

## JAPANESE ENCEPHALITIS VIRUS INFECTION IN FETAL MICE AT DIFFERENT STAGES OF PREGNANCY I. STILLBIRTH

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Received June 23, 1981; revised October 20, 1981

*Summary.* — The relationship between the stage of pregnancy at the time of Japanese encephalitis (JE) virus inoculation and the frequency of stillbirth was investigated in mice. Pregnant mice inoculated at nine to sixteen days before parturition demonstrated a higher frequency of stillbirth (38%, 12/32) than mice similarly inoculated at one to eight (20%, 6/30) or at seventeen to twenty (14%, 2/14) days respectively before parturition. Stillbirth frequency in the control group was 1.4% (1/72). Five out of thirteen stillborn of mothers inoculated at nine to sixteen days before parturition were positive in virus isolation tests, and three others showed congenital malformations, anencephaly, cephalocele and underdevelopment below the waist.

*Key words:* Japanese encephalitis virus; mice; pregnancy fetal infection; stillbirth; malformation

### Introduction

Fetal disturbances caused by Japanese encephalitis (JE) virus infection during pregnancy have been reported in humans and animals. JE virus was isolated from brain, liver and placental tissues of fetuses aborted by pregnant women suffering from JE (Chaturvedi *et al.*, 1980). Among domestic animals, it is well known that JE virus infection in pregnant swine caused epizootic abortion and stillbirth (Kitaoka *et al.*, 1950; Burns, 1950; Shimizu *et al.*, 1954). In experimental animals, abortion, stillbirth and early death after birth among pregnant hamsters affected by JE virus infection has been reported (Takehara *et al.*, 1975; Tamura *et al.*, 1977). In mice, fetal infection in pregnant mother affected by JE virus infection has been described (Fujisaki *et al.*, 1976). However, the relationship between the stage of pregnancy at the time of JE virus infection of the mother and the frequency of stillbirth has not been clarified yet. This study was carried out to analyze the relationship between the stage of pregnancy at the time of JE virus inoculation and the frequency of stillbirth in mice.

### Materials and Methods

*Mice.* ICR mice reared at our laboratory were used.

*Virus.* JaGAR-01 strain of JE virus has undergone five intracerebral passages in suckling mice.

*Virus inoculation.* The virus was inoculated intravenously to pregnant mice in a single dose of  $10^{2.7}$  LD<sub>50</sub>/0.2 ml.

Table 1. Effect of intravenous inoculation of pregnant mice with JE virus on fetuses

Determination	Interval between JE virus inoculation and parturition			Control
	1-8 days A	9-16 days B	17-20 days C	
No. of mothers	30	32	14	72
No. of mothers with stillbirth (%)	6 (20 %)	12 (38 %)	2 (14 %)	1 (1.4 %)
No. of offsprings	347	365	181	857
No. of stillborn (%)	9 (2.6 %)	25 (6.8 %)	2 (1.1 %)	3 (0.4 %)
No. of offsprings per litter	11.6 ± 2.6*	11.4 ± 2.3	12.9 ± 2.0	11.9 ± 2.5
No. of stillborn tested for JE virus	4	13	2	—
No. of stillborn-JE virus isolated (%)	0	5 (38 %)	0	—

\* standard deviation.

*Stage of pregnancy at the time of inoculation.* The day of parturition of each mouse was taken as day 0, and the number of days between JE virus inoculation and parturition was counted. Four groups of pregnant mice were used in the experiments, one being the non-inoculated control group and three (A, B and C) being the inoculated groups. The mice of group A were inoculated at one to eight days, of group B at nine to sixteen days and of group C at seventeen to twenty days before parturition.

*JE virus isolation.* The test was carried out by intracerebral inoculation of suckling mice with the brain emulsion of each stillborn mouse. Those which showed typical symptoms were tested by the direct fluorescent antibody technique in order to identify JE virus antigen in the brains. FITC conjugated IgG prepared from rabbit anti-JE serum was used. Rabbits were immunized with the JaGAR-01 strain of the virus.

