Congenital Japanese B Encephalitis Infection of Swine. (18285)

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In Japan a large number of stillbirths was reported among swine in various stages of gestation during the summer months of 1947 and 1948. This was especially prevalent during August and September, with a morbidity approximating 60 to 70% of the pregnant animals. The disease was observed from northern Hokkaido to Kyushu, the southernmost island of Japan. A majority of the pregnant females delivered their dead young at or near the expected date of delivery. In some instances, live, dead and moribund piglets were observed in the same litter. Concomitantly, there occurred among equidae and humans an outbreak of Japanese B encephalitis, which was one of the largest ever recorded (1948).

Through questioning, it was ascertained that a disease among swine with comparable symptomatology occurred during the great epidemic of Japanese B encephalitis in Japan during 1935. No published report is available. The high incidence of inapparent infection with the virus of Japanese B encephalitis has been demonstrated among swine in a survey of antibodies for this agent. These data and the simultaneous occurrence of epidemics in swine, horses and humans suggested the possibility of an interrelationship(1).

In this study a neurotropic virus was isolated from typical swine cases and was identified as the virus of Japanese B encephalitis.

General aspects. Various stages of fetal development of the aborted animals were observed in a given litter. Mummified fetuses, together with normally developed ones, were observed. Subsequent to delivery, most of the young which appeared normal in development were weak and unable to stand; to a lesser extent some, which appeared normal at birth, later developed symptoms of central nervous system involvement. At autopsy, a high percentage of the young had either an encephalomalacia or internal hydrocephalus with the cerebral cortex appearing extremely thin in some cases. Histopathologically, a nonpurulent encephalitis was found among a number of cases. A large amount of exudate was observed in the pleural, pericardial or peritoneal cavities. In contrast to the offspring, no abnormalities were observed among the maternal animals, although some owners noticed anorexia and a slight fever of short duration occurring at varying periods of pregnancy.

Isolation of virus. Materials. During the last week in July, 1948, a litter of 5 pigs was delivered near Hachioji, a city in Tokyo Prefecture. One of the newborn was delivered moribund; 3 of the pigs were too weak either to stand or nurse and expired within 48 to 72 hours. The remaining animal appeared normal in size and development and grew through adolescence without any apparent abnormalities.

The 3 affected piglets were autopsied immediately after death, and fragments of brain were preserved in glycerine saline solutions for biological study (V-4948, V-4949 and V-4950).

Pathologic anatomy. Macroscopic findings. Subcutaneous tissues showed advanced evidence of edema with the skeletal muscle fibers having a turbid appearance. The thoracic and mesenteric lymph nodes showed inflammatory swelling. A large amount of reddish colored fluid was observed in the thoracic and abdominal cavities. Serous membranes contained petechial hemorrhages. Multiple small areas of necrosis were present in the liver and spleen. The stomach contained a small amount of yellow stringy material. The meninges were intensely congested and the encephalon was edematous and extremely soft and friable. On sectioning, the encephalon was hyperemic with numerous extravasations of blood. Congestion of the spinal cord

1. Hosoya, H., Matumoto, M. and Iwasa, S., *Japan. J. Exp. Med.*, 1950, v20, 587.

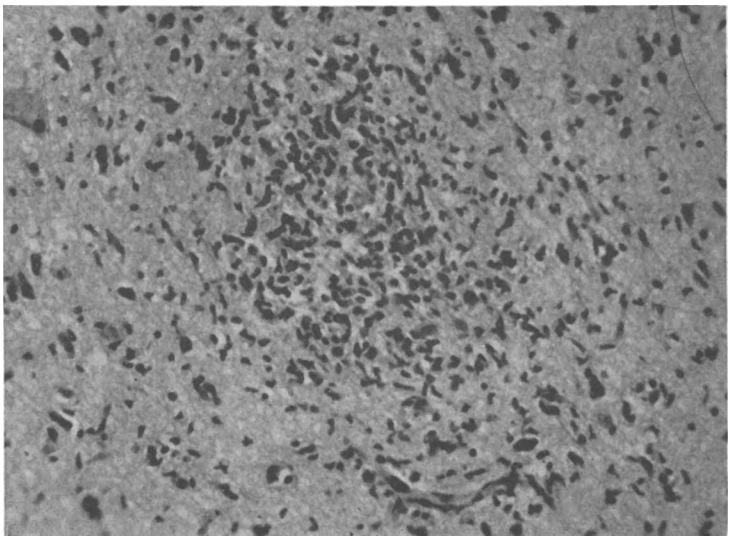

FIG. 1.
Subcortical area showing glial nodule. H and E. ×260.

