Production of Rinderpest Tissue Culture Live Vaccine

By AKIRO SONODA

Second Research Division, National Institute of Animal Health
(Yatabe, Ibaraki, 305 Japan)

Rinderpest is an acute, febrile, highly contagious viral disease of most artiodactyla such as cattle, water buffaloes, sheep, goats, deer and swine. Especially, the disease of cattle has been one of the most devastating of all infectious diseases in the world with high morbidity and mortality.

In the past, the disease prevailed three times throughout Japan during about fifty years between 1872 and 1922 and approximately 80,000 animals were lost. The disease has not occurred in Japan since 1923, but it is still common in some areas of Asia and Africa, inflicting great damages on animal husbandry.

In enzootic areas, the disease rarely spreads among pigs, sheep, goats and camels, and it has been known that wild animals such as wild pigs, deer, antelope and giraffes are also susceptible to the disease.[24] Japanese and Korean native cattle are highly susceptible to rinderpest. Therefore, if the disease invades from abroad, it is in danger of spreading throughout the country in a short period.

Although rinderpest has not occurred among animals in Japan since 1923, a tissue culture live vaccine has been produced by National Institute of Animal Health and the doses for 100,000 animals are stored for emergency use.

The outline of the tissue culture live vaccine which was developed by the tissue culture technique in Japan is given in this paper.

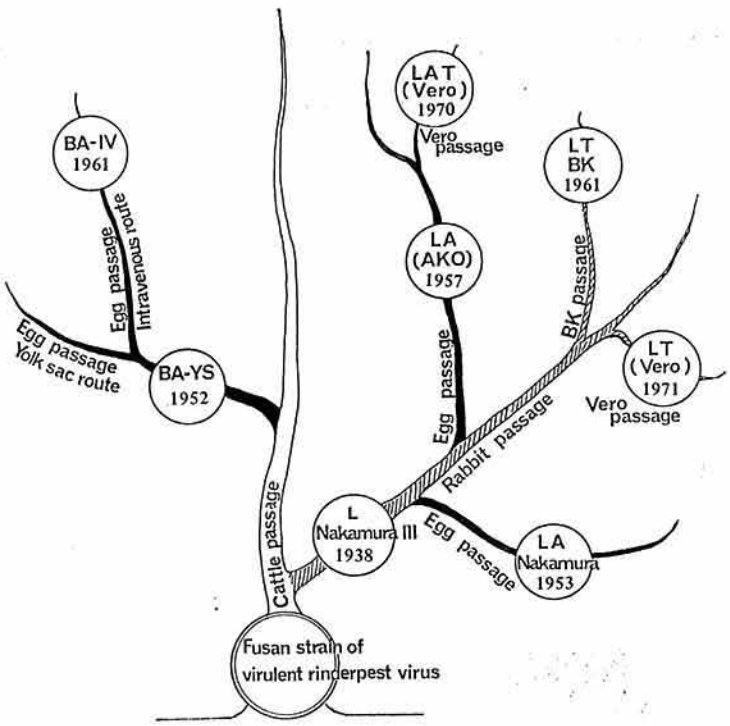
History of rinderpest vaccine in Japan

Preventive measures against rinderpest in Japan were studied and improved since the severe occurrence of the disease late in the 19th century. In the early period, injection of serum from convalescent cattle or injection of immune serum were attempted to protect cattle from the disease and then, serum-virus simultaneous injection method of immunization was used.[23] After that, inactivated vaccines were devised using the pulp of spleen and lymphnodes from experimentally infected cattle treated with inactivating chemicals such as phenol, glycerol and toluol.[12,13] They were, however, too much costly and the immunity they induced lasted for only a short period. Thereafter, many studies have been done to develop living virus vaccine capable of conferring active and solid immunity for long period on animals.

In Japan, starting from the Fusan strain of virulent virus, various attenuated viruses have been developed for the purpose of finding a suitable vaccine virus for indigenous Japanese black cattle.

Fig. 1 is the schematic presentation of developmental history of various attenuated rinderpest viruses which were established with the Fusan strain as starting virus.[36]

Nakamura et al. were the first to attempt the attenuation of the Fusan strain of virulent rinderpest virus by using the method of serial passage of the virus in rabbits. In 1938, they established the so-called Nakamura-III strain of lapinized (L) virus.[15] Since then, the Nakamura-III strain has been used widely for the control of rinderpest in many countries.[1,2,9,25] However, cattle indigenous to Japan and Korea are extremely susceptible to rinderpest virus.[4,17] So that sometimes they are involved in clinical infection and die



L	:	The Nakamura-III strain of lapinized virus.
LA-Nakamura	:	The Nakamura line of LA strain of lapinized-avianized virus.
LA-AKO	:	The AKO line of LA strain of lapinized-avianized virus.
LAT	:	The Vero cell adapted strain of lapinized-avianized virus.
LT-BK	:	The bovine kidney cell adapted strain of lapinized virus.
LT-Vero	:	The Vero cell adapted strain of lapinized virus.
BA-YS	:	The yolk sac line of BA strain of avianized virus.
BA-IV	:	The intravenous line of BA strain of avianized virus.
BK	:	Bovine kidney cell culture.

Fig. 1. Phylogenetic tree of attenuated variants originated from Fusan strain of virulent rinderpest virus

after inoculation with L virus.[3]

In order to develop a more attenuated strain than the Nakamura-III strain avianizations of L virus were attempted through serial passages in eggs by intravenous inoculation in the chorioallantoic membrane. Two strains of lapinized-avianized (LA) virus, LA strain by Nakamura et al.[19] and LA–AKO strain by Furutani et al.,[6,7] were developed in 1953 and 1957, respectively.

On the other hand, the direct avianization of virulent virus was attempted for the same purpose and two strains (BA–YS, and BA–IV) which showed a similar degree of attenuation to LA virus were developed from the Fusan strain of virulent virus by Ishii et al.[8] in 1952 (BA–YS) and by Isogai et al.[11] in 1961 (BA–IV), respectively. On the other hand, there are tissue culture adapted strains of lapinized (LT)[10] and lapinized-avianized (LAT)[27] viruses, respectively, at present.

Tissue culture live vaccine

Since Japanese native cattle are highly susceptible to rinderpest, the LA virus, which is

the lowest in pathogenicity for animals among all the attenuated rinderpest viruses, has been used as a seed virus for the preparation of the vaccine now for emergency use in Japan.

Previously, a living virus vaccine had been prepared in Japan by making the LA virus multiply in embryonating eggs, and stored for emergency use. Many research workers confirmed that the LA virus is safely and effectively applied as a vaccine for Japanese cattle.[5] There were, however, some critical points from the standpoint of expense, potency, safety and preservability etc., in the production of this vaccine.

The research on tissue culture live vaccine was started to develop a vaccine that is easy and economical to produce and high in keeping quality. It has been known that LA virus can grow in several kinds of cells, as shown in Table 1, especially the virus multiply well in skin and muscle cells of chick embryo (CE) and Vero cells which have been established from African green monkey kidney cells (Table 1).[10,27] Accordingly, a study on tissue culture vaccine was carried out for many years to make it possible for LA virus to multiply in Vero cells or CE cells.

Table 1. Susceptibility of various cell cultures to lapinized-avianized rinderpest (LA) virus

Cell type	Virus growth	Cytopathic effects
Bovine embryo kidney	+	+
Bovine testis	+	+
Bovine leukocyte	±	±
Rabbit kidney	−	−
Chick embryo	+	+
Swine embryo kidney	+	+
Vero cell	+	+

In 1976, a new type of tissue culture live vaccine using LA virus multiplied in Vero cells was devised by the author and his associates.[27,28,30] Since then the tissue culture live vaccine has been produced replacing egg vaccine. The product of this vaccine which is packed with pure nitrogen gas is prepared, by freeze-drying, from an emulsion of tissue culture of Vero cells infected with AKO strain of LA virus.

1) Ingredients and quantity

The vaccine consists of a freeze-dried suspension of living attenuated virus and a required diluent. One vial of the freeze-dried live vaccine is to be used for twenty doses after dissolving into 20 m*l* of the required diluent.