### Results

The percentage of mothers with stillbirth and the percentage of stillborn among newborn were higher in those groups inoculated with JE virus than in the control group (Table 1). Among the inoculated groups, group B demonstrated the largest percentage of mothers with stillbirth and the largest percentage of stillborn among newborn in relation to the total number of offsprings. There were, however, no significant differences in the average number of offsprings per litter by the time of virus inoculation. Among the stillborn in group B, there was one case of anencephaly (Fig. 1), one case of encephalocele (Fig. 2) and one case of underdevelopment below the waist (not shown). JE virus was isolated from the brains of five out of thirteen stillborn in group B (Table 1). Eight pregnant mice of group B were subjected to laparotomies one to two days before expected parturition. In one of these mice one live fetus was found, the other eight fetuses having died at various stages of growth. Some of the latter were dark in color and much smaller than the normal live fetuses (Fig. 3). In another pregnant mouse, three of the litter were of normal size but their entire skin surface was wrinkled (Fig. 4).

### Discussion

There were no significant differences in the average number of offspring per litter between three groups of pregnant mice inoculated with JE virus at different stages of pregnancy and one control group of non-inoculated pregnant mice. Group B, inoculated at nine to sixteen days before parturition, showed the highest rate of stillbirth and the highest rate of stillborn among newborn. Furthermore, JE virus isolation test of these stillborn led to the conjecture that such stillbirth were due to fetal JE virus infection. All stillborn of the groups inoculated at one to eight (group A) and at seventeen to twenty (group C) days before parturition were negative in JE virus isolation test. This indicated that the influence of JE virus infection on fetuses differed in accordance with the stage of pregnancy at which JE virus was introduced to the mother animals. Morphological abnormalities and abnormal appearance of skin colour were also observed in stillborn of mothers in group B.

It has been reported that a higher incidence of fetal infection occurred when C3H mice were inoculated with JE virus on the seventh day of pregnancy (Fujisaki *et al.*, 1976). Regarding hamsters, the highest stillbirth rate caused by fetal JE virus infection was observed among those inoculated on the fifth day of pregnancy (Takehara *et al.*, 1975). Fetal disturbances caused by other arbovirus infections during pregnancy have been reported in humans and animals. In regard to humans, cases with western equine encephalomyelitis virus infection have been reported (Mendvy, 1943; Shinefield and Townsend, 1953). Among experimental animals, when mice were intravenously inoculated with St. Louis encephalitis (SLE) virus, the highest incidence of fetal SLE virus infection and stillbirth occurred among those inoculated on the seventh to twelfth day of pregnancy (Andersen and Hanson, 1970; 1975). When mice were infected with tick-borne encephalitis virus on the eighth day of pregnancy, fetal infection occurred in fetuses of all pregnant mothers (Molnárová and Mayer, 1980). These reports indicate that the effects on fetuses of arbovirus infection during pregnancy differed according to the stage of fetal development at the time of inoculation, and our results correspond to these results.

The death of fetuses and the occurrence of mummified fetuses dark in colour also have been found to occur in swine infected with JE virus (Kitaoka *et al.*, 1950; Burns, 1950; Shimizu *et al.*, 1954). Such morphological abnormalities as anencephaly, encephalocele and underdevelopment below waist as seen in our experiment with JE virus also indicate that various malformations may occur due to JE virus infection during pregnancy.

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*Description of Figures* (Plate XXXVI):

*Figs. 1–4.* Fetal disturbances caused by JE virus infection in mice during pregnancy (the least scale in one millimeter).

*Fig. 1.* Anencephaly

*Fig. 2.* Encephalocele

*Fig. 3.* A live fetus (upper left) and eight fetuses having died at various stages of growth.

*Fig. 4.* Fetuses revealing wrinkled skin