# Avian Encephalomyelitis—Review of Recent Japanese Works

By HIKOKICHI TSUBAHARA

Chief, Viral Products Section, Biological Products Division,
National Institute of Animal Health

It was thought that this disease did not exist in Japan, but since the country also saw the mass outbreak of it in 1963, the investigations and studies on this subject have been done earnestly. The outlines of these studies are mentioned here.

In the spring of 1963 Miura *et al.*[10] discovered a case extremely similar to the disease, judging from the symptoms, and reported it to be thought as avian encephalomyelitis. However the histopathological and virological proofs could not yet be obtained. Then in the fall of 1963 there was occurrence of this disease in the imported chicks under inspection,[1] and the case was histopathologically and virologically proved to be definitely avian encephalomyelitis, and received a great deal of attention as the first of the cases in Japan. After that, in 1964 and 1965 the disease occurred successively in the flocks of home-produced chicks.[2),4),6),15),16)] Since 1966 it seems that there are outbreaks of the disease everywhere, not described particularly.

The disease had its onset chiefly in chicks, less than 30 days of age. In an extreme case 100% of the chicks in a flock showed the symptoms of the disease, but the usual morbidity was less than several score %. Some of chicks, more than 50 days old, showing a symptom of weak leg, were found to be diagnosed histopathologically as avian encephalomyelitis.[15]

That the occurrence of the disease came to be seen suddenly in Japan since 1963 gives us an impression that avian encephalomyelitis first invaded this country at that time, but it is not supported by the results of serological surveys. Miura *et al.*[11] proved that in the blood serums collected and preserved in 1953 and 1956 there already existed those having neutralizing antibody. The survey which was conducted from 1963 to 1965 gained the results that the disease already existed in the nation-wide scale. Namely Iwai[5] and Miura *et al.*[9),12)] surveyed 124 breeding flocks of 32 hatcheries in 22 prefectures (Japan is divided in 46 prefectures) and reported that in each prefecture there was flocks having neutralizing antibody, and that only 11% of the tested flocks were estimated to be uninfected. Tsubahara[18] also surveyed 84 breeding flocks of 39 hatcheries in 21 prefectures, obtaining much the same results. Moreover, as to flocks for egg-producing, surveyed 78 farms in 8 prefectures, 66% of them was revealed clearly to be infected. For the first invasion of encephalomyelitis in 1963 the disease is thought to spread too widely in this country.

Horiuchi[2] conducted histopathological studies on the cases of both natural and experimental infections and emphasized the importance of swelling of the spinal cord nerve cells, accompanied with central tigrolysis, for differential diagnosis. Yamagiwa *et al.*[20] examined changes of the nerve cells in detail, discerned that the etiologic agent of this disease chiefly attacked the large motor neurons which underwent degenerative changes and diminished reducing the number of the cells, and proposed that the disease should be called poliomyelitis. Moreover, through the

electron microscopic observations of motor neurons, they found expansion of the lumen of rough-surfaced endoplasmic reticulum of motor neurons and vacuolization of them, investigated the normal structure to diminish and change to small vacuole, and considered the relation of the phenomenon to the changes, called chromatolysis or central tigrolysis, seen by the optical microscopes.[21]

For serological tests, the embryo susceptibility test and neutralization test, are being conducted using eggs, and development of a *in vitro* method is being tried. Sato *et al.*[17], partially refining and concentrating the cerebral emulsion of infected chicken embryos, produced the antigen, carried out the complement fixation test using the non-heated serum of the immune bird, and obtained the result being positive. They, however, pointed out that the antigen was dubious to be applied practically because the antigen titers of cerebral emulsions varied in every lot produced. Ikeda[3] conducted the agar-gel-diffusion method, using the cerebral emulsion of infected embryos as the antigen, and found a specific precipitin line to be formed between the antigen and the immune blood serum. Since if the blood serum is low in neutralization titer the reaction does not occur, there is room for further improvement in this method.

