STUDY ON EQUINE ENCEPHALOMYELITIS
EPIZOOTICA

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In 1942 we reported on the studies of pathological anatomy of the Equine Encephalomyelitis Epizootica which had occurred in North China during the year 1940. Pathologically, it was similar to the epizootic as seen in Japan from 1935 to 1938, in which we found the intranuclear inclusion bodies, the so called "Joest-Degen's Corpuscle", in the nuclei of the ganglion cells and also cytoplasmic inclusion bodies, the so called "Ichii's Corpuscle", in the protoplasma of nerve cells of Cornu Ammonis of the brain of sick horses. However, we found that contrary to the epidemic in Japan, the inflammatory changes in the cases of prolonged processes were less remarkable than in those cases which died during the acute stage. For this reason, the disease was diagnosed as "Equine Encephalomyelitis Epizootica" in North China until the causative agent could be identified as similar to the Japanese horse encephalitis in the same manner as the Japanese B encephalitis by virus isolation.

From the middle of July 1945, there occurred a large epizootic of horse encephalitis in wide spread districts of North China. We were able to study clinically, pathologically, anatomically, epidemiologically and virologically, several cases during the outbreak. But in as much as it was the time of surrender for Japan, we could not continue the study and were unable to carry the virus to Japan at the time of demobilization.

1. Epidemiological Findings:

The epizootic first occurred in Taian, which is in the southern part of China, North China, from 10 July 1945, in which more than 15 horses were infected. In the areas around Peiping, more than 35 horses were infected from 23 July. As the clinical symptoms of the sick horses were somewhat slight or atypical, as will be described later, they were diagnosed as encephalitis only at the depot where excellent veterinary officers had been assigned.

The summer of 1945 was very wet, rainy and humid and there was an abundance of mosquitoes. The peak of the temperature was between 7-10 July. In this epizootic, contrary to that of the summer of 1940, more Chinese horses suffered than the Japanese horses, of which only several cases were observed. The termination of this epizootic was unclear in that sick horses continued to appear until the early part of September. The diagnosis on dead horses was "Hind Trunk Paralysis" and was recorded as such from the time of seizure until the beginning of October.

The occurrence of an epidemic of encephalitis among humans was questionable, though the Japanese army doctors of the epizootic areas stated that there were no patients in North China. But we heard from people in the demobilization centers that there was a
severe epidemic of human encephalitis in Manchuria in the summer of 1946. On the other
hand, we observed 3 young pigs which were suffering from encephalitis at the end of
October in the same year and in the same area.

2. Clinical Findings:

The following three forms were observed; a) severe form, b) mild form and c)
atypical form.

a) Severe Form: Animals were found to be sick and carrying a temperature of 40
degrees C, decreased or complete absence of appetite, standing in a stupor which suddenly
reversed itself in that they became very nervous which can best be described as three
types, as follows:

1. The delirious type: animals showed blindness or weak sight, clonic and tonic convul-
sion with opistotonus, spasm and tremors of muscle, gnashing of teeth, trismus, dilatation
of pupils, nystagmus, gnawing and excessive salivation and when forced to move, did so
violently displaying a lack of coordination of the hind legs and at the same time moving
in a circle always in the same direction. The symptoms were repeated and eventually
the animals changed to the next type or died of heart paralysis between the first to
third day, although a few of them began to eat 6 to 10 days following the original onset
of the symptoms.

2. The paralytic type: after a slighter or severe delirious stage, the animals laid down
on the ground with clonic and tonic convulsions, the so called “swimming or wagon like
movements”, where they eventually became comatous and remained so until death. Less
than half of them survived and upon regaining consciousness 5 to 10 days following the
comatous or lethargic stage, they began to eat green grass but still displayed extreme
weakness of the hind legs. Occasionally some sick animals without signs of deliriousness
were observed.

3. Mixed type: During the convulsion period, the animals laid on the ground, but
they sometimes woke up and regain their feet under their own power. It may be the
severest of the delirious type.

b) Mild Form: The first symptoms were high temperatures of more than 39,5 degrees
C, and decreased appetite, followed by a sleepy expression, lowered head and weak loins.
Most of them showed slight convulsions with gnashing of teeth or trismus and circular
movement, blindness or weak sight and then a lethargic sleep in which food was still
being retained in the mouth. In this form it was observed varying degrees of nervous
symptoms, from slight stupor to convulsive conditions. Most of them recovered in 4 to
10 days from the initial onset of symptoms.

c) Atypical Form: Two types of this form was observed. We could not see any
signs of prodromal symptoms in either type. One form showed colic like symptoms with
high temperature and slight sounds of peristalsis of the intestines on examination; the
other type was a spontaneous lumbar paralysis. The animals reclined on the ground
without any prodromal signs of illness. Sometimes they showed clonic and tonic convul-
sions or they tried to stand, and were very weak; the reflexes of the joints was accelerated
but reflexes to the impulse of sticking needles in the skin was decreased; there was no sensitive area around the lumbar region or its underlying spinal cord. These two types and the mild form appeared in latter stages of epidemic; the atypical form was suggested as encephalitis by the time period of their appearance and proved by histological examination.

