日本脳炎顯性感染誘発の一要因としての脳脊髄線虫症に関する実験的研究I(獣医學)

Cerebrospinal Nematodiasis as a Provoking Factor in Japanese Encephalitis: Experimental Approach (I)

Hiroshi MOCHIZUKI, Tamotsu TOMIMURA and Takenori OKA

Department of Veterinary Science, College of Agriculture

(Received for Publication Oct. 6, 1953)

In the recent epidemic years of Japanese encephalitis (1947 to 1948) Sugawa, Mochizuki and Yamamoto (1949) demonstrated the lesions of parasitic encephalomalacia in 4 horse brains out of 54 cases which were diagnosed as Japanese encephalitis histopathologically. As to the relationship between these two diseases, however, they obtained no conclusion.

Shoho (1951), Innes and Shoho (1952) alluded to the possibility of the parallel adjuvancy of these two diseases in horse, and suggested that a nematode could play a role of destroyer of the blood-brain barrier permitting virus invasion.

The following reasons are likely to refrain the authors from reaching a satisfactory interpretation of the possible relationship of these two diseases on the basis of the field materials so far obtained. 1) As the lesions of cerebrospinal setariasis are usually very confined, there is an excusable reason for their being overlooked especially in large animals as horse. So it appears very difficult to deny setariasis decisively in any equine case. 2) The lesions of Japanese encephalitis seems to spread all over the brain and spinal cord following the development of encephalitic symptoms, although the lesions have some affinity to interbrain and endbrain (Sugawa *et al.*, 1949). Consequently it is also difficult to decide the place where the initial lesion occurs. 3) Tanaka *et al.* (1945) demonstrated 9 cases of subclinical cerebrospinal setariasis out of 100 serum horses. As to Japanese encephalitis, an extremely frequent occurrence of subclinical infection of men and animals is well-known. Therefore complication of the two diseases might exist frequently without any causal correlation.

These studies were designed as an experimental approach to the question of whether the balance between clinical and subclinical Japanese encephalitis might be tipped in favour of the former by the cerebrospinal migration of nematode larvae.

Materials and Methods

The Nakayama strain*, a standard of Japanese encephalitis virus, was used in all experiments. This strain was isolated in 1935 in Tokyo by Kasahara *et al.*

* The authors wish to express their gratitude to Drs. Okuno and Tsujimoto, Research Institute for Microbial Diseases, Osaka University who kindly donated the virus strain.

(1935). Since then it has been maintained by intracerebral mouse passages. As mice were fairly tolerate to this strain in extracerebral inoculation (Tsubahara *et al.*, 1951), it was favourably used for experimentation. Virus suspensions were prepared from the heavily infected mouse brain. The tissues were homogenized, the suspension centrifugalized for 15 minutes at 2000 r.p.m. to eliminate larger tissue particles. Subcutaneous inoculation was applied, as it could easily produce subclinical infection.

As a parasitic factor the authors chose *Toxocara canis* larvae which caused cerebrospinal nematodiasis in mice easily as Yokogawa (1923), Tiner (1953a) and Sprent (1953) indicated. The eggs employed were obtained from uteri of worms and were cultured in moist animal-charcoal longer than 3 weeks at room temperature in warm seasons before being used. They were administered to mice through fine plastic tubes directly into stomach after a desirable dilution of embryonated eggs were prepared.

The mice used were 7 to 11 g in weight. They were inoculated subcutaneously with 0.3 ml of each suspension of decimal serial dilutions 3 to 4 days after feeding of eggs, and were observed for the following 10 days. When mice died or became moribund, their brains were cut longitudinally, and halves of them were fixed in a 10% hot formalin for histological examination. The remaining halves were cut to the identification of each part of brain and were pressed between slide- and cover glass in order to count the number of larvae migrated into the brain. In some cases, recovery of the virus was tried by intracerebral inoculation into mice. All survivors were killed 10 days after the virus inoculation, and their brains were examined in the same manner as the above-mentioned.

Results

Symptoms and macroscopical changes of brain due to cerebral migration of T. canis larvae: According to Yokogawa (1923), mice died with symptoms of hemoptysis, paresis, hemiplegia and circling 3 to 6 days after feeding of *T. canis* eggs (number of eggs fed were not described). Tiner (1953a), however, reported that mere feeding of eggs did not kill mice, and the maximum length of *T. canis* larvae in the rodent brain was less than 0.5 mm. Sprent (1953) also described that more than 100 *T. canis* larvae were demonstrated in the mouse brain for 1 to 6 months after feeding of eggs without producing notable symptoms in mice.