In order to demonstrate antigenic materials in the tissues of sick birds, Miyamae *et al.*[13),14)] tried to apply the direct fluorescent antibody method; namely, they investigated the cases of both natural and experimental infections, using a fluorescent antibody preparation (dyeing titer: 320 times), which was raw gamma globulin from the immune chicken serum labelled by fluorescin isothiocyanate. In the cases showing the symptoms the brains and spinal cords were positive highly in the fluorescent antibody method, but those in the uninfected birds were negative. The birds showing no symptom among the infected flock were rarely positive in the fluorescent antibody method. Fluorescent reactions were hardly demonstrated in the viscera of infected birds. The fluorescent site in brain was the plasma of the cells positive to Nissl's stain. It tended to difficulty to detect the specific fluorescent antigen in the sick chick that passed considerable days after onset of symptom, and so they concluded that the method was worth applying as a diagnosis in the early stage of this disease.

Miura *et al.*[8] inoculated to normal chicks and embryos with the cerebral materials of infected chicks of which ages in day varied when the disease had its onset. In chicks, inoculated with the cerebral emulsion of a sick chick 2 days after birth, the latent period of the disease was short, the incidence high; some of inoculated embryos came to show changes after several embryo passages. On the other hand, in chicks that were inoculated with the cerebral emulsion of chick diseased 12 and 18 days after birth respectively, the latent period was long and the morbidity rate was low, and even after several passages changes in the embryos were not demonstrated. These findings proposed a problem that the invasiveness of virus would vary according to the age in day when the bird showed the symptoms.

Tsubahara *et al.*[19] isolated the virus from a case of the disease in 1965, and investigated the pathogenicity of the virus which was from the embryos in a few passages. No abnormality was demonstrated in the infected embryos but the hatched chicks showed the symptoms several days after hatching. Baby chicks were easily infected by either inoculation intra-cerebral or oral, and the indicence was high; chicks more than 28 days old were easily infected by oral administration, but had no specific symptom such as leg weakness, tremor, and so on, and were clinicaly normal. The oral administration to the laying hens brought about temporary decrease of eggs. The virus first appeared in the liver and spleen about 5 days after oral administration, and a few days later they came to be recovered from the central nerves. In the embryos and chicks 4 days of age, the virus in the central nerve increased, but as age in day became older, the recovery of the virus came to be hard and the histopathological features of the disease were not discernible. When laying

hens were administered, the virus transfered to the eggs 5~10 days after administration. These results seems to confirm that in nature this disease chiefly is seen in the chicks less than 30 days old.

For preventing of the outbreaks of this disease in younger chicks, the parental antibody plays an important part. Matsukura *et al.*[7] investigated how long the antibody durated. It was cleared that, the parental antibody transferred to chicks for a long period of time after infection, and that the transferred antibody could be detected from chicks within 5 or 6 weeks old. This indicates that as long as the parent bird is immunized against the disease, the parental antibody protects the chicks during the most dangerous period when the disease has its onset.

The above-mentioned findings are the outlines of the recent studies on avian encephalomyelitis in Japan. For the present prophylaxis, live vaccine is administered the hens, 10~16 weeks of age, that are expected to become breeding birds, and the chicks are produced from the immunized breeding birds only. Thus the occurrence of the disease in the chick days is well prevented. For the virus to be used as live vaccine, utilizing the character of this virus that can hardly invade the central nerve in older chicks, the near one with those in nature is applied. However, as the propagating ability of the virus in the central nerve still remains, there is room for further improvement in this point.

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## Present Address of Authors

Chikatsune, Masateru: Branch Office of National Animal Quarantine, Kobe, Hyogo
Horiuchi, Teiji: National Institute of Animal Health, Kodaira, Tokyo
Ikeda, Sumio: Tohoku Branch, National Institute of Animal Health, Hichinohe, Aomori
Iwai, H.: Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido
Kurogi, Hiroshi: Kyushyu Branch, National Institute of Animal Health, Kagoshima, Kagoshima
Matsukura, Toshihiko: Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido
Miura, Shiro: ditto
Miyahae, Takeo: ditto
Odagiri, Yoshiharu: Faculty of Agriculture, University of Osaka Prefecture, Sakai, Osaka
Sato, Gihei: Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido
Tsubahara, Hikokichi: National Institute of Animal Health, Kodaira, Tokyo
Yamaigwa, Saburo: c/o Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido