Clinical and Hematological Studies on Hemolytic Icterus of Foals

Mitsuo Sonoda*, Hiroshi Noda**, Kōsaku Kobayashi*† and Yoshimitsu Maede*

Ten cases of hemolytic icterus of foals were studied clinically and hematologically. The results obtained are summarized as follows.

1. The mode of occurrence of the disease in question and the clinical signs of diseased foals coincided with those of the hemolytic icterus of foals reported already.

2. Anemia, high icterus index, appearance of erythrophages and sideroleukocytes, and reduced red blood cell fragility were characteristic findings in the blood.

3. The red blood cells of diseased foals were hemolyzed in the hemolytic test. They were positive for the direct Coombs’ test.

4. Erythrophages and slight or almost perfect destruction of red blood cells were seen by electron microscopy.

For many years, the sporadic occurrence of a disease of newborn foals with severe anemia and icterus and marked high fatality as main signs has been experienced by veterinary practitioners in the Hidaka district of Hokkaido, which is the most famous district for the production of Thoroughbred horses in Japan.

Hosoda et al. (1959-1960)1–5) carried out serological studies on this disease. As a result, they clarified that the disease was identical with hemolytic icterus of newborn foals, which had been reported by some workers in other countries6–11).

Up to the present time, there have been many published papers1–19) to deal with clinical, immunological, and immunohematological studies on the disease, however, only few hematological findings of the disease were reported in detail20).

Recently, the present authors performed clinical and hematological studies on the disease in the Hidaka district to clarify the actual state of the disease furthermore.
In this paper, the results of the studies are described.

**Materials and Methods**

1. The cases studied

Ten newborn foals affected naturally with the disease in question during a period of 1969 to 1971 were provided for the studies. They were all of Thoroughbred. The diagnosis of the disease was based principally on clinical history, clinical signs, and hematological and immunohematological findings.

2. Clinical and laboratory examinations

When information on the onset of the disease was received from the farm owner or attendant, clinical examination and collection of specimens for laboratory studies, as well as therapeutic treatment, were initiated as soon as possible.

3. Methods for hematological examinations

The examinations conducted were red and white blood cell counts by the Thoma-Zeiss method, packed cell volumes by the micro-hematocrit method, osmotic fragility of red blood cells by a descending series of saline solutions, icterus index by the comparative method*, sideroleukocytes in thick smear films fixed in formaldehyde gas and stained with Prussian blue and carbol-fuchsin solutions, erythrophages in thick smear films of leukocytes stained with Giemsa stain, differential counts of leukocytes, and morphological abnormalities of red blood cells in blood films stained with Giemsa stain.

4. Immunohematological tests

Hemolytic test: This test was carried out for detection of hemolysin, employing 1 drop of rabbit serum as complement, 2 drops of twofold dilution of mare's serum, and 1 drop of a 2.5% patient's red blood cell suspension in 0.9% saline. Reaction was allowed to take place at room temperature over a period of 3~6 hours.

Agglutination test: Red blood cells from patients were washed 3 times. One drop of a 5% suspension of them in 0.9% saline was added to 2 drops of twofold dilution of mare's serum contained in an 80 by 7 mm test tube. After shaking, the cells were allowed to settle at room temperature for 3~6 hours.

Direct Coombs' test: Red blood cells from patients were washed 3 times. One drop of a 5% suspension of them in 0.9% saline was added to 1 drop of anti-equine γ-globulin rabbit serum. The mixture was incubated at room temperature for 15 minutes, centrifuged at 800~1,000 rpm for 5 minutes. Then the results of the reaction were read.

5. Electron microscopy of blood cells

Fresh whole blood was harvested from the affected foals and leukocytes were collected from the blood plasma of the same foals by centrifugation. They were fixed in 1% buffered osmic acid solution at pH 7.4 for 45 minutes, dehydrated in an acetone series, and embedded in Epon 812. Sections were cut by a Porter-Blum Mt-1 ultra-microtome, and photographed by a JEM 7 type electron microscope.

