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PROTECTION OF PIGS AGAINST MOSQUITO-BORNE JAPANESE ENCEPHALITIS VIRUS BY IMMUNIZATION WITH A LIVE ATTENUATED VACCINE

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Pigs were vaccinated with an attenuated Japanese encephalitis virus (JEV) vaccine and challenged with virulent JEV, either by subcutaneous injection or by exposure to infected mosquitoes. The vaccinated pigs developed circulating antibodies to JEV. After challenge they did not develop viremia detectable by inoculation of their serum in suckling mice. They were also unable to transmit virus to mosquitoes fed on their skin. In contrast, unvaccinated pigs, whether challenged by injection or by mosquito bites, developed viremia and did transmit virus to mosquitoes which were allowed to bite them. Transmission seemed possible for only 3 days post-infection.

Japanese encephalitis virus (JEV) vaccine pigs

INTRODUCTION

Japanese encephalitis (JE) is prevalent in summer in southeast Asia (Korea, China and Japan). In the southern part of Japan, a few cases occur every summer; the virus can also be isolated from mosquitoes. The disease is often fatal, and in cases where it is not, the patients are left with sequelae.

Control of the spread of JE by extermination of infected mosquitoes is impractical. An alternative approach has been suggested by studies indicating that pigs constitute an important amplifier host to the virus. Artificial immunization of the pig population could protect them from widespread infection during the season of high prevalence. Although the true reservoir of the virus would not directly be affected by this strategy, spread of the virus to man may be significantly reduced.

In fact, over the last 10 years, public health authorities in Kyoto City have attempted to reduce the prevalence of JE by inoculating all pigs (about 20,000) in the area with the live attenuated vaccine. However, at present there is insufficient experimental evidence to show that this strategy is truely effective in reducing the prevalence of JE in general.

We have already conducted some open field investigations in order to examine the effect of the pig vaccination [10]. The present study is intended to investigate the inhibitory effects of the live attenuated vaccine administration on virus proliferation in pigs bitten by mosquitoes artificially infected with JEV.

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EXPERIMENTAL

JEV-free pigs 1–2 months old were obtained from a non-endemic area in Japan. It was confirmed in preliminary experiments that they had no history of JEV infection.

Live attenuated vaccine (strain M-PK/L, Biken Laboratories, Japan) which was prepared according to the methods of Inoue [2,4,9], was used. The vaccine was inoculated subcutaneously in 2 ml of buffer solution.

JEV-infected mosquitoes were obtained by dipping larvae of colonized *Culex tritaeniorhynchus* for 30 min in a solution containing 10^6 suckling mouse lethal dose 50% ($SMLD_{50}$) of JEV (strain JaGAr-01, isolated in Gunma, Japan) [3]. In order to assess the infectivity rate, 22 out of the 826 available mosquitoes were chosen at random 7 days post-infection. Each insect was placed in a saran foil cage together with a chicken. After 60 min the mosquitoes were caught and homogenized individually in 1 ml phosphate-buffered saline. After centrifugation the supernatant fluids were tested for infectivity by intracerebral injection in suckling mice. Serum was obtained from the chickens before exposure to the mosquitoes and again 3 and 10 days post-exposure. Infectivity of the serum on day 3 was tested by intracerebral injection in mice. Hemagglutinin inhibition (HAI) antibody was determined on the samples obtained before and 10 days after exposure. These tests showed that 15/22 mosquitoes contained virus detectable by direct inoculation in mice; all these plus one extra mosquito (total 16/22) were able to transmit the virus to chickens, as evident from the presence of viremia and development of HAI antibody.

For exposure to mosquito bites, the pigs were kept lying on their back in an air-conditioned room at 28°C and 80% humidity. A cage (25 × 25 × 40 cm) made of saran foil containing the mosquitoes was placed on the animals' abdomens, which allowed the insects to bite through the saran net. The cage was covered with a black vinyl wrapper and was left in contact with the pigs for about 90 min.

After being challenged by the infected mosquitoes, the unvaccinated and vaccinated pigs were bled daily for 7 days. Undiluted sera were inoculated intracerebrally into suckling mice in order to determine prevalence of viremia. Furthermore, serially diluted sera were injected into mice and the virus titer in each serum sample was determined in LD_{50}/ml.

In order to assess the ability of the infected pigs to transmit JEV to uninfected mosquitoes, 60–100 of JEV-free mosquitoes were allowed to bite the infected pigs according to the methods described above. The mosquitoes were then kept at 28°C and 80% humidity for 1 week, and their infectivity was examined.