We observed about 25 cases of this form and received 8 reports of clinical findings. Morbid cases died within 6th day of the disease and the mortality rate was about 25%. Cases that survived showed signs of recovery on the 3rd to 8th day after onset of symptoms. We were not able to establish the morbidity and mortality rate of the atypical form.

3. Pathological Findings:

Six autopsies were carried out by our group. Macroscopically there was no causative changes of death. The meninges was severely congested, and the brain and spinal cords also showed a reddish congestion with petechial hemorrhages in the gray matter. The spleen was slightly swollen and the liver, kidney and heart muscles showed a moderate to severe cloudiness. We had examined microscopically 12 cases on their CNS, and each case showed a different degree of nonpurulent inflammatory changes as follows; degeneration of nerve cells, neuronophagia, diffuse proliferation of neuroglia cells, glia nodules, and remarkable perivascular cell infiltration, etc. These elementary changes of inflammation was widespread throughout the gray matter of CNS. On the section of Cornu Ammonis of telencephalon, which were stained by Lentz’s stain after fixation by Zenker’s fluid, we observed, in the nuclei of the nerve cells, pink reddish corpuscles of almost similar size or slightly smaller than the nucleolus, inclusion bodies, surrounded by a narrow stainless hole. The characteristics of these corpuscles were identical to the description of the so called “Joest-Degen’s Corpuscle” which has been found at first at the same part of the horse brain as Bornia’s disease, the German horses encephalitis, and recently was also found in the Japanese and North China horse encephalitis. On the other hand, in the cytoplasm of the nerve cells on section, inclusion bodies were discovered of almost similar size as the nucleolus, sometimes connecting with the nucleus and stained homogenously reddish by eosin. These cytoplasmic inclusion bodies were identical to the so called “Ichii’s Corpuscle” which were found in the nerve cells of the Japanese horse encephalitis by Dr. Ichii in 1937.

4. Virological Findings:

Our experiment consisted of the inoculation of brain tissue from 8 horses known to have encephalitis intracerebrally into mice. These horses died between 28 July and 28 August, and two of the horses were transported from Taian and five from Fundai and one from Shinyeig, nearest Peiping. Four cases belonged to the paralytic type and were observed to be lying on the ground with weak tremor to convulsions of the muscles, but one case showed similar symptoms with severe clonic and tonic convulsions and struggling,
while the other three cases showed a delirious type with very severe nervous symptoms. Two cases died on the 2nd day, four on the 3rd day and 2 cases on the 4th day after onset of the symptoms.

The brain tissues from the sick horses were removed under sterile conditions and placed in a solution of 50% glycerin-water and refrigerated for 3 to 30 days; at the time of inoculation, we made a 10% suspension of gray matter of the cortex and base parts of the brain in saline and injected intracerebrally into mice.

We inoculated a brain tissue suspension of Horse No. 2 to 2 Chinese colts, 2 rabbits, 2 guinea-pigs and mice intracerebrally with only the mice showing positive reactions through virus isolation in sectioning of the brain, while other animals remained healthy for six weeks.

With the spoiled brain tissues, we centrifuged the saline suspension at first 5 minutes 1000 cpm. then twice each 10 minutes with at 3000 cpm. We examined the sterility by putting each 0.5 cc of all suspensions into ordinary bouillon media and incubated 5 days longer at 37 degrees C. Results of the isolation tests of causative agent is described in table 1.

Table 1. The results of the isolation tests of causative agents.