One vial of the freeze-dried live vaccine consists of following ingredients;

LA virus in Vero cell culture fluid	1 m*l*
Sucrose (extra pure)	100 mg
Sodium L-glutamate (extra pure)	10 mg
Gelatin	10 mg
Kanamycin sulfate	0.02 mg

One vial of the required diluent contains 20 m*l* of a solution of following ingredients;

Sodium chloride (extra pure)	8.1 g
Potassium phosphate, Monobasic (extra pure)	0.254 g
Sodium phosphate, Dibasic (extra pure)	1.286 g
Phenol Red (extra pure)	10 mg
Refined water	1,000 m*l*

The reconstituted vaccine should contain a virus titer of more than 10^3 $TCID_{50}$ or 10^3 EID_{50} per dose.

2) Seed virus of the vaccine

The AKO strain of LA virus has been used as an original seed of this vaccine. The original seed is prepared and stored by the National Institute of Animal Health. It is of sufficient volume to permit multiple use for several years. This method makes it possible to reproduce the vaccine at the same passage level necessary for demonstrating consistency. The AKO strain is inoculated intravenously into 11 to 12-day-old embryonating chicken eggs. Five days after the inoculation, the infected embryos are harvested and then the virus suspension is prepared from the infected embryos after discarding the head, wings and legs. The virus suspension can be freeze-dried with suitable stabilizers and stored at a temperature lower than −20°C or, if not freeze-dried, stored below −60°C. The original seed should be free from detectable ex-

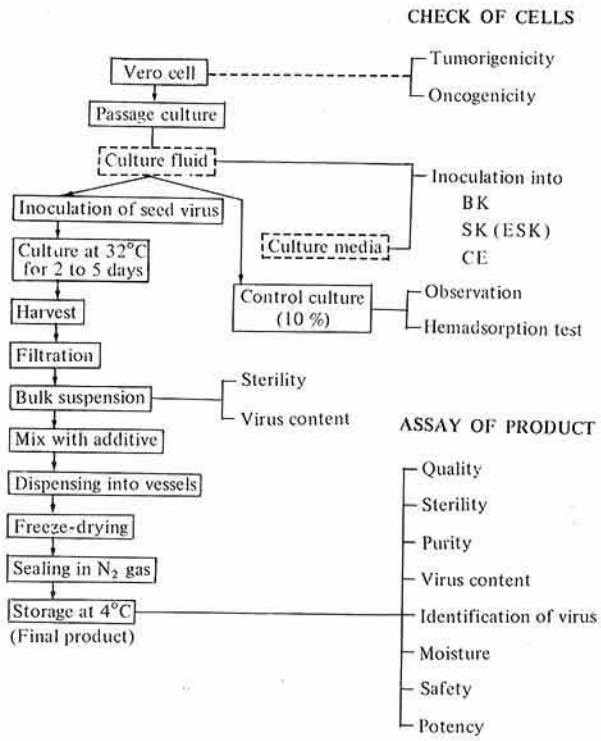


Fig. 2. Outline of mass production and quality control of rinderpest tissue culture vaccine

traneous microorganisms and satisfy all the requirements for seed virus. The original seed which has been freeze-dried with suitable stabilizers and sealed *in vacuo* should show no fall in infectivity over periods of at least five years at temperatures below −20°C. The original seed should contain at least $10^{5.0}$ $TCID_{50}$ per m*l* or 10^{4} EID_{50} per 0.05 m*l*.

The seed virus to be directly used for manufacturing the vaccine, is prepared with serial passages of original seed virus in Vero cell culture. The seed virus, however, should not be of more than five tissue culture passages from the original seed.

The seed virus should be tested for sterility, purity and virus titer, and should contain at least $10^{5.0}$ $TCID_{50}$ per m*l* or $10^{4.0}$ EID_{50} per 0.05 m*l*.

3) Production

The outline of the manufacturing process and assay of tissue culture live vaccine of rinderpest is shown in Fig. 2.

Vero cells that multiplied and formed cell monolayer in rolling bottle are used for the manufacture of the vaccine.

At least 5% of the bottle culture is set aside as control. On the day of inoculation with the seed virus these control cultures are incubated without the inoculation under the same conditions as the inoculated cultures and kept for at least 7 days, to examine cytopathological changes. The cell fluids from such control bottles are tested for adventitious virus by inoculating them to cell cultures of bovine kidney, swine kidney and chicken embryo. The cell sheet of the control are examined for presence of hemadsorption viruses by addition of guinea-pig and goose red blood cells.

The cell maintenance medium which contains at least 10^{3} $TCID_{50}$ per m*l* of seed virus is added into cell culture bottles, to replace

old cell fluids.