**Results**

1. Clinical history

History of affected foals: All the foals were born normally at full term. They appeared to be healthy at birth and got up soon after birth, being nursed strongly in 30 minutes to 2.5 hours. They continued to be active. The initial signs of hemolytic icterus appeared to have escaped observation in many instances. The careful attendants of two stables, however, took notice of abnormal attitudes of foals, such as shivering of the whole body, acceleration of respiratory rate, remaining to lie down

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*Icterus index comparator manufactured by Igaku Shoin Ltd., Tokyo.*
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Table 1. Clinical findings

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Day of onset after birth</th>
<th>Day of examination after birth</th>
<th>Temperature (°C)</th>
<th>Pulse</th>
<th>Anorexia</th>
<th>Dullness</th>
<th>Anemia</th>
<th>Icterus</th>
<th>Palpitation</th>
<th>Acceleration of breath</th>
<th>Hemoglobinuria</th>
<th>Termination</th>
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<td>1</td>
<td>3</td>
<td>4</td>
<td>39.4</td>
<td>130</td>
<td>+</td>
<td>+</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Recovery</td>
</tr>
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<td>2</td>
<td>5</td>
<td>5</td>
<td>39.6</td>
<td>160</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>*</td>
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</tr>
<tr>
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<td>3</td>
<td>3</td>
<td>38.8</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>#</td>
<td>+</td>
<td>Recovery</td>
</tr>
<tr>
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<td>4</td>
<td>4</td>
<td>38.6</td>
<td>120</td>
<td>+</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>Recovery</td>
</tr>
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<td>4</td>
<td>4</td>
<td>38.9</td>
<td>110</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>#</td>
<td>+</td>
<td>+</td>
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</tr>
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<td>3</td>
<td>39.0</td>
<td>102</td>
<td>+</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>#</td>
<td>+</td>
<td>Recovery</td>
</tr>
<tr>
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<td>3</td>
<td>3</td>
<td>39.3</td>
<td>130</td>
<td>+</td>
<td>#</td>
<td>+</td>
<td>#</td>
<td>+</td>
<td>+</td>
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<td>Recovery</td>
</tr>
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<td>38.8</td>
<td>108</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Recovery</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>2</td>
<td>39.6</td>
<td>120</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
<td>Recovery</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>4</td>
<td>40.3</td>
<td>160</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>*</td>
<td>Death</td>
</tr>
</tbody>
</table>

* The urine was not examined.

for several hours after nursing.

From 2 to 4 days after birth, such symptoms as weakness, dullness, infrequent nursing, anemia, and icteric coloration of the visible mucous membranes became so clear that the abnormalities of the foals were discovered surely by attendants in all cases.

History of mares: According to the owners and farm veterinarians, 6 mares (Nos. 1, 5, 6, 8, 9 and 10) out of the ten had no history of giving birth to foals affected with hemolytic icterus. The remaining 4 mares had a history of giving birth to one (Nos. 2 and 3), two (No. 4), and three (No. 7) icteric foals in the past.

2. Clinical findings

The first clinical examination of affected foals was conducted by the authors as soon as possible upon receiving information on the abnormalities of the foals; that is: just on the day or the next day of the onset.

The main signs of the affected foals at that time are given in Table 1. They were anorexia, weakness and dullness, anemia and icterus of the visible mucous membranes, pounding heart beat, accelerated breathing, and hemoglobinuria. Generally, body temperature was slightly or considerably high, and pulse rate and respiratory cycle showed an increase.

Changes in clinical signs and the course of disease thereafter were rather variable, depending on the severity of disease and the time of examination of individual cases. After all, one of the affected foals died on the 7th day of disease and the other nine recovered in 20 to 30 days.

An example which took a typical course of disease is given below. Changes in clinical symptoms in it are as follows.