<table>
<thead>
<tr>
<th>No &amp; Name</th>
<th>Living Place</th>
<th>Date of onset</th>
<th>Form of disease</th>
<th>Date of death</th>
<th>Date of inoculation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Inga</td>
<td>Tainan</td>
<td>28 &quot;</td>
<td>mixed</td>
<td>30 &quot;</td>
<td>&quot; &quot; &quot;</td>
<td>Pos.</td>
</tr>
<tr>
<td>5. Nangun</td>
<td>Tainan</td>
<td>26 &quot;</td>
<td>paralytic</td>
<td>27 &quot;</td>
<td>29 &quot;</td>
<td>Neg.</td>
</tr>
<tr>
<td>6. Ogo</td>
<td>Shiheng</td>
<td>30 July</td>
<td>delirious</td>
<td>1 &quot;</td>
<td>2 &amp; 9 &amp; 29 &quot;</td>
<td>Pos.</td>
</tr>
<tr>
<td>8. Kengiku</td>
<td>&quot;</td>
<td>25 &quot;</td>
<td>paralytic</td>
<td>28 &quot;</td>
<td>&quot; &quot; &quot;</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

All suspensions of brain tissues were inoculated intracerebrally, each between 7 to 10 mice, but No. 2 and No. 8 were also inoculated into 2 rabbits and 2 guinea-pigs intracerebrally at the same time as the mice were inoculated.

As is shown in this table, we were able to isolate 3 strains of causative agents from the brain tissue of No. 1, 2 and 6 sick horses only through mice inoculation.

a) The clinical findings of infected mice: In the first generation, 17 mice inoculated with suspension of No. 1 sick horse brain, there were 5 that died between the 9-16 days after inoculation and which showed partial or typical nervous symptoms. Among 27 mice inoculated with the suspension of brain tissue of No. 2 sick horse, 5 died between 7 to 13 days after inoculation with or without partial encephalitic symptoms and 2 of the 5 showed typical histological changes in their brains. Of the 10 mice inoculated with brain tissue suspension of No. 6 sick horse, 8 died in 3 to 4 days after inoculation as a result of contamination, but 1 of the 2 remaining mice showed on the 7th day, partial symptoms of encephalitis; this mouse was sacrificed and studied histologically, with
typical changes of encephalitis being observed, but unfortunately we lost the mice brain tissue preserved in glycerine, and so we could not succeed in completing any more transfers.

Except the 1st generation, mice inoculated with virulent brain tissues showed nervous symptoms after 4 to 11 days. We wanted to know the relationship between the body weight of mice during the incubation period. We used 40 mice and the average incubation period was 6 days; 15 of them with body weight of more than 18.5 gms, showed the average incubation of 6.6 days, whereas 25 with less body weight showed average period of 5.8 days. There was some evidence that the large mice showed the more prolonged incubation period. The onset of encephalitis was seen to be mostly a decreased movement, but sometimes the rough hair predominated before stillness.

In the infected mice, slight tremors of body appeared and trismus or gnashing of teeth, gradually increased in their degree as also did the convulsions. Several hours after, more severe convulsions began to show with circular like movement as a center with one side of hind leg, and then overturning themselves at the peak of the convulsion. There was not found opistotonus which is usually observed commonly in the mice infected with Japanese B encephalitis.

The convulsions became more and more severe, and then the animals began forced movements; they would run straight into the cage or they jumped to 25 cm; after this they recovered almost to the normal state, though some teeth gnashing or tremor still remained. In this stage, no movements were observed. The period of these convulsions lasted about 5 to 15 minutes, varying in each case and in each stage of sickness; the convulsions at the end stage the periods were the longest and the interval was the shortest. It was very clear that the exciting symptoms were more severe than that of the infected mice of Japanese B encephalitis, but tremor of these mice was not so remarkable as that of St. Louis encephalitis. About 24 to 48 hours after the exciting stage, paretic or paralytic symptoms appeared as in other encephalitis strains; sick mice could not walk owing to the paresis of the hind legs, but the fore legs showed symptoms of extreme convulsive movements, with death occurring within 24 hours.

b) Pathological findings of the brain of infected mice: Macroscopically, there was only a moderate congestion of the infected mouse brain, but microscopically the non-purulent inflammation of the brain was observed. In the brain sections of the infected mice, the regressive changes were of a very high degree but reactive changes of proliferation of the glia cells and perivascular infiltration was somewhat slighter. The nerve cells showed heavy degeneration such as vacuolization of nuclei or protoplasm, and took irregular forms and carryorrhaxis, sometimes held in the nuclei "Joest-Degen's Corpuscle" like intranuclear inclusion bodies. The proliferation of migrating cells around the nerve cells were slight. In the gray matter of the brain, there was seen slight proliferation of glia cells holding fewer leucocytes and small glia nodules. The perivascular infiltrations were also slighter and were seen only in a slight increase of cells in the perivascular lymph vessels.
On the pathologic-anatomical findings, the changes in character as to reactions were slight compared to the heavy clinical symptoms; these changes were slighter than St. Louis encephalitis and were more sure than that of Japanese B encephalitis.