After the virus inoculation, bottles are then maintained at about 32°C with slow mechanical rolling. When the remarkable cytopathogenic effect (CPE) appears in all the cell sheets in which the highest level of virus activity is to be maintained, from 2nd to 5th day of virus inoculation, the cell fluid containing infected cells is harvested and a part of it is used as a sample for safety testing as described below. The pooled virus suspensions is then passed through sterile wire gauze with a 120–150 mesh (the bulk suspension).

The bulk suspension is prepared from a number of single harvests collected on the same day with addition of the equal volume of suitable stabilizer. The stabilizer for the vaccine contains 10% of sucrose, 1% of sodium L-glutamate, 1% of gelation and 0.002% of kanamycin. The bulk suspension is then tested for sterility, virus titer and purity. After testing, the bulk suspension is dispensed into sterile vials with 2 m*l* of volume and lyophilized. After freeze-drying, the vials are filled up with pure nitrogen gas sterilized through filter before sealing.

The required diluent is prepared by mixing 28 m*l* of 1/15 M potassium phosphate, 72 m*l* of 1/15 M sodium phosphate, 900 m*l* of 0.9% sodium chloride and 1 m*l* of 1% phenol red. The pH is adjusted to 7.2–7.4. The mixture is dispensed in 20 m*l* amounts in vials, sealed with rubber stopper, and then sterilized by autoclaving.

4) Assay

Two points must be checked for this vaccine. Firstly, it is necessary to examine the cells used for the production of vaccine, and secondly the inspection of the product itself must be made.

Those cells must be checked by practicable methods to confirm the normality of the cells and the absence of any adverse agent. Vero cell line to be used for vaccine production must also be examined for the absence of tumorigenicity and oncogenicity to confirm that they are cells of an established line.

As shown in Fig. 2, the final product shall meet the following tests according to the "minimum requirements for biological products for animal use" issued by the Japanese Government.[36]

(1) Property test and moisture test of the dried vaccine, before suspending into the attached diluent.

(2) Potency test, sterility test, identity test, marker test, purity test, safety test and test for virus content of the vaccine, after suspended into the attached diluent.

It is regarded as final product only when it successfully passes these tests mentioned above and stored at 4–10°C.

The more details of these tests are as follows;

Property test: The dried vaccine shall be a dried mass of a grey-white color with a light yellowish brown tint. The attached diluent shall have such components as appropriate to it and contain no foreign substance. When the dried vaccine is added with the diluent and shaken, it is easily made a homogeneous suspension with reddish orange color. The suspension should contain no coarse particles or foreign bodies. Moreover, there should be no remarkable differences in the properties of the dried vaccine and the prepared suspension among different final containers on test.

Moisture test: The moisture of the dried vaccine should not be more than 3% when determined by the desiccation method at 60°C under reduced pressure (Abderhalden's method).

Sterility test: The reconstituted vaccine (prepared suspension) should not contain any living microorganisms which can be detected at all.

Virus content test: The prepared suspension should be potent enough to produce LA virus infection in chick embryos when it is inoculated intravenously to them in a dose of 0.05 m*l* of 1:10 dilution, and/or, the prepared suspension should have a virus titer of more than $10^{3.3}$/0.1 m*l* $TCID_{50}$ in cultures of Vero cells or chick embryo cells or bovine kidney cells.

Identity test: The vaccine virus is neutralized well by known reference antiserum of

rinderpest.

Safety test: The prepared suspension should not induce any abnormal changes in small laboratory animals inoculated or any pronounced reaction in cattle inoculated.

Potency test: The vaccine should be proved to possess an antibody titer not lower than 1:10 in serum dilution when subjected the neutralization test with serum harvested three weeks after inoculation of the vaccine.

Marker test: Two homogeneous suspensions of 1:10 and 1:100 dilutions are prepared from the dried vaccine by using the attached diluent and 0.05 m*l* each of resulting suspension are inoculated into the vein of the chorioallantoic membrane of 11- to 12-day-old of embryonated eggs. More than 10 eggs are used for each dilution.

All the inoculated eggs shall be reincubated at 38°C for 5 days. When the eggs are opened 5 days after inoculation, splenomegaly (a spleen weighing more than 15 mg) can be noticed in more than 20% of the surviving embryos.