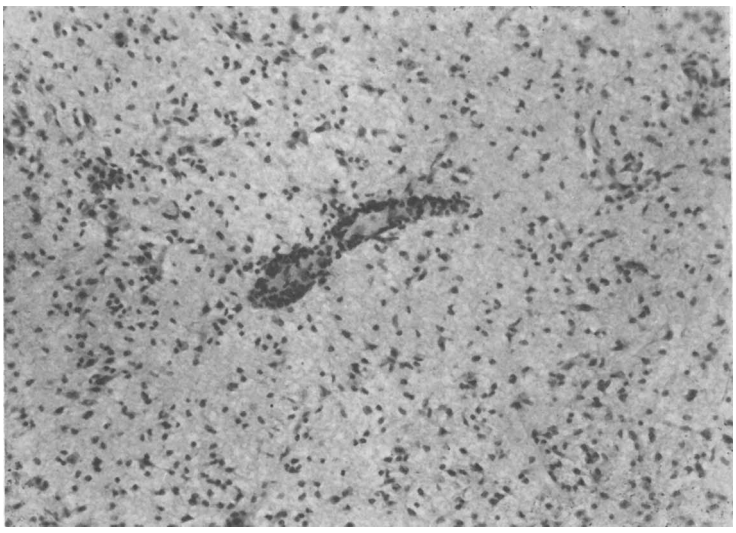

FIG. 2.
Cerebral peduncle showing perivascular cuffing and interstitial infiltration. H and E. ×160.

was also noted. Small areas of softening in the substance of the brain and in the walls of the ventricles were present. The subarachnoid space was filled with a slightly turbid fluid.

Microscopic findings. Histopathologically, changes seen were typically those associated with a virus encephalitis (Fig. 1 and 2), and were diffusely distributed throughout the cortex, white matter, basal ganglia and brain stem. In scattered areas neurones showed changes ranging from those of slight acute cell degeneration to advanced destruction with karyolysis and cytolysis with neuronophagia. There were also numerous small vessels throughout the tissues showing perivascular

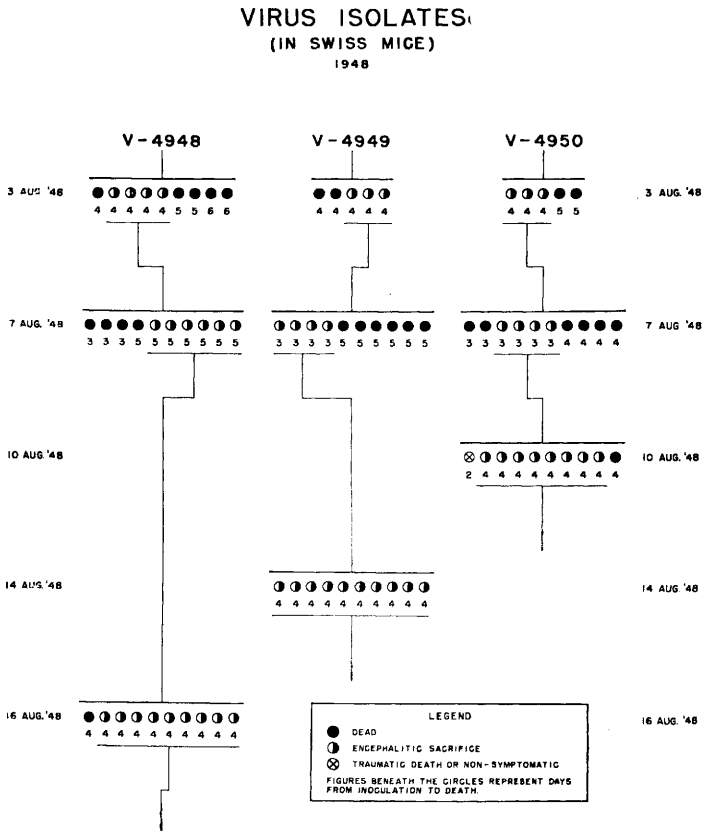

FIG. 3.

cuffing with round cells. Focal areas of glial nodules composed of proliferating microglia and round cells were scattered through both white and gray substance. In the latter area, remnants of degenerated neurones could occasionally be identified.