After preliminary experiments with more than 30 mice, it was also confirmed that mice scarcely showed notable symptoms by feeding of less than 2000 eggs. Only a slight depression and inappetence were evidenced. Intracerebral hemorrhage resulted in a rare occurrence of death. By feeding of more than 3000 eggs, however, tachypnea, paresis and more frequent death occurred especially in young mice 1 to 6 days after feeding apparently attributable to remarkable hemorrhage of lungs and brain. Cerebral migration of the larvae and meningeal hemorrhage began to appear in 2 to 3 days after feeding. The hemorrhage became remarkable in 4 to 9 days, and thereafter brownish spots of meningeal hemosiderosis took place of hemorrhage. Meningeal hemorrhage was moderate in its extent by feeding of about 2000 eggs, slight by about 1000, and very slight or none by less than

500 macroscopically (see fig. 1). Five to 10 percent of the total dose given generally reached the brain. Encapsulated larvae were first observed on the 11th day after feeding in the subcutaneous tissue. This finding almost corresponds to Sprent (1952) who found first them on the 12th day.

Experiment 1: Nineteen mice were fed with about 2000 embryonated eggs respectively August 24, 1953, and 15 of them and other 10 untreated mice were inoculated subcutaneously with the virus August 28. The interval between both treatments was 4.4 days. The result obtained was shown in Table 1 and 2. A significant difference of mortality was observed between experimental and control groups.

Experiment 2: Twenty mice were fed respectively with about 2000 embryonated eggs, 10 with about 1000, 10 with about 500 September 12, 1953, and they were all inoculated with the virus September 15, the interval being 3.2 days. Ten mice without feeding of eggs were inoculated subcutaneously and other 20 intracerebrally with the virus. The result obtained was shown in Table 3 and 4. A significant result was again obtained even in a group of mice which had received only 500 eggs, later having shown very slight meningeal hemorrhage.

Histopathology: Detailed histopathological findings will be reported in the authors' next report. All died or killed in extremis, except accidental deaths, showed typical encephalitic lesions. Many of survivors which received both virus inoculation and feeding of eggs showed moderate encephalitic lesions, while almost all survivors which received virus inoculation only showed very few, slight lesions, if any.

Discussion

The result recorded above demonstrated that the cerebral migration of *T. canis* larvae was considerably effective as a provoking factor in experimental Japanese encephalitis of mice. As the virus and the eggs were administered separately and the virus was inoculated after some of larvae had already migrated into the brain, provocation seemed to be caused primarily by the destruction of the blood-brain barrier. Further experiments seem necessary in order to decide whether or not there might exist other reasons, such as the adjuvancy of virus and larvae in the provocation, besides the simple destruction of the barrier.

The idea of cerebrospinal nematodiasis has developed from the researches on diseases caused directly by a cerebrospinal migration of nematodes. Since the brilliant works of the Research Committee of Government General of Korea on the Ovine Lumbar Paralysis (1939 to 1944), many Japanese workers have contributed to the elucidation of ovine, caprine and equine cerebrospinal setariasis. As to cerebrospinal ascaridiasis Tiner (1949) demonstrated that skunk and raccoon ascarid utilized mice and other rodents as intermediate hosts during their life cycle, and recently (1953b) reported that raccoon ascarid caused fatal central nervous system damages in certain North American wild rodents as endemic infection. Thus the direct injury by cerebrospinal nematodiasis has been established, and some other related diseases of unknown etiology may be classified into the same category in future.

