

Comparison between the Antigenicity of Two Rinderpest Vaccine Strains using the Neutralization Test

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Rinderpest (RP) virus is classified into the Morbillivirus genus belonging to Paramyxovirus family. It is generally considered that RP virus has only one serotype. However, it has been reported by some researchers that there are some antigenic differences among RP virus strains [1, 4, 6, 9]. In this report, the two RP vaccine strains, Kabete "O" (RBOK) strain [5] and lapinized and avianized (LA-AKO) strain [2] were compared by neutralization test (NT) using the sera from calves vaccinated with each live vaccine. RBOK strain is most commonly used in RP epidemic countries worldwide, while Vero cell-adapted RBOK strain was recently developed for vaccine use [3]. On the other hand, the LA-AKO strain has been used in Japan as a tissue culture vaccine produced in Vero cells [8].

RBOK strain which had undergone 95 passages through bovine kidney (BK) cells was kindly supplied by the Animal Virus Research Center, Pirbright, England, and subcultured once in BK cells in our laboratory. LA-AKO strain has been stored as an original vaccine seed which had undergone 456 passages through embryonating chicken eggs in our laboratory and further 5 passages in Vero cells.

In this experiment, Vero cells were used for infectivity titration, NT, and the propagation of both strains. The growth medium used for the Vero cells was Eagle's minimum essential medium (MEM) containing 10% tryptose phosphate broth (TPB) and 10% calf serum while the maintenance medium was Eagle's MEM containing 10% TPB and 2% calf serum.

Two calves weighing 120 and 150 kg were used for experimental infection. They were subcutaneously inoculated with each strain. RBOK ($10^{4.25}$ 50% tissue culture infectious dose) or LA-AKO ($10^{4.00}$ TCID₅₀) and bled every 7 days up to 21 days. The sera obtained were stored at -20°C and inactivated at 56°C for 30 min before use.

NT was performed using both the virus dilution and serum dilution methods. The virus dilution method was carried out as follows. The immunized sera taken from calves 21 days after inoculation and normal calf serum were diluted 1:10 with maintenance medium. Equal quantities of the diluted serum thus prepared and the serial tenfold virus dilutions were then mixed. After storage at 4°C for 2 hrs, 0.1 ml of each of these mixtures

was inoculated into Vero cell cultures using 8 cultures per mixture. After the adsorption period of 1 hr at 37°C, 0.5 ml of maintenance medium was added to all the culture tubes, and they were incubated in a roller drum at 37°C for 7 days. Infectious titers of each sample were calculated using the Behrens-Kärber method, and expressed a TCID₅₀/0.1 ml. Neutralization indices (NI) were expressed by differences in the logarithms of TCID₅₀ between samples of immunized sera and normal serum.

For the serum dilution method, the procedure described by Sonoda [7] was modified slightly before use. Sera were sequentially diluted twofold from 1:5 to 1:1280 in a maintenance medium. 0.3 ml of each serum dilution was then mixed with an equal volume of virus solution containing 200 TCID₅₀/0.1 ml. After storage at 4°C for 2 hrs, 0.1 ml of the mixture was inoculated into a tube of Vero cell culture. Four tubes were used for each dilution. The conditions of both adsorption and incubation were identical to those of the virus dilution method. The neutralizing antibody titer in the serum dilution method was expressed as the reciprocal of the highest dilution which had inhibited cytopathic effect in 2 or more of 4 tubes.

The results of NT by the virus dilution method are shown in Table 1. Anti-RBOK serum neutralized the more strongly homologous strain, but not so much the LA-AKO strain. The difference in the NI of anti-RBOK serum against both strains was 3.250. On the other hand, NI of anti-LA-AKO serum against both strains were almost identical. NI of this serum was found to be a little higher against the homologous as opposed to the heterologous strain. The difference in the NI of anti-LA-AKO serum against both strains was only 0.875.