Isolation of filterable agent. Within 24 hours subsequent to autopsy, each glycerinated brain specimen (V-4948, V-4949 and V-4950) was ground in a mortar and a 10% emulsion prepared, using as diluent 10% rabbit serum in saline. This suspension was centrifuged at 3000 r.p.m. for 15 minutes. The supernate was inoculated intracerebrally in 0.03 ml amounts into 5 to 10 two-week-old mice. Two strains of white mice were used for each specimen; one group was a Bagg-Swiss strain procured in the United States and reared under screen. The second group of mice was purchased locally from Saitama Prefecture animal farms, located approximately 40 miles north of Tokyo. Japanese B encephalitis is considered to be endemic in this area.

Results. The results of isolations in Swiss mice were similar to those obtained in Saitama mice. Both groups of mice (Saitama and Bagg-Swiss) of the first passage exhibited definite signs of encephalitis within a 3 to 6 day incubation period. Subsequent serial passages were readily accomplished by intracerebral inoculation of the infected brain emulsions (Fig. 3). Brain tissue of passage mice was examined histologically and revealed a nonpurulent encephalitis.

Neutralization tests. Intracerebral neutralization tests were carried out with 0.2 ml volumes of undiluted and unheated serum against an equal amount of 3 or 4 ten-fold dilutions of brain suspensions from mice infected with the swine isolates and with viruses of Japanese B encephalitis (Nakayama) and

TABLE I. Identification of Virus Isolates as Japanese B Encephalitis by Neutralization Tests.

Virus	Immune serum (rabbit)	Dilutions of the virus								Log LD_{50}	Neutralization log index
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}		
V-4948	JBE*	—	1/4†	0/4	0/4	0/2	0/4	—	—	2.75	5.0
	SLE*	—	—	4/4	2/4	1/4	1/4	1/4	—	5.75	2.0
	Control	—	—	4/4	4/4	4/4	2/4	3/4	—	7.75	—
V-4949	JBE	—	2/4	0/4	0/4	0/4	0/4	—	—	3.0	4.0
	SLE	—	—	4/4	2/4	1/4	1/4	1/4	—	5.75	1.25
	Control	—	—	4/4	2/4	4/4	2/4	2/4	—	7.0	—
V-4950	JBE	4/4	4/5	3/5	2/4	0/5	—	—	—	4.4	2.8
	SLE	—	—	5/5	4/4	5/5	4/5	2/4	—	7.8	<1.0
	Control	—	—	5/5	5/5	5/5	2/5	1/3	—	7.2	—
JBE (Nakayama)	JBE	4/4	5/5	5/5	1/5	0/5	—	—	—	4.6	3.65
	SLE	—	—	4/4	4/4	2/4	0/3	0/4	—	6.0	2.25
	Control	—	—	4/4	4/4	4/4	4/4	3/4	0/4	8.25	—
SLE	JBE	—	—	4/4	3/4	1/3	1/4	2/3	—	6.5	2.0
	SLE	—	4/4	1/4	0/4	0/3	0/4	—	—	3.75	4.75
	Control	—	—	4/4	3/3	4/4	4/4	4/4	—	8.5	—

* JBE—Japanese B encephalitis; SLE—St. Louis encephalitis.
† The numerator denotes No. of mice which died; the denominator denotes No. of mice inoculated.

St. Louis encephalitis, making a series of final virus dilutions ranging from 10^{-4} to 10^{-8}. The serum-virus mixtures were incubated for 2 hours at 37°C in a water bath and finally were inoculated intracerebrally into Bagg-Swiss mice, 4 or 5 mice per dilution, each mouse receiving an inoculation of 0.03 ml. A normal rabbit serum known to be negative for Japanese B virus was included as a control in each test. The neutralization log index, representing the ratio between LD_{50} endpoint of the control and the immune serum, was calculated in the usual manner. Tabulated results are shown in Table I.

Complement fixation tests. Antigens of isolates, V-4948, V-4949 and V-4950, and St. Louis encephalitis (SLE) were prepared by high speed centrifugation. Ten percent mouse brain suspensions in physiologic saline containing 2% inactivated serum from normal guinea pigs were centrifuged horizontally at 2000 r.p.m. for 30 minutes after over-night incubation in the refrigerator. The supernates, subsequent to freezing and thawing, were then centrifuged at 15,000 r.p.m. for one hour in the angle head of an "International" centrifuge under refrigeration with solid CO_2. To the clear supernates, merthiolate was added to give a final concentration of 1:10,000. These were stored at 5°C. Antigen of the Nakayama strain of Japanese B encephalitis was prepared by the benzol extraction method of Espana and Hammon (2). Normal brain antigen for control purposes was processed in a similar manner. Complement fixation tests were performed in a manner similar to that of Casals and Palacios (3) with over-night incubation of the serum-antigen-complement mixtures, and complement titrations in the presence of antigens at 5°C. Four units of virus antigen contained in 0.25 ml were used in the test. Immune guinea pig serum was employed routinely as a positive control. Results are summarized in Table II.

