Inoculation Tests of Blood of Foals Affected with Hemolytic Icterus on Normal Horses

Mitsuo SONODA*, Yoshimitsu MAEDE*, Ryoji HATA* and Haruhiko SHIBATA*

Blood samples were collected from four foals affected with typical hemolytic icterus and having sideroleukocytes and inoculated intravenously to four normal horses, respectively. These horses were examined clinically and hematologically for 35 days and at the end of the observation period, from them serum samples were collected for complement fixation and immunodiffusion tests and liver biopsy specimens for histopathological examination. Thereafter, all the horses were held under clinical observation for 60 days or more. No findings suggestive of equine infectious anemia were obtained from any of the four inoculated horses at any time of the observation period.

It is well known1-6) that foals affected with hemolytic icterus show anorexia, dullness, anemia and icterus of visible mucous membranes, palpitation, reduced erythrocyte count, and high icterus index in the clinical and hematological examinations. These findings are very similar to those of equine infectious anemia. Recently, the authors7) clarified that in addition to these findings, sideroleukocytes were seen in the peripheral blood of the diseased foal. Such finding as the appearance of sideroleukocytes has given rise to a very important problem in equine medicine, because, in Japan, horses having over a definite number of sideroleukocytes in the peripheral blood are to be diagnosed as equine infectious anemia and forced to be either isolated strictly or slaughtered by law for the eradication of the disease.

In the cases of hemolytic icterus reported in the previous paper7), sideroleukocytes were detected, but it was not as certain whether there were complications with equine infectious anemia or not. Therefore, the present studies were carried out to confirm this point by inoculation tests of blood.

In the present paper, the results of the studies are described.
Materials and Methods

Foals with hemolytic icterus: Whole blood samples from four foals diagnosed as typical hemolytic icterus were provided for inoculation tests. The diagnosis of the disease was made on the basis of the clinical history, mode of onset, clinical signs, such as anorexia, anemia, icterus, quick shallow breathing and palpitation, and specific hematological findings, such as low red blood cell count, high icterus index, severe reduction of erythrocyte fragility and positive Coombs’ test. In these foals, sideroleukocytes were detected at the rate of 0.5 to 52 per ten thousand leukocytes in thick smear films fixed in methanol.

Horses for inoculation tests: Four horses were used for inoculation tests of blood. They were checked to be normal in health by the clinical and hematological examinations and histopathological observation of liver biopsy specimens before inoculation.

Method of blood inoculation: Ten ml of whole blood from each of the four diseased foals was injected intravenously to each of the four normal horses.

Methods of observation: The inoculated horses were examined clinically every day, and hematologically every 4 to 8 days. For the check of sideroleukocytes, thick smear films of leukocytes were fixed in both formalin gas and methanol, and stained first with Prussian blue and then with carbol-fuchsin. At the terminal stage of observation, serum samples were collected from the horses and checked by complement fixation and immunodiffusion tests, and liver tissue samples were obtained by biopsy for histopathological examination.

Periods of observation: The above mentioned tests were carried out in all the horses until 35 days after inoculation. Thereafter, all the horses were held under clinical observation for 60 days or more.

Results

Clinical findings of each horse are shown in Figs. 1-4, and histological findings of liver tissues in Figs. 5-8.

Findings of case No. 1.

Before inoculation, the mare was positively normal in the clinical, hematological, and histological examinations. Over the period of observation after inoculation, she showed no abnormal clinical signs at any time. Her body temperature ranged from 37.3° to 38.7°C throughout the period.

In the blood examination, the red blood cell count increased to 10.04 millions on the 10th day. At the end of the observation period, it was 10.20 millions, which was the highest count during the observation period. No side-
Inoculation Tests of Blood of Icteric Foals

Table 1. Foals with hemolytic icterus

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Clinical signs</th>
<th>Red blood cell count (millions)</th>
<th>Sideroleukocytes*</th>
<th>Icterus index (units)</th>
<th>Reduction of erythrocyte fragility</th>
<th>Direct Coombs' test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>4.56</td>
<td>1.5</td>
<td>80</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>2.40</td>
<td>52</td>
<td>200</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>2.28</td>
<td>4</td>
<td>45</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>1.49</td>
<td>0.5</td>
<td>120</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Number per 10,000 leukocytes.

Table 2. Horses used for inoculation test

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Foal No. which provided blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hybrid Percheron</td>
<td>1</td>
<td>Female</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Hybrid Percheron</td>
<td>1</td>
<td>Female</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Hybrid Percheron</td>
<td>1</td>
<td>Male</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Thoroughbred</td>
<td>8</td>
<td>Male</td>
<td>4</td>
</tr>
</tbody>
</table>

roleukocytes were recognized at any time of blood examination. Both complement fixation and immunodiffusion tests were negative on the 35th day (Fig. 1).

Histopathological examination of liver tissue specimens collected on the 32nd day after inoculation failed to reveal any change suggestive of equine infectious anemia.

Therefore, it was impossible to make a positive diagnosis of equine infectious anemia on this mare (Fig. 5). During the following 60 days, the horse did not show any clinical change.

Findings of case No. 2.

In the clinical examination, no abnormal signs were observed during the entire period of observation. The body temperature fluctuated between 37.0°C and 38.7°C, being in the normal range. The red blood cell count before inoculation was 7.82 millions. On the 17th day after inoculation it increased to 9.84 millions, which was the highest count throughout the period of observation. At the end of the observation period, it was 8.60 millions.