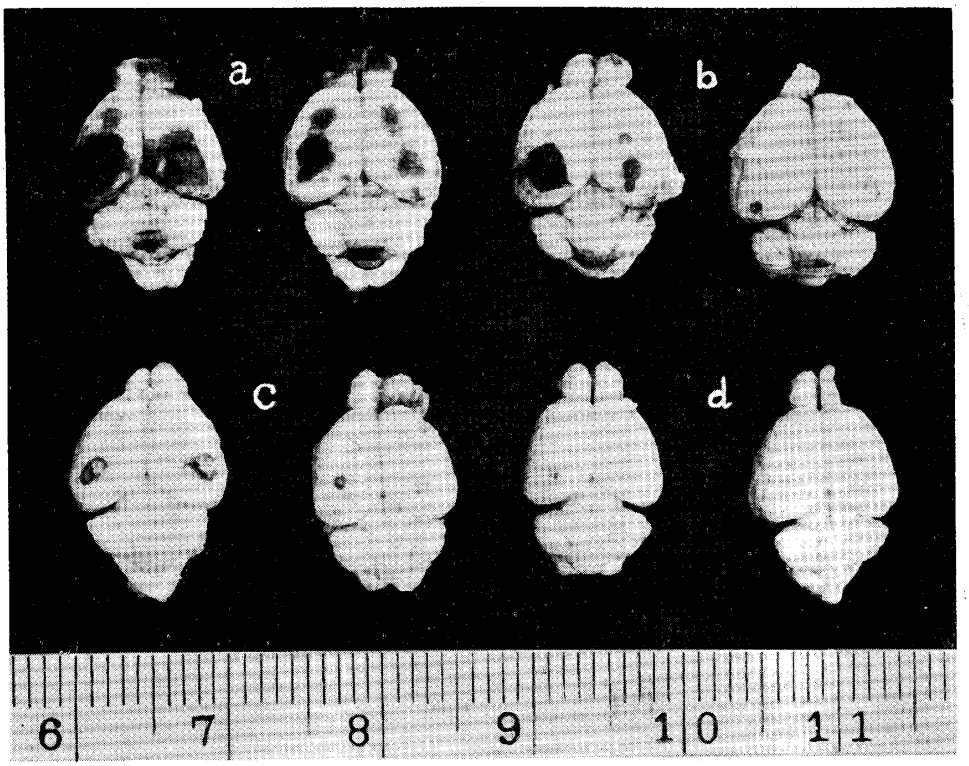


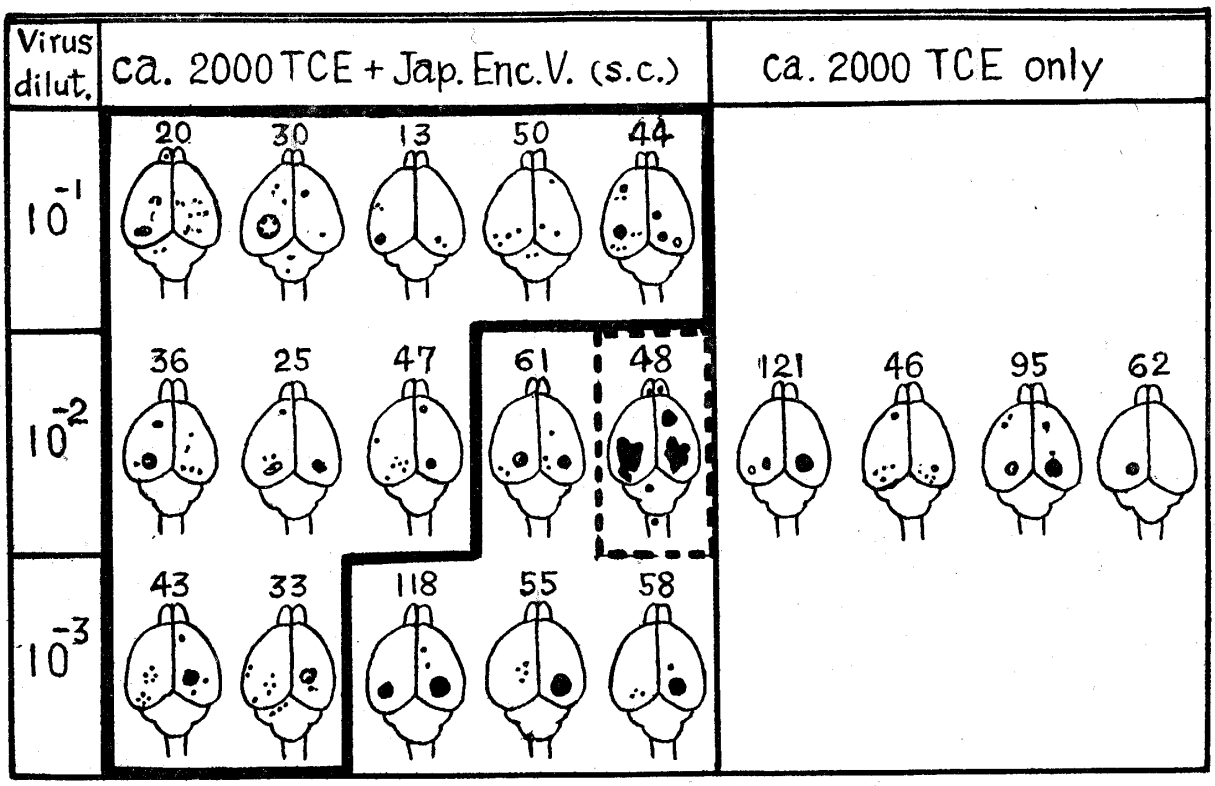
Fig. 1. Brains of mice which had received about 5000 (a), 3000 (b), 2000 (c) and 500 (d) of embryonated eggs of *Toxocara canis*. The extent of meningeal hemorrhage nearly corresponded to the number of eggs given. Both of (a) died 4 days and the remaining 6 were killed 6 days after feeding of eggs.

Table 1. Results of experiment 1

Inoculated s.c. with Jap. Enc. V.			Fed with about 2000 TCE only
Virus dilution	Without feeding of TCE	4.4 days after feeding of about 2000 TCE	
10^{-1}	●● ○○○ 5 6 10 10 10	●●●●● 4 4 5 5 6	
10^{-2}	○○○○○ 10 10 10 10 10	●●●◑⊗ 4 4 5 10 1	○○○○ (11) (11) (14) (14)
10^{-3}		●●◑⦶⦶ 6 6 10 10 10	
-log(LD50)	< 0.9	> 2.6	−

Notes. ●: Died or killed in extremis, fatal nervous symptoms. ◑: Killed, moderate nervous symptoms ⦶: Killed, after recovering from slight nervous disorder. ○: Killed, no symptoms. ⊗: Died, no encephalitis. Numerals under the ring marks indicate days died or killed after virus inoculation, and those in brackets after feeding of eggs. TCE: Embryonated eggs of *Toxocara canis*. ∗: Recovery of virus was tried by intracerebral inoculation to mice with success.

Table 2. Extent of meningeal hemorrhage and hemosiderosis in experiment 1.



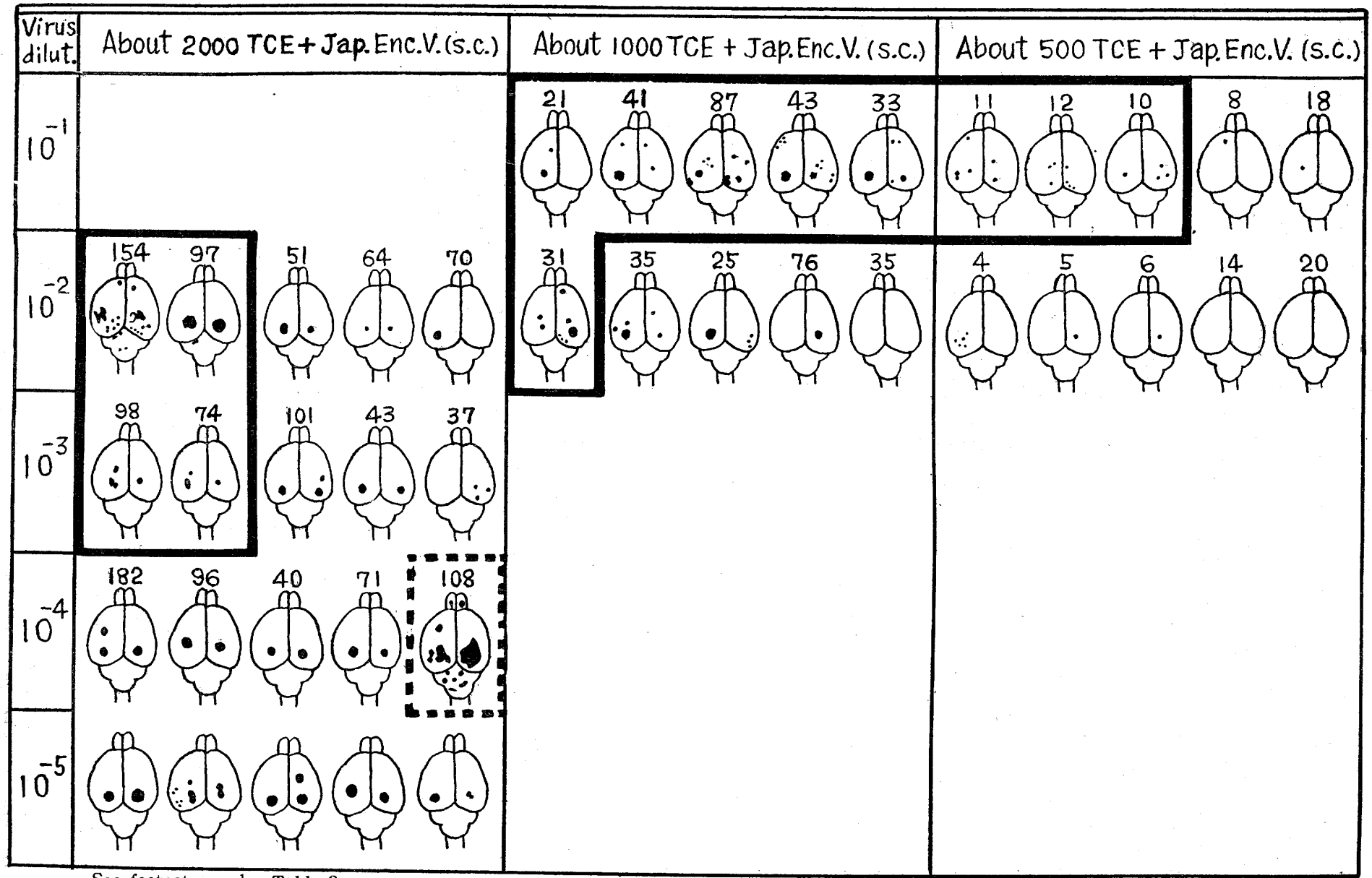
Notes. Numerals indicate number of ascarid larvae found in one side of brain. Brains within thick line correspond to ●, and in broken line to ⊗ in Table 1.

