboratory experiments suggested that the GPE^- virus is safe enough for field use. In 1967 and 1968, GPE^- virus was inoculated experimentally to a total of 64,325 pigs in the field. As a result, it was confirmed that this vaccine virus was very safe and highly immunogenic. Then, a nation-wide field test on this vaccine was conducted in 1968. Results of the test were in perfect agreement with those reported previously.

Safety

To determine how safe the GP vaccine is, the piglets were injected with ten doses of the vaccine. They were observed and their temperature, leukocyte counts and virus titer in the blood were recorded every day. None of the vaccinated animals exhibited clinical reactions, leukopenia, or viremia. A test was set up to determine whether the vaccine virus spread from vaccinated to unvaccinated contact pigs. After one month of contact with vaccinated animals control pigs remained free from neutralizing antibodies against hog cholera virus. The results indicated that the vaccine virus did not spread to the unvaccinated susceptible pigs. However, evidence was presented to show that the vaccine virus spread to susceptible pigs by contact with vaccinated ones with respiratory symptoms.

The vaccine virus multiplied mostly in tonsils and lymph nodes, and only small amounts of the virus were demonstrated in the spleen and lungs over a short period. Localized focal necrosis was apparent in the lymphatic tissue, but not in other tissues of the body. As a result, limited multiplication of vaccine virus was present in the body of vaccinated pigs.

Immunity

Ten piglets of one month of age born to nonimmune dams were injected intramuscularly with a dose of vaccine. Of them, 2 piglets were challenged with virulent hog cholera virus every day until 5 days after injection. The piglets challenged from the 3rd to 5th day after vaccination exhibited slight febrile response but remained normal thereafter. Four piglets challenged within 2 days after vaccination died with hog cholera symptoms. The experiment indicated that immunity with GP vaccine was established 3 days after injection.

Antibody response was observed in piglets injected with the GP vaccine. Following vaccination, the neutralizing antibody titer started to increase 2-3 weeks after injection and reached maximum values after 1-2 weeks. The titer persisted for more than 2 years at the same level.

Effect of maternal antibodies on immunization: It is known that virus neutralization by specific maternal antibodies appears to be an important factor interfering with active immunization. In the inoculation with crystal violet vaccine, it was demonstrated that pigs with a maternal antibody titer of 10 or above at the time of vaccination were not vaccinated effectively. Such interference can be overcome by use of GP vaccine. No apparent decrease in antibody production by GP vaccine occurred in pigs with a maternal antibody titer lower than 8. A decrease to a variable extent, however, was observed in pigs with a maternal antibody titer of 8 and above. The decrease was remarkable in pigs with a titer of 128 and above. In approximately 50% of inoculated pigs with a maternal antibody titer of 128 and above, no antibodies were detected 5 months after vaccination. Therefore, maternal antibodies at a higher titer prevented inoculated pigs from showing a take of vaccine.

In conclusion, the GP vaccine induced protection as early as the third day after injection, and although such a type of resistance seemed to be due to interference rather than to the presence of antibodies, it blended smoothly into the period of active immunity. Immunity following vaccination with the GP vaccine was found to be solid at 3 years, the longest period tested.

Production

The seed lot system has been adopted for manufacturing the GP vaccine. The master seed is prepared by the National Institute of Animal Health. It shall be of sufficient volume to permit multiple use for 5 years. This method makes it possible to reproduce the vaccine at the same passage level necessary for demonstrating consistency. The master seed shall have been shown to be safe and potent in pigs by field studies with experimental vaccines. The virus seed for the production of vaccine shall be not more than of two tissue culture passages from the master seed virus. A commercial maker may produce one lot consisting of 500,000 doses of vaccine from 30 ml of master seed virus.

Guinea-pigs shall be maintained in quarantine for at least one month prior to use. Each animal shall be examined by necropsy and if there are any significant gross pathological lesions, the kidney shall not be used in the manufacture of vaccine. At least 20% of the bottle culture shall be set aside as control. The control shall be examined microscopically for cell degeneration for 14 days. The cell fluids from such control bottles shall be tested for adventitious virus by inoculation to pig and cell cultures of swine testicle and kidney. The cell sheet of control shall be examined for presence of hemadsorption viruses by the addition of guinea-pig and chicken red blood cells.

After virus inoculation, bottles shall be maintained at a temperature of less than 30°C with slow mechanical shaking. After harvesting and removal of samples for safety testing to be described below, the pool shall be passed through sterile filters with sufficiently small porosity to ensure bacteriologically sterile filtrates. The virus pool shall be tested for sterility, safety, potency, identity, virus titer, *in vitro* marker and purity. The filtered virus suspension is lyophilized with addition of equal volume of suitable additive. The additive contains 10% lactose, 0.3% polyvinylpyrrolidone, and 0.002% kanamycin per milliliter. The lyophilized vaccine shall be tested for purity, virus titer, vacuum, moisture content, sterility, property, safety, potency, contact infection and marker.