Discussion. The occurrence of an increased incidence of stillbirths and death of newborn in swine populations has been observed previously, but to the best of my knowledge its relationship to Japanese B encephalitis has not been suggested. The correlation between epidemics of the disease and equine epizootics has been recently noted (4-6). Meiklejohn *et al.* (7) reported the experimental infection with Japanese B enceph-

2. Espana, C., and Hammon, W. McD., *J. Immunol.*, 1948, v59, 31.

3. Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, v74, 409.

4. Burns, K. F., unpublished data.

5. Burns, K. F., Tigertt, W. D., and Matumoto, M., *Am. J. Hyg.*, 1949, v50, 27.

TABLE II. Identification of Isolates as Japanese B Encephalitis Virus by Complement Fixation Tests.

Immune sera	Antigens	Dilutions of immune serum									
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	NMB*	Sal.*
JBE Nak.*	V-4948. U	—	—	—	4	4	4	±	0	0	0
Guinea pig	V-4949. U	—	—	—	4	4	4	±	0	0	0
56°C, 20 min.	V-4950. U	—	—	—	4	4	4	±	0	0	0
	JBE Nak. (1:16)	—	—	—	4	4	4	4	3	0	0
	SLE* (1:2)	—	—	—	0	0	0	0	0	0	0
SLE	V-4948. U	3	±	0	0	0	0	—	—	0	0
Mouse	V-4949. U	3	±	0	0	0	0	—	—	0	0
60°C, 20 min.	V-4950. U	3	±	0	0	0	0	—	—	0	0
	JBE Nak. (1:16)	2	0	0	0	0	0	—	—	0	0
	SLE (1:2)	4	4	3	0	0	0	—	—	0	0

* JBE Nak.—Japanese B encephalitis, Nakayama strain.
SLE—St. Louis encephalitis.
NMB—Antigen control: normal mouse brain.
Sal.—Mixture with physiological salt solution instead of antigen to check on anticomplementary properties of the serum.
Complement fixation is regarded as 4; different degrees of partial fixation as 3, 2, or 1. ±, trace. 0—No fixation or complete hemolysis. U = Undiluted.

alitis virus of 3 pigs, approximately 4 months of age with the production of fatal encephalitis in 2 of the 3 animals following the intravenous injection of the virus. It is of interest to note that they were unable to recover the virus from the dead pigs.

The concurrence of this disease in swine and the Japanese B encephalitis epidemic of 1948 suggested an identical etiologic agent. Histopathologic study and virus isolates confirm this viewpoint.

It is believed that this is the first demonstration of congenital damage of the nervous system by any of the arthropod-borne encephalitis viruses.* During recent years the subject of congenital damage to the nervous system by various infectious agents has become important in medicine. The effects of congenital infection with German measles virus and toxoplasma in human beings have received special consideration in recent years. In human congenital toxoplasmosis, the situation is similar to that described in swine in the present communication, namely, the mother shows either no or very negligible evidence of infection, while the central nervous system of the fetus is severely affected.

Summary. 1. A congenital disease of swine is reported which results in stillbirth or death during the neonatal period. The chief pathologic changes are those of a nonbacterial encephalitis. 2. This disease showed a temporal relationship to epidemics and equine epizootics of Japanese B encephalitis. 3. A virus identified as that of Japanese B encephalitis was recovered from the brain of 3 piglets which died 48 to 72 hours after birth. 4. Congenital damage of the nervous system by an arthropod-borne encephalitis virus has thus been demonstrated in swine.

6. Burns, K. F., and Matumoto, M., Survey of Animals for Inapparent Infection with the Virus of Japanese B Encephalitis, *Am. J. Vet. Res.*, 1949, v10, 146.

7. Meiklejohn, G., Simpson, T. W., and Stacey, I. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 359.

* Medovy, H. (*J. Pediat.*, 1943, v22, 308) reported that during the 1941 epidemic of western equine encephalitis in Canada, the disease appeared in 2 infants (cases 14 and 15) 3 and 4 days after birth respectively, with simultaneous mild febrile illnesses in the mothers. While it is possible that the mothers may have been infected antepartum and transmitted the virus to the children by way of the placenta, it is also possible that infection of both mother and child may have occurred postpartum.

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