The results of NT by the serum dilution method are shown in Table 2. Anti-RBOK sera (calf No. 86-2) obtained every weeks were tested against both the homologous (RBOK) and heterologous (LA-AKO) strains. On the 14th day, the neutralizing antibody was seen to appear against the RBOK strain but not against the LA-AKO strain, while on the 21st day, antibodies

Table 1. Neutralization index using RBOK strain and LA-AKO strain

Immunized calf serum		Virus strain	
		RBOK	LA-AKO
Anti-RBOK	(86-2, 21d) ^{a)}	4.375	1.125
Anti-LA-AKO	(86-11, 21d)	3.125	4.000

a) Calf number and days after inoculation.

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Table 2. Development of neutralizing antibody in calves inoculated with RBOK strain or LA-AKO strain against both strains

Calf No.	Virus strain inoculated	Days after inoculation	Neutralizing antibody titers against	
			RBOK strain	LA-AKO strain
86-2	RBOK	0	<5 ^{a)}	<5
		7	<5	<5
		14	20	<5
		21	80	5
86-11	LA-AKO	0	<5	<5
		7	<5	<5
		14	20	40
		21	20	40

a) Reciprocal of the highest serum dilution that inhibited a cytopathic effect.

were detected against both strains. The neutralizing antibody titer was high (80) against the RBOK strain but low (5) against the LA-AKO strain. Meanwhile, the neutralizing antibody of anti-LA-AKO sera (calf No. 86-11) was also detected against both strains on the 14th day, and the titers against both viruses were relatively similar, that against the homologous strain only being twice of the heterologous strain.

Several investigators demonstrated some antigenic differences among RP virus strains by NT, using immunized rabbit sera [1, 6] or bovine sera [4]. In addition, Tokuda [9] reported some antigenic differences even among attenuated strains derived from a virulent strain. In this investigation, the difference between the RBOK and LA-AKO strains was detected by cross neutralization tests. It is notable that while anti-RBOK serum showed low cross reactivity with LA-AKO strain, anti-LA-AKO serum reacted sufficiently with RBOK strain. This seemed to be a one way cross reaction.

LA-AKO strain might be more suitable as the virus for the immunization, because it is capable to provoking the antibodies against both strains. Moreover, anti-LA-AKO serum would be preferable to anti-RBOK serum for the detection or identification of RP virus. However, the LA-AKO strain is not appropriate for use as the virus to detect neutralizing antibody, because a low titer in the serum of cattle infected with the RBOK strain might be

not detected by this strain.

These results suggested that the strains used for vaccine production or neutralization test had to show wide cross reactivity with field viruses or be capable of detecting low antibody titers. Appropriate strains should be selected based on a consideration of the antigenicity of field viruses. In the future, details of variations in the antigenic determinants producing the neutralizing antibody should be analyzed using monoclonal antibodies against RP viruses.

REFERENCES

1. De Boer, C. J. 1961. *Arch. Virol.* 11: 534-543.
2. Furutani, T., Kataoka, T., Kurata, K., and Nakamura, H. 1957. *Bull. Natl. Inst. Anim. Health* 32: 117-135.
3. Mariner, J. C., House, J. A., Sollod, A. E., Stem, C., van den Ende, M., and Mebus, C. A. 1990. *Vet. Microbiol.* 21: 195-209.
4. Plowright, W. 1963. *Res. Vet. Sci.* 4: 96-108.
5. Plowright, W. and Ferris, R. D. 1962. *Res. Vet. Sci.* 3: 172-182.
6. Rossiter, P. B., Taylor, W. P., and Crowther, J. R. 1988. *Vet. Microbiol.* 16: 195-200.
7. Sonoda, A. 1971. *Natl. Inst. Anim. Health Q. (Jpn.)* 11: 64-74.
8. Sonoda, A. 1983. *JARQ* 17: 191-198.
9. Tokuda, G., Fujisaki, Y., and Watanabe, M. 1967. *Jpn. J. Vet. Sci.* 29: 79-80 (in Japanese).