No sideroleukocytes were present at all at any time of hematological observation. Both complement fixation and immunodiffusion tests were negative on the 35th day (Fig. 2).

Histopathological examination of liver tissue specimens obtained on the 32nd day after inoculation revealed no findings suggestive of equine infectious

![Fig. 3. Findings of case No. 3](image)

Remarks. Lb : Liver biopsy, IB : Inoculation with blood, and N : None.

![Fig. 4. Findings of case No. 4](image)

Remarks. LB : Liver biopsy, IB : Inoculation with blood, and N : None.
All the liver tissue specimens were obtained from the 4 inoculated horses by biopsy at the end of the observation period. They showed no histopathological changes suggestive of equine infectious anemia when stained with hematoxylin and eosin and examined at a magnification of x 280.
anemia (Fig. 6).

Thereafter, no clinical changes were observed during the following 60 days.

Findings of case No. 3.

Before inoculation, the horse was perfectly normal in the clinical examination. The red blood cell count was 7.96 millions. No sideroleukocytes were seen at all. Histopathological examination of liver biopsy specimens revealed no findings suggestive of equine infectious anemia. Over the period of observation, no abnormal clinical signs were exhibited. The body temperature was always within the normal range from 37.7°C to 38.6°C. The red blood cell count began to increase on the 17th day. On the 25th day, it reached 12.35 millions, which was the highest count throughout the period of observation. Then, it decreased to 7.40 millions on the 35th day after inoculation. No sideroleukocytes were seen at any time of hematological examination. The serum of the 35th day was negative for both complement fixation and immunodiffusion tests (Fig. 3).

Histopathological examination of liver tissue specimens obtained on the 32nd day after inoculation revealed no findings of equine infectious anemia (Fig. 7).

By clinical observation, no changes were seen during the following 65 days.

Findings of case No. 4.

Before inoculation, the horse was confirmed to be normal and healthy by the clinical and hematological examinations and histopathological study of the liver tissue.

Over the period of observation after inoculation, no abnormal clinical signs were manifested. The body temperature was always normal, fluctuating between 37.0°C and 38.0°C. The red blood cell count was in the normal range of 8.16 to 10.2 millions. No sideroleukocytes were found at any time of observation. The serum of the 35th day was negative for both serological tests (Fig. 4).

Histopathological examination of the liver tissue specimen obtained on the 33rd day revealed no findings of equine infectious anemia (Fig. 8).

For the following 60 days, the horse did not show any clinical change.

Discussion

As equine disease in which sideroleukocytes are observed in the peripheral blood, strangles\(^{10}\), purpura haemorrhagica\(^{10}\), onion poisoning\(^{11}\), and piroplasmosis\(^{12}\) are already known, in addition to equine infectious anemia.

By the present studies, it was certified that the appearance of sideroleukocytes in the peripheral blood in the case of hemolytic icterus of foals, which is one of the immunohemolytic diseases\(^{1,4}\) had no connection with equine infectious anemia.

Recently, the authors also found the appearance of sideroleukocytes in the peripheral blood of dogs\(^{13}\) and horses\(^{14}\) with experimental immunohemolytic anemia.

In fact, it is not so difficult to make a differential diagnosis of each of the above-mentioned four diseases having sideroleukocytes from equine infectious anemia, because they have signs characteristic of each disease.

In hemolytic icterus of foals, however, the clinical and hematological findings are very similar to those of equine infectious anemia. It is very difficult to differentiate one disease from the other without taking detailed history, clinical examination, red blood cell fragility test, and Coombs' test into consideration.

In Japan, collective examinations are carried out on horses in the field for the eradication of equine infectious anemia. It is the actual state of these examinations, however, that only the appearance of sideroleukocytes in thick smear films fixed in methanol and the reduction of red blood cell count are
used as keys for the clinical diagnosis of the disease, and that detailed clinical and other hematological examinations are perfectly neglected in general.

Under such circumstances it may be reasonable to say that foals affected with hemolytic icterus are in a dangerous condition of being diagnosed erroneously as equine infectious anemia to be slaughtered.

From the results of the present studies, the authors would like to draw a conclusion that hemolytic icterus of foals is one of the equine diseases in which sideroleukocytes are seen in the peripheral blood. In addition, they propose that when sideroleukocytes are detected in the peripheral blood of foals which are 2 months old or younger\(^7\), a strict differential diagnosis of hemolytic icterus and equine infectious anemia must be made in the foals, taking detailed history and the results of clinical, hematological, and immunohematological examinations\(^7\) and immunoserological tests\(^5,9\) into consideration.

**Acknowledgements**

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**References**

溶血性黄疸に罹患した子馬血液の健康馬への接種試験

其田三夫*・前出吉光*・秦 良治*・柴田治彦

さきに著者らは、溶血性黄疸に罹患した子馬の血液中に、担鉄細胞の出現することを報じた。今回は、このような担鉄細胞の出現が伝貧の合併と関係あるか否かを検討するために4例の定型的な臨床例から得た血液を、それぞれ1例の健康馬に接種し、臨床学的ならびに血液学的観察を行なった。第32〜35日目には血清の寒天ゲル内沈降反応および補体結合反応ならびにバイオプシーにより得た肝の病理組織学的検査を実施し、更にその後60日以上一般臨床所見を観察した。その結果、4例の接種馬はいずれも伝貧の徵候を示さなかった。