ECOLOGY

Sexual Transmission of Tick-Borne Encephalitis Virus in Laboratory Mice

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Mating of males infected with tick-borne encephalitis virus (strain Sofyin) with intact females results in a marked decrease in the weight of embryos and an almost threefold increase in embryonal mortality. Viral RNA was identified by the polymerase chain reaction in embryonal tissues of 2 out of 11 litters. In one case the infection of the progeny was confirmed by biological test.

Key Words: *tick-borne encephalitis virus; route of transmission*

Stable circulation of tick-borne encephalitis (TBE) virus in natural foci is due primarily to transmission of the causative agent from *Ixodidae* to warm-blooded animals and vice versa [5,7]. On the other hand, the monitoring of parasitic systems indicates that many-year fluctuations in the number of TBE-positive animals in the populations of small mammals do not correlate with the number of ticks or their virusophoria [3]. Hence, the virus may circulate in populations of mammals by routes other than through ticks. For example, transplacental infection of embryos with TBE in experimentally infected pregnant females has been described [8].

In this study we elucidated the role of infected males in infection of progeny of laboratory mice.

MATERIALS AND METHODS

Swiss autbred mice aged 3-3.5 months were obtained from the vivarium of *Vektor* Research and Production Unit 4 weeks before experiments. The animals were kept at 20-22°C in standard cages, 5 females and 1 male per cage, 14 h light and 10 h darkness, and fed *ad libitum*.

Experimental males (n=28) were infected by subcutaneous injection of 0.25 ml of TBE virus (strain Sofyin) in medium 199. The dose of 100 LD_{50} for intracerebral injections to 2-week mice. After 7 days, 2 females in estrus were placed in to cage with an infected male. Vaginal smears for assessing the stage of estral cycle were analyzed 2-3 h before mating. The females were left in cages with males overnight for 15-16 h. The efficacy of mating was assessed by the presence of a vaginal plug, and the day when it was detected was considered as day 1 of gestation. Pregnant females were kept in separate cages and decapitated on day 16. Corpora lutea in the ovaries and the number of implantation sites were counted, the number and weight of embryos free from amniotic membrane were recorded. The same scheme of mating was used to evaluate the reproductive efficacy of 35 control males injected sterile medium 199 a week before mating.

On day 8 postinoculation, the infection of males was verified by testing blood and brain samples. Embryonal infection was assessed in a mixed sample

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of tissues from one litter on day 16 of gestation. The virulent form of the agent was tested in the brain of males and embryonal tissues in 2-week mice [2]. Viral RNA was detected by the polymerase chain reaction and reverse transcription [1]. The titer of antibodies to the TBE virus envelope protein E was determined in blood samples by solid-phase dot enzyme immunoassay (dot-immunoassay) [6].

RESULTS

Reproductive activity of pregnant females mated with experimental males was much lower than that of those mated with controls, although the proportion of pregnant females was the same (Table 1). This difference is due to a significant increase in pre- and postimplantation embryonal loss in females mated with infected males. In mammals with multiple pregnancies, the embryo body weight is inversely proportional to the number of embryos [4]. In our experiment, the number of embryos per female and embryo body weight were significantly lower in the group of mice mated with infected males than in controls.

Early antibodies to TBE virus were detected in 48.6±9.4% of experimental males. Viral RNA was found in 58.3±9.3% of brain samples. Biological tests with brain tissues from males were negative. This may be explained by an extremely low (for subcutaneous infection) infecting dose.

Viral RNA was identified in embryonal tissues from two litters; in one of them a virulent form of TBE virus was detected, as evidenced by positive biological test. The females with infected progeny had been mated to the same male with a high titer of anti-TBE antibodies. The virulent form of TBE virus (positive biological test) was found in the tissues of embryos which, judging from their size, died on days 12-14 of gestation. This fact may be regarded as an additional proof that the higher embryonal mortality after mating with infected males is caused by TBE virus.

Hence, mating of healthy females with infected males leads to an increase in embryonal mortality, which may be caused by infection of the progeny with TBE virus. The possibility of sexual transmission of TBE virus can be regarded as another ecological mechanism maintaining this parasitic system.

TABLE 1. Reproductive Characteristics of Females Mated with Control and Infected Males ($\bar{X}\pm SE$)

Characteristics	Males	
	controls	infected with TBE virus
% of pregnant females	11.4±3.8 (70)	19.6±5.3 (56)
Number of corpora lutea	12.6±0.5 (8)	11.5±0.8 (11)
Number of embryos:		
implanted	11.1±0.7 (8)	7.8±0.8 (11)*
live	10.1±0.8 (8)	5.3±1.7 (11)*
Embryonal loss:		
preimplantation	11.9±3.2	31.7±4.1**
postimplantation	9.0±3.0	32.6±5.1**
Embryonal weight	754±10 (81)	693±15 (45)**

Note. *$p<0.05$, **$p<0.001$ versus the control; the number of animals is given in parentheses.

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