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## FURTHER OBSERVATIONS ON THE TRANSMISSION OF EQUINE INFECTIOUS ANEMIA VIRUS TO SHEEP\*

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In the previous paper<sup>7)</sup>, MIURA et al. described some interesting facts concerning the appearance of the virus in circulating blood and the persistence of the virus in tissues of lambs that were subcutaneously inoculated with equine infectious anemia (E. I. A.) virus.

The present report deals with attempts to trace the later fate of E. I. A. virus in tissues of experimental lambs and with some discussion on the susceptibility of sheep to this virus.

### EXPERIMENT

In as much as the blood sample which was collected from the lambs on the 14th, 21st and 28th days after the inoculation of E. I. A. virus was proved free from this virus, further experiment on the virulency of the lamb blood was left over from the previous experiments. To search for the persistence of E. I. A. virus in lamb body, 400 ml of the organ emulsion added with 1,000 uts. of penicillin and 500 uts. of streptomycin per ml was injected subcutaneously into a horse numbered No. 9 (foal) that was ascertained not infected with E. I. A. prior to the experiment. The organ emulsion was prepared in the same way as recorded in the previous paper after storage at  $-30^{\circ}\text{C}$  for 281 days, from lamb No. 4 slaughtered on the 77th day after the administration of E. I. A. virus.

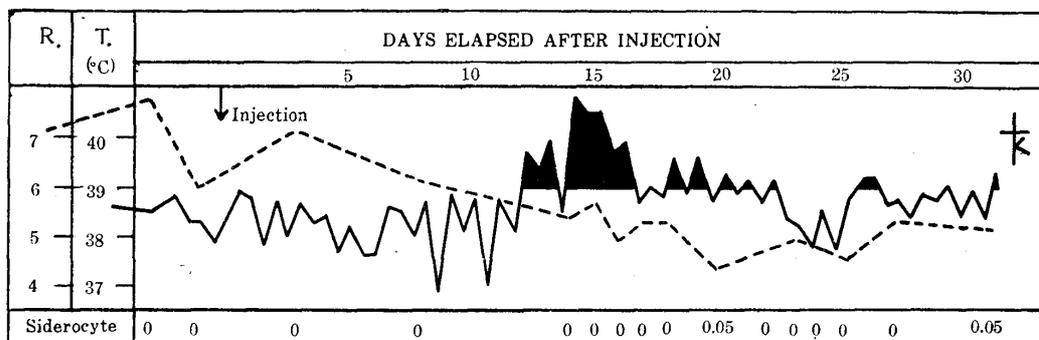
Horse No. 9, as indicated in chart 1, developed a temperature higher than  $39^{\circ}\text{C}$  lasting for about a week. Erythrocytes diminished in number from 6~7 million to 5 million or less and a small number of siderocytes was demonstrated in jugular vein blood on the days succeeding the febrile attack. At that time, anemic and icteric discolourations of mucosae appeared and petechiae also developed on the sublingual mucosa and gums; furthermore cardiac disturbances, such as cloudiness and stippling of the 2nd sound, were recognized. Though biopsies were conducted 3 times during the period of the 14th~21st days of the test, neither the histological findings in liver nor splenograms suggested the infection of E. I. A. On the 32nd day, the horse was subjected to post-mortem examinations, however, histopathological findings did not show the changes indicative of this disease. Bacterio-

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logical examinations on the several organs of the animal resulted negative so far as common aerobes were concerned.

CHART I. Horse No. 9



Note R.: Erythrocyte number in million  
 T.: Body temperature  
 Siderocyte: Siderocyte number in 10,000 leucocytes

The present workers diagnosed horse No. 9 as E. I. A. from the clinical and hematological findings in spite of the absence of histopathological evidence. Thus, it is confirmed that the E. I. A. virus was contained in the lamb organ as long as 77 days after inoculation apart from the questions whether the viruses multiplied or merely harboured therein.

#### DISCUSSION

While a number of reports in regard to negative results on transmission of E. I. A. virus to sheep have been published, the Japanese Commission considered sheep slightly susceptible on the basis of certain experimental results showing some clinical responses and appearance of E. I. A. virus in blood in the animal subsequent to exposure to the virus. THEILER et KEHOE and BALOZET also detected the virus in blood of the animals receiving E. I. A. virus. AUERMAN and HARING also reported the slight susceptibility of lambs from their clinical responses after subcutaneous inoculation with virulent blood. However, none of the previous investigators has sought E. I. A. virus either in circulating blood of sheep day by day after the administration of the virus or in tissues at all. Moreover, none of them investigated the relations between the clinical responses and the appearance of the virus in blood. In addition to these facts, the recent advances in experimental virology seem to require a reinvestigation of the views based upon the results of earlier experiments.

As previously reported<sup>7)</sup>, the authors detected E. I. A. virus in organs of each of the lambs sacrificed on the 3rd, 10th or 28th days subsequent to subcutaneous inoculation with the virulent serum. The virus was also found in the circulating

blood of the lamb within the period of the 5~10th days after the administration of the virus. Moreover, histopathological changes analogical with those of E. I. A. observed in 1 out of 5 experimental lambs suggested infection of this animal with E. I. A. However, the blood collected on the 14th, 21st and 28th days was proved avirulent, and there was failure to certify the virulency of blood withdrawn on the 1st~3rd days. On the other hand, some clinical findings, such as fever increase and erythrocyte diminution, were recognized not only in experimental lambs but also in control animal.

In the present experiments it was confirmed that the emulsion prepared from several organs of the lamb on the 77th day of the test contained the active virus.

Among these experimental results, the appearance of the E. I. A. virus in circulating blood within the period of the 5~10th days of test would be considered as a suggestive fact for the occurrence of transient viremia. In addition to this, some histopathological changes analogous with those of chronic E. I. A. and the persistence of the virus for as long as 77 days in lamb tissues, may indicate the possible propagation of the virus in the animal body.

#### SUMMARY AND CONCLUSION

In order to supplement the previous work, the authors performed some experiments. The result obtained is as follows: Equine infectious anemia (E. I. A.) virus was demonstrated in the mixed emulsion prepared from several organs of a test lamb (No. 4) that was slaughtered on the 77th day after subcutaneous inoculation with the serum virus.

Basing their consideration on the results of the previous and present experiments, the authors discussed the susceptibility of the sheep. It was concluded that it may be possible to cause a subclinical infection in lamb with E. I. A. virus.

#### APPENDIX

According to DONATIEN et LESTOQUARD and GERLACH, artificial inoculation with ovine infectious anemia (O. I. A.) virus induces, in soliped, a disease which is closely similar to E. I. A. so far as symptoms, blood and pathological changes are concerned; subclinically infected sheep may also be encountered in infected area. As previously reported<sup>7)</sup>, the present authors observed some temperature increases and erythrocyte diminutions in test lambs. Therefore, in spite of no reports on the occurrence of O. I. A. in Japan, it seems desirable to ascertain whether the causative agent with which the experimental horses were infected is none other than E. I. A. virus administered to lambs for inoculum or O. I. A. virus, contaminant, originated from the animals.

In order to solve this question, as far as possible, some investigations were conducted as briefly described in the following.

(1) Lamb No. 8 (10-month-old Corriedale) was injected subcutaneously with 50 ml of