Vaccinated pigs, whether infected by subcutaneous injection or by exposure to infected mosquitoes, did not develop viremia detectable by either direct inoculation of serum in mice or by re-exposure to uninfected mosquitoes. In contrast, unvaccinated pigs developed viremia and transmitted virus to mosquitoes that were allowed to bite them. As can be seen in Table 1, viremia appeared on the first day after either type of challenge. It reached its highest level on the second and third day and then rapidly disappeared. On

TABLE 1

Viremia in JEV-infected pigs as tested by inoculation of serum in new-born mice and by assay of infectivity of mosquitoes fed on the pigs[a]

Pig No.	Infected by	Viremia on days:					
		1	2	3	4	5	6
1	Subcutaneous injection	1.3[b]	3.2	3.8	1.3	n.d.	n.d.
		(0)[c]	(33)	(6)	(0)	(0)	(0)
2	Mosquito-bites	3.2	3.7	2.2	1.3	n.d.	n.d.
		(9)	(19)	(0)	(0)	(0)	(0)
3	Mosquito-bites	2.6	4.2	1.5	n.d.	n.d.	n.d.
		(0)	(27)	(14)	(0)	(0)	(0)

[a] The experiment also included eight pigs vaccinated against JEV 3–4 weeks beforehand; two of these were challenged by subcutaneous injection of the virus, six by exposure to infected mosquitoes. None of these developed signs of viremia by any of the two criteria used in the experiment.

[b] Log_{10} LD_{50}.

[c] % Mosquitoes infected after feeding on the pig.

the second day post-exposure each pig was able to transmit virus to a considerable percentage of mosquitoes to which they were exposed. Concordant with the decreasing viremia the ability to transmit virus to mosquitoes was reduced to zero after about 4 days.

The antibody responses of all pigs were examined with neutralization (NT) and hemagglutination inhibition (HAI) tests. The NT tests were done by plaque reduction assay according to Oya and Okuno [7] using primary chick embryo fibroblasts. HAI tests required prior removal of normal inhibitors and agglutinins from the serum samples by treatment with acetone and chick red blood cells. For the determination of NT and HAI antibodies, virus and HA antigens of the JaGAr-01 strain were used.

HAI antibody appeared immediately after vaccination and reached an average titer of 1/320 after 1 week. A transient rise in titer was observed after challenge of vaccinated pigs by infected mosquitoes (Fig. 1). This was considered to be a booster effect. The booster effect was perhaps less apparent in pigs challenged by subcutaneous injection of JEV. The results of NT tests (Fig. 2) were basically similar to those of HAI tests, although the booster effect in NT antibody production resulting from the mosquitoes was slightly more pronounced than that in HAI antibody production. HAI and NT titers rose rapidly when the unvaccinated pigs were challenged by either infected mosquitoes or subcutaneous injection. The JEV serum antibody titers were 4–6 times higher after either type of challenge by virulent JEV than after vaccination.

CONCLUSION

Our study shows that unvaccinated pigs are highly susceptible to JEV infection when they are exposed to bites from infected mosquitoes. Administration of an attenuated JEV

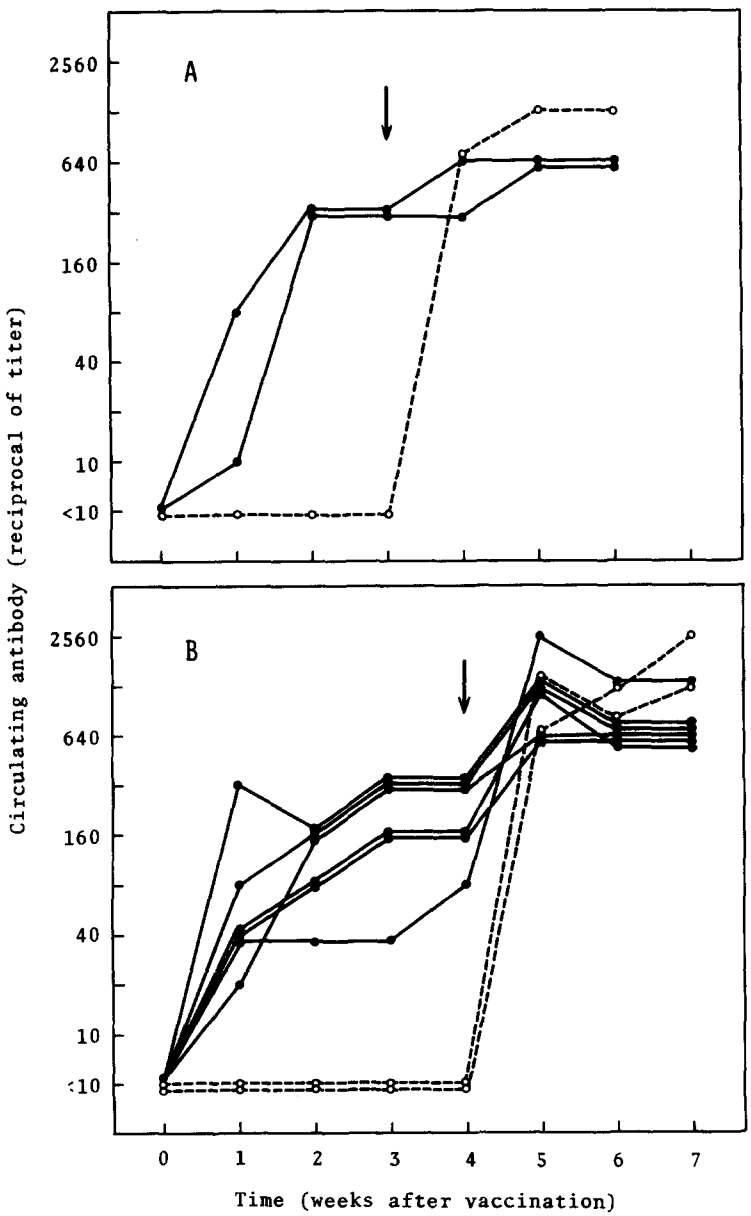

Fig. 1. Development of circulating HAI antibody in vaccinated and unvaccinated pigs, challenged with JEV. A) Challenge by subcutaneous injection. B) Challenge by exposure to JEV-infected mosquitoes. ●, Vaccinated pigs; ○, unvaccinated pigs; arrow indicates time of challenge.

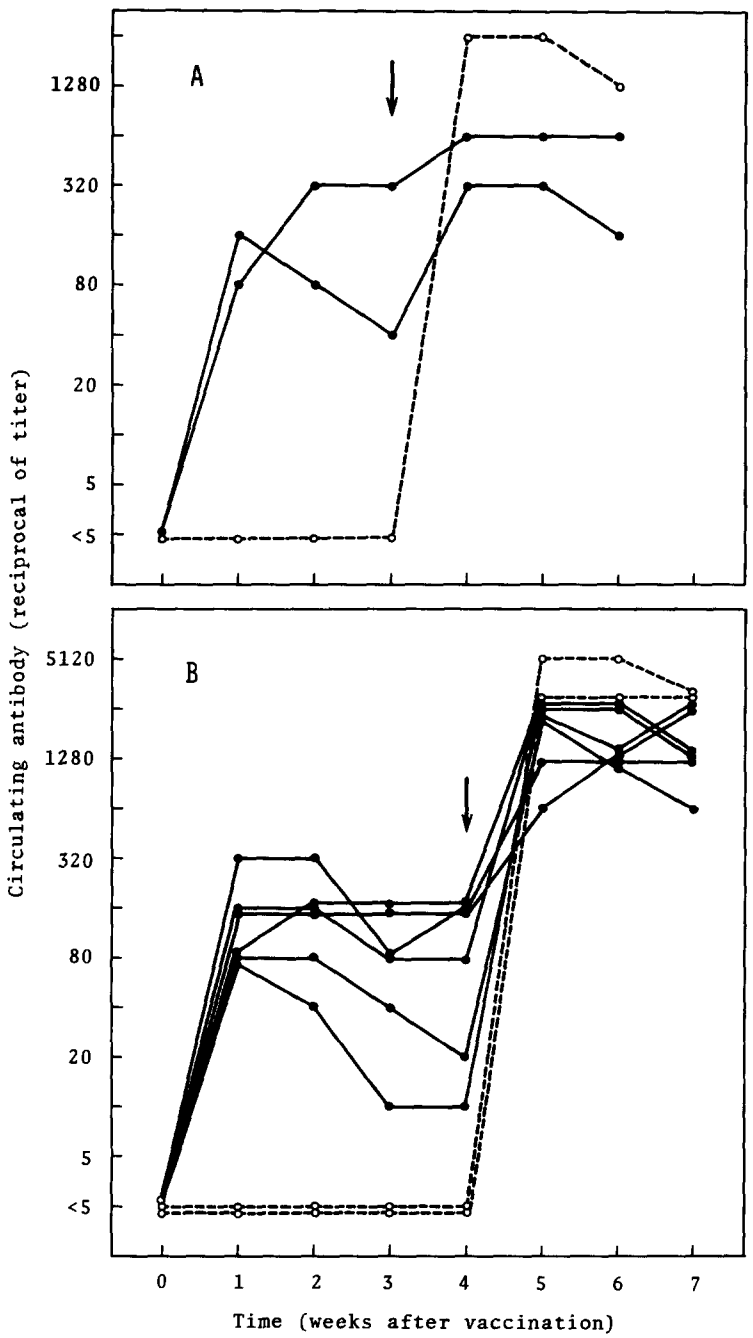

Fig. 2. Development of circulating NT antibody in vaccinated and unvaccinated pigs, challenged with JEV. A) Challenge by subcutaneous injection. B) Challenge by JEV-infected mosquitoes. ●, Vaccinated pigs; ○, unvaccinated pigs; arrow indicates time of challenge.

vaccine resulted in development of circulating HAI and NT antibody. High titers were maintained for over 7 weeks. These pigs were found to completely resist infection by the virulent JEV strain whether inoculated subcutaneously or by mosquito bites. Significantly, no viremia developed and the pigs were unable to infect mosquitoes that were allowed to bite them. Although in the present study resistance to infection was studied for a period of only 3–4 weeks, the maintenance of high antibody levels for over 7 weeks suggests that resistance to infection will also persist for at least 7 weeks. In this context it is of interest that in other studies high antibody titers have been reported to persist for about 80 days [4].

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