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Effect of complete adjuvant on vaccination of live attenuated Japanese encephalitis vaccine to swine for preventing viremia. 39. Epidemiological studies of J.E

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Abstract

In an attempt to eliminate Japanese encephalitis virus in natural surroundings, pigs having maternal antibody were given inoculation of live-attenuated Japanese encephalitis vaccine and injection of Freund's complete adjuvant simultaneously. Titer of hemoagglutination inhibiting antibodies of pigs inoculated with live attenuated vaccine and complete adjuvant, was higher than that inoculated with vaccine alone and its titer persisted.

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**EFFECT OF COMPLETE ADJUVANT ON VACCINATION OF
LIVE ATTENUATED JAPANESE ENCEPHALITIS VACCINE
TO SWINE FOR PREVENTING VIREMIA (REPORT 39 OF
EPIDEMIOLOGICAL STUDIES OF J. E.)**

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Many ecologic studies (1, 2) of Japanese encephalitis (JE) virus have recognized that swine in Japan is naturally infected in high incidence and is an important natural source of virus for *Culex tritaeniorhynchus* proved to be the vector mosquitoes. Therefore, it is first necessary to prevent viremia in swine to eliminate the natural source of infection.

Freund's complete adjuvant was widely used for yielding high antibody in rabbits and guinea pigs (3—6). And we have reported that the swine inoculated inactivated JE vaccine supplemented with complete adjuvant showed higher titers of hemoagglutination inhibiting (HI) and neutralizing (NT) antibody, than those swine inoculated JE virus alone (7). And also we described that occurrence of viremia in swine caused by natural infection can be protected by antibody induced by inoculation of vaccine (8).

On the other hand, KODAMA, SASAKI, and INOUE (9) inoculated pigs with live-attenuated mutant strain (Mukai strain) of JE virus and found that the strain produced no detectable viremia and was able to evoke both HI and NT in colostrum deprived pigs after a single subcutaneous injection. However, the antibody response in the swine having maternal antibody by the use of live-attenuated vaccine (Mukai strain) is found to be absent (10).

In the present study, the effect of complete adjuvant on antibody response of swine, having maternal antibody and receiving inoculation

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with a new strain of live-attenuated vaccine was investigated. This report describes briefly our results.

MATERIALS AND METHODS

Materials

JE vaccine: Live-attenuated vaccine prepared by tissue culture was donated by Dr. KAWAKUBO of Nissei Research Institute. The titer showed 10^9 /ml TCID₅₀ units for HK cells.

Pigs: Pigs, 1.5 month old, showing 1:40 of HI antibody titer were employed. These animals were kept on Wake district, Okayama Prefecture, where JE virus is epidemic every year.

Method

Vaccination: In the group inoculated with JE vaccine alone, pigs were inoculated once with 3 ml live-attenuated JE vaccine intramuscularly in the neck. In the group inoculated with JE vaccine and injected with complete adjuvant, pigs were inoculated with 3 ml live-attenuated JE vaccine in the neck, and injected simultaneously with 3 ml complete adjuvant intramuscularly, on the other side of the neck.

Collection of blood: The blood of pigs was obtained from auricular vein at one-week interval after inoculation.

Determination of HI antibodies in the serum: Antibody titers in the sera of pigs were tested by the technique of the HI test, following the modified method of CLARK and CASALS (11). The hemagglutination antigen used was JaGAR strain (Takeda Co.) purified with ether after suckling mouse brain passage.

RESULTS

Serum samples were obtained before inoculation and on day 9, 19,

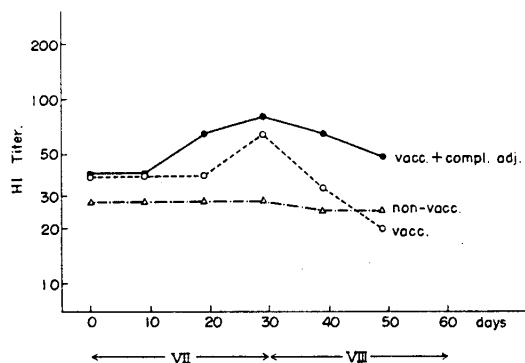


Fig. 1 Changes in HI antibody titers in the pigs inoculated with live-attenuated vaccine, injected with or without Freund's complete adjuvant simultaneously.