c) General biological characters of the causative agent: The agent was not able to cultivate in the ordinary artificial culture media, but we could pass this agent to the 7th generation with almost similar incubation periods, clinical symptoms and pathological changes. By the filtration test of the brain emulsion and of infected mice at the 3rd generation, the agent passed through the Berkefeld N filter easily. At the 5th generation, the titer of the virus contained in the brain was $10^{-6}$ dilutions. We tried to get powdered virus of the titer containing $10^{-6}$ dilution from skim milk emulsion of the mice brain in putting it into the desiccator in the electric refrigerator, using a rotary pump and salted ice. But the instruments were so unsatisfactory that it took about 10 days to dry it perfectly, and the loss by the drying process was larger than expected and final powder had only $10^{-1}$ dilution virulence. The infected mice brain which was preserved in 50% glycerin aqueous solution maintained its original virulence for more than 2 weeks under 4 degrees C. After the third passage, we inoculated it intracerebrally to 2 rabbits of about 800 g weight and 2 guinea-pigs of about 150 g weight with the control of 5 mice; all mice died in 5 to 6 days after inoculation under typical symptoms, but the rabbits and guineapigs remained well for more than 45 days.

5. Summary and Discussion:

1. There should have been a large epizootic of horse encephalitis in the summer of 1945 in North China, but only a small number of them were reported due to the clinical findings being relative atypical.

   By our observation, we found that Chinese horses were more susceptible than the Japanese horses. The occurrence of encephalitis did not occur in humans during that year.

2. The climate during the summer was very rainy and more humid than usual with the peak of the temperature occurring two weeks prior to the epizootic; during this time there was an abundance of mosquitoes.

3. We were able to classify the disease into three forms, namely, the mild, severe and the atypical. The severe form could be divided into three types. The atypical form assumed both the colic type and the lumbar paralysis type. The mortality rate was about 25%.

4. As for the pathological-anatomical findings, we could observe in all parts of CNS of sick horses, the elementary changes of non-purulent inflammation, and the so-called "Joest-Degen's Corpuscle" and "Ichii's Corpuscle" like inclusion bodies, both in the ganglion cells of the Cornu Ammonis of the telencephallon of the sick horses.

5. We were able to isolate three strains of virus from the brain tissue of eight horses. These viruses would pass through the Berkefeld N filter easily and mice were susceptible in the titer of $10^{-6}$, but rabbits and guinea-pigs showed no susceptibility.

6. The clinical symptoms of the infected mice were very severe and continued over long
periods, but the the histological changes of proliferation characters were relatively slighter and regressive changes were more marked; ganglion cells of the Cornu Ammonis showed carryorrhexia and contained intranuclear inclusion bodies like granules. Comparing with the results of the studies on the epizootic of horse encephalitis in 1940, the results of this epizootic were almost similar in nature, but as for horses, there were very few of Japanese origin in North China owing to the difficulties of transportation. This occurred during the time when the Japanese horses had been more sick from encephalitis than the Chinese horses, and the Japanese Army arranged for Mongolian horses while the remaining Japanese horses remained for two more summers in North China.

We believe by these facts that the virus of the horse encephalitis isolated by us would be similar to the causative agent of the epizootic of horse encephalitis occurring in the summer of 1940.

We could not identify the virus as the causative agent of this epizootic or compare it with the virus of the Japanese horse encephalitis, Japanese B encephalitis, the St. Louis encephalitis or louping-ill of sheep immunologically, as it was just the time of surrender of Japan, and also we had no strains of encephalitis in our laboratory at that time.

But we believe that the virus isolated by us was the causative agent because we could isolate the same virus twice from the same brain tissues of 2 cases of encephalitis horses by similar method after a time interval.

We wish it to remain by the name of Equine Encephalitis Epizootica in North China for the following three reasons, until the virus of this disease can be identified immunologically in the future.

1. There was no epidemiological evidence that the virus of the horse encephalitis is identical to the encephalitis virus occurring in humans.

2. The clinical symptoms of the infected mice were more severe than those observed in Japanese equid encephalitis and Japanese B encephalitis.

3. The histological changes of the brain tissue in the infected mice were slightly different as that seen in Japanese B encephalitis.

Judged from the animal experiments, it resembles more, the virus of Japanese B encephalitis than any other virus of encephalitis, although we could not prove this by cross immunity tests.

Finally we wish to thank Major M. W. Scothorn, D. V. M. for his special support in preparing this report.