Table 3. Result of experiment 2

Virus dilution		Inoculated with Jap. Enc. V. only	Inoculated subcutaneously with Jap. Enc. V. 3.2 days after feeding of: About 2000 TCE	About 1000 TCE	About 500 TCE
10^{-1}	Subcutaneously	○○○○○ 10 10 10 10 10		●●●●● 5 5 5 6 8	●●●*◐◐ 5 5 7 10 10
10^{-2}	Subcutaneously	○○○○○ 10 10 10 10 10	●●◐○○ 6 6 10 10 10	*●○○○○ 5 10 10 10 10	○○○○○ 10 10 10 10 10
10^{-3}			●●*○○○ 5 5 10 10 10		
10^{-4}	Intracerebrally	●●●●● 4 5 5 5 5	○○○○⊗ 10 10 10 10 5		
10^{-5}	Intracerebrally	●●○○○ 6 6 10 10 10	○○○○○ 10 10 10 10 10		
10^{-6}	Intracerebrally	○○○○○			
10^{-7}	Intracerebrally	○○○○○			
$-\log(LD_{50})$		s. c.------ — i. c.------ 4.9	2.3	> 1.7	< 1.1

See footnotes under Table 1.

Table 4. Extent of meningeal hemorrhage and hemosiderosis in experiment 2



See footnotes under Table 2.

Beautyman and Woolf (1951) reported an ascarid larva in the brain of a child in association with acute anterior poliomyelitis, and discussed the relationship of larval migration to genesis of poliomyelitis but without any decisive conclusion. Here remains the question of the second type of injury which might be caused by cerebrospinal nematodiasis. The authors have confirmed that their experimental approach to the question gives a significant support to the assumption that cerebrospinal nematodiasis might facilitate virus invasion into the central nervous system where specific defence is provided, irrespective of the appearance of the clinical manifestation of direct injury by nematode.

The authors' results may not always be applied immediately to the relationship between cerebrospinal setariasis and Japanese encephalitis of horse. Because much difference was noted between setariasis and the authors' experimental ascaridiasis in size, number and pathway of migrating larvae. It seems, however, very probable that a similar provocation exists, as the destruction of the blood-brain barrier did occur commonly not only in clinical cases but also in subclinical ones of setariasis (Tanaka *et al.*, 1945). Perhaps there may be such a latent setariasis that becomes injurious on account of the facilitation of invasion of the neurotropic virus.

The relationship of *T. canis* larvae to Japanese encephalitis in the authors' experiments seems quite different from that of swine lung worm to swine influenza (Shope, 1941), that of *Trichinella spiralis* to lymphocytic choriomeningitis (Syverton *et al.*, 1947) and that of *Heterakis gallinae* to blackhead (Tyzzer, 1926). The pathogenic agents, swine influenza virus, choriomeningitis virus and *Histomonas meleagridis*, were carried in a masked form by the worms through their life cycle. It is considered, however, that the role of *T. canis* larvae in these experiments should never be underestimated, merely because they do not carry Japanese encephalitis virus and have no significance as the reservoir of infection.

Conclusions

1. Cerebral migration of *Toxocara canis* larvae is demonstrated to be considerably effective as a provoking factor in experimental Japanese encephalitis in mice.
2. The provocation seems to be caused primarily by the destruction of blood-brain barrier.
3. These results give a significant support to the assumption that cerebrospinal setariasis might provoke a clinical infection of Japanese encephalitis in horse.

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