TABLE 1. VARIATION IN TITER OF HI ANTIBODY IN THE CASE OF LIVE-ATTENUATED VACCINE WITH OR WITHOUT COMPLETE ADJUVANT TO SWINE

methods	dete (D/M) ¹⁾ days after inocul. ²⁾	2/7 0	11/7 9	21/7 19	31/7 29	10/8 39	20/8 49
live attenuated vaccine + complete adjuvant	No. 1	40	40	40	40	40	20
	No. 2	40	40	80	80	80	80
	No. 3	40	40	80	160	80	80
	math. average	40	40	67	93	67	60
	n of 10×2^n	2.0	2.0	2.7	3.0	2.7	2.3
	Log-mean ³⁾	40	40	65	80	65	49
live-attenuated vaccine	No. 1	40	40	40	40	20	20
	No. 2	40	40	40	80	40	40
	No. 3	40	40	40	80	40	<20
	math. average	40	40	40	67	33	20 ⁴⁾
	n of 10×2^n	2.0	2.0	2.0	2.7	1.7	1.0 ⁵⁾
	Log-mean	40	40	40	65	33	20
non-vaccinated	No. 1	40	40	40	40	40	40
	No. 2	20	20	20	20	20	20
	No. 3	20	20	20	20	20	20
	No. 4	40	40	40	40	20	20
	math. average	30	30	30	30	25	25
	n of 10×2^n	1.5	1.5	1.5	1.5	1.3	1.3
Log-mean	28	28	28	28	25	25	

1) day/month 2) days after inoculation 3) mean of Log scale

4) calculated below 20 as zero 5) calculated below 20 as 10

29, 39 and 49. The results are shown in Fig 1 and Table 1.

Non-vaccinated group :

Level of HI titer of pigs was constant during the experiment.

The group receiving live-attenuated vaccine :

On 19 days after inoculation, pigs showed no detectable HI antibody response. On 29th day, the level of antibody attained its maximum, showing 1 : 67 antibody titer in average, then decreased gradually, and titer showed 1 : 33 on 39th day and 1 : 20 on 49th day.

The group receiving live-attenuated vaccine and adjuvant :

On 9th day from inoculation, pigs showed no detectable antibody response, and on 29th day, level of the antibody attained its maximum, showing 1 : 80 of antibody titer, then decreased very gradually and on 39th day, titer showed 1 : 65 and on day 49th, 1 : 49.

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DISCUSSION

In the previous report, we described that vaccination using inactivated JE virus vaccine supplemented with complete adjuvant yielded higher antibody response in pigs having a trace of or lacking maternal antibody than those inoculated JE virus alone. However, antibody response of pigs with maternal antibody was not recognized by the above method.

Up to date, the effect of live-attenuated vaccine has not been found in young pigs with maternal antibody. In this experiment, tendency of antibody response was found even in the young pigs. As the live-attenuated vaccine prepared by KAWAMURA was used instead of the MUKAI strain, the former will be more effective in producing antibody than the latter.

Data also indicate that the vaccination of live-attenuated vaccine and simultaneous injection of complete adjuvant in pigs yields higher titer of HI antibody and maintained at high level. Therefore, it is concluded that this method of simultaneous adjuvant injection is useful for inducing and maintaining high antibody titer for the vaccination of pigs using live-attenuated JE vaccine.

CONCLUSION

In an attempt to eliminate Japanese encephalitis virus in natural surroundings, pigs having maternal antibody were given inoculation of live-attenuated Japanese encephalitis vaccine and injection of Freund's complete adjuvant simultaneously. Titer of hemoagglutination inhibiting antibodies of pigs inoculated with live-attenuated vaccine and complete adjuvant, was higher than that inoculated with vaccine alone and its titer persisted.

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