Assay

The final product shall meet the following requirements.

Property test: The dried vaccine shall be a dried mass of light-orange grayish-white color. The diluent shall have such components as appropriate to it and contain no foreign body. When the diluent is added to the dried vaccine and shaken, a homogeneous suspension of reddish orange color shall be produced. The reconstituted vaccine shall not contain any coarse particles or foreign bodies. There shall be no marked difference in property of the content among the final containers.

Vacuum extent: Non-electroid discharge shall be carried out in each final container of the dried vaccine, in the dark, with Tesler coil set in a distance of less than 5mm. **Moisture test:** The moisture of the dried vaccine shall be less than 4 per cent. **Sterility test:** The reconstituted vaccine shall be free from any viable bacteria which can be detected. **Virus content test:** The reconstituted vaccine shall show a virus titer of more than 10^3 $TCID_{50}$ per pig dose in swine testicle cell culture. **Identity test:** The vaccine virus shall be neutralized by known specific antiserum of hog cholera. **Safety test:** No test pigs inoculated with 100 doses of the vaccine shall manifest any abnormal changes caused by the product for 14 days. **Test for contact infection:** No infection shall be induced by cohabitation. **Potency test:** The product shall be so potent that pigs inoculated subcutaneously with 1/1,000 dose of the restored vaccine can withstand challenge with 10^5 MLD of virulent virus two weeks later. **Marker test:** The marker test shall consist of the tests for E-marker, T-marker and G-marker. Test for E-marker: When the restored vaccine is inoculated into swine testicle cell culture and when the culture is challenged by Newcastle disease virus, the virus of vaccine shall not enhance cytopathogenic effect of Newcastle disease virus which is proper to the virulent virus. Test for T-marker: When the restored vaccine is inoculated into guinea-pig kidney cell culture and incubated at 30°C and at 40°C, the virus titer of culture fluid shall be higher at 30°C than at 40°C. Test for G-marker: In the test, the virulent hog cholera virus of 20th passage level in swine testicle cell culture shall be prepared as control. When the restored vaccine and virulent virus are respectively inoculated into guinea-pig kidney cell culture and incubated at 30°C, the difference in virus titer of culture fluids of vaccine and virulent viruses shall be 10^2 $TCID_{50}$ or above.

Comparison with lapinized hog cholera virus

A comparison between the GP vaccine and the vaccine prepared from lapinized LPC strain (more than 800 passages in rabbits) indicated that both strains were safe to use and provided a high degree of immunity. From the comparative studies on the pattern of viral multiplication in the body of pigs, both vaccine viruses were detected from almost the same organs. The virus was found most consistently in the tonsils and continued to show a higher titer than in other organs. The lymphatic tissues were major sites for virus replication. Virus was recovered from feces and urine of 6 of 12 pigs inoculated with the GP vaccine within 10 days post-inoculation, but no virus was recovered in 6 pigs vaccinated with the lapinized virus. No pathogenic recovery was found when both strains were respectively passed for 20 serial back passages in SPF pigs. No significant differences in morbidity and mortality between either vaccine were observed in the field test using approximately 1,000 pigs.

Practical application

In Japan, the GP vaccine was introduced for nation-wide vaccination in 1969. The vaccine was prepared in a freeze-dried form by lyophilization. A sterile diluent was supplied to restore the vaccine for use. One milliliter was injected into one pig, regardless of body weight. As the $TCID_{50}$ seemed to be 1 log lower than the titer determined by the pig inoculation test, one dose of vaccine contained 10,000 pig infectious doses. Under dark refrigerated conditions, the vaccine was found to be stable at least 2 years after lyophilization.

There was no significant difference in antibody response between pigs vaccinated by the subcutaneous route and by the intramuscular route. Intranasal inhalation was less effective in causing antibody response of vaccinated pigs. On the basis of nation-wide survey of the maternal immunity in piglets, the appropriate time of vaccination in piglets was found to correspond to the age of one month, at least.

The vaccine has been inoculated practically to more than seventy million pigs including pregnant sows up to date. No overt post-vaccinal side effect has been reported after application of the GP vaccine. Annual outbreaks of hog cholera have decreased markedly after the application of the GP vaccine. Only 16, 4, 10, 2 and 17 outbreaks were observed in 1971, 1972, 1973, 1974 and 1975 respectively. No outbreak has been recorded since December 1975.