5) Application and dosage

The tissue culture live vaccine is prepared in a freeze-dried form by lyophilization. The vaccine is used to afford the active immunization of animals such as cattle, water buffaloes, sheep and goats, and pigs against rinderpest.

A sterile diluent is supplied to restore the vaccine for use. The official expiration period of this vaccine is specified as two years from the date of manufacture when it is stored under dark refrigerated condition, although it was proved that LA rinderpest virus can be stored stably for at least 6 years.[34,35]

Before inoculation the content of a dried vaccine vial should be dissolved in the diluent of one vial (20 m*l*). Dilution of the vaccine must be carried out immediately before administration and the reconstituted vaccine should be kept on ice and used within several hours after the dissolving. One m*l* is injected subcutaneously into an animal, regardless of body weight.

The vaccination is harmless. Vaccinated animals do not excrete virus.

Sonoda et al. compared the efficacy of the tissue culture live vaccine and the conventional egg vaccine using Holstein-Friesian and Japanese Black cattle. It was clarified that all the animals exhibited a similar reaction to the inoculation, regardless of the type of vaccine inoculated, and that they were immunized within 24 days after the inoculation[32]. It has also been confirmed in their experiment that Yorkshire pigs which were inoculated subcutaneously with 10^2 $TCID_{50}$ (1/10 of dosage) or 10^4 $TCID_{50}$ (10 times of regular dose) of the vaccine were immunized 21 days after the inoculation, without manifesting any clinical reaction.[33]

It is considered that the vaccine will confer sufficient immunity on cattle and be safely applicable to Yorkshire pigs.

Practical application of vaccine

Recently, several different types of attenuated living vaccine are widely used in the world. The various living vaccines which were prepared from attenuated viruses, such as goat-adapted or caprinized (C), rabbit adapted or lapinized (L), lapinized-avianized (LA) and tissue culture adapted viruses,[22,30] are used according to the species and breed of animals.

In ascending order of attenuation for cattle, the C virus is the shallowest one among attenuated viruses and then follows lapinized and tissue culture passaged L viruses. LA or BA strains are the most attenuated among them.

On the other hand, it has been known that the susceptibility of animals to rinderpest varies according to the species and breeds.

Nakamura suggested by data obtained either in experiments or in field experiences that some breeds of cattle, buffaloes and pigs may be graded in the order shown in Fig. 3 as to the susceptibility to different strains of rinderpest virus.[14,20,21]

The caprinized virus is safe and strongly immunogenic in native cattle in Southeast Asia, zebu cattle, Egyptian buffalo and York-

shire pig, but often produces fatal reaction in other animals. The lapinized virus is safe and effective in Asian water buffalo, European cattle and Asian cattle, but has some risk of provoking severe reaction in Holstein-Friesian and Japanese and Korean cattle.[25,26,31] The LA virus is applicable with safety to all animals.[5,29,32]

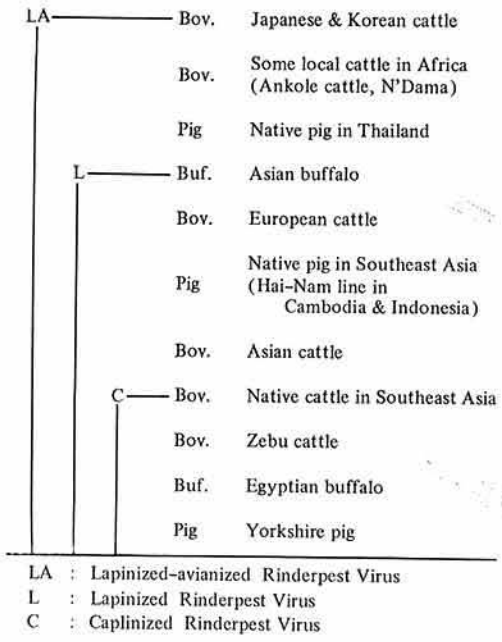


Fig. 3. Applicability of live virus vaccines to animals of different susceptibility.

It is advisable to carry out some preliminary experiments to check the safety and efficacy of the vaccine for local animals to which the vaccine is to be applied for the first time.

It is also recommendable that in a certain area in the world where rinderpest has occurred, a vaccine suitable to susceptibility of the respective animals kept in that area should be used after being selected from living vaccines produced by attenuating virus to various degrees.

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