Case No. 3. A male was born on March 23, 1970. At the time of birth, he was very good in health and began to be nursed actively 30 minutes after birth. Seven hours after nursing, the stable attendants noticed slight shivering of muscle of the whole body, although the foal was nursed normally. On the 3rd day after birth, he showed clear dullness, accelerated breathing, and infrequent nursing. Thereupon, the attendants called the authors, who drove to the farm and examined the foal clinically. Specimens were collected from blood and urine for laboratory examination.
Clinical examination revealed the following findings: a temperature of 38.8°C, pulse rate of 100 per minute, acceleration of heart beat, respiratory rate of 60 per minute, weakness and dullness, and continuing to lie down for a long time.

The urine was dark red in color, containing no red blood cells in the sediment. It was strongly positive for the benzidine test. Conjunctiva, sclera, and mucous membrane were very pale and icteric. He was separated from his dam and nursed by artificial milk for 3 days. Then he was returned to his dam.

Beginning with the first day of disease, he was treated with blood transfusion and injection with large amounts of glucose and electrolyte solutions, as well as thiamine, hepatica and cortisone derivatives.

By these therapeutic treatments, such signs as weakness and hemoglobinuria disappeared in a few days. The remaining signs disappeared gradually. After all, he recovered on the 21st day after the onset of disease.

3. Hematological findings

The results of the first hematological examination of the diseased foals are shown in Table 2.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Erythrocyte count (Mill.)</th>
<th>Packed cell volume (%)</th>
<th>Red cell fragility</th>
<th>Anisocytosis</th>
<th>Erythrocyte phagocytosis</th>
<th>Icterus index (unit)</th>
<th>No. of sidero-</th>
<th>Leukocyte</th>
<th>Differential leukocyte count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.78</td>
<td>16</td>
<td>0.85</td>
<td>+</td>
<td>++</td>
<td>180</td>
<td>42</td>
<td>18,600</td>
<td>0.5 79.0 15.5 5.0 0 0</td>
</tr>
<tr>
<td>2</td>
<td>2.60</td>
<td>12</td>
<td>0.85</td>
<td>+</td>
<td>+</td>
<td>70</td>
<td>16</td>
<td>7,200</td>
<td>0 68.0 30.0 2.0 0 0</td>
</tr>
<tr>
<td>3</td>
<td>4.39</td>
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<td>0.90</td>
<td>+</td>
<td>+</td>
<td>120</td>
<td>46</td>
<td>9,600</td>
<td>13.0 59.0 28.0 0 0</td>
</tr>
<tr>
<td>4</td>
<td>1.47</td>
<td>11</td>
<td>0.85</td>
<td>+</td>
<td>+</td>
<td>130</td>
<td>8</td>
<td>11,000</td>
<td>0 77.0 19.0 4.0 0 0</td>
</tr>
<tr>
<td>5</td>
<td>5.60</td>
<td>22</td>
<td>0.90</td>
<td>+</td>
<td>+</td>
<td>70</td>
<td>6</td>
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<td>0 78.0 20.0 1.0 0 1.0</td>
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<td>0.90</td>
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<td>+</td>
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<td>12,600</td>
<td>2.0 79.0 16.0 2.0 0 1.0</td>
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<td>7</td>
<td>4.56</td>
<td>19</td>
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<td>+</td>
<td>+</td>
<td>80</td>
<td>2</td>
<td>9,600</td>
<td>2.0 77.0 15.5 5.5 0 0</td>
</tr>
<tr>
<td>8</td>
<td>2.40</td>
<td>12</td>
<td>1.00</td>
<td>+</td>
<td>+</td>
<td>200</td>
<td>80</td>
<td>10,600</td>
<td>0.5 89.0 8.5 1.5 0 0.5</td>
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<td>0.85</td>
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<tr>
<td>10</td>
<td>1.49</td>
<td>7</td>
<td>0.85</td>
<td>+</td>
<td>+</td>
<td>120</td>
<td>0.5</td>
<td>8,700</td>
<td>21.5 63.5 13.5 1.5 0 0</td>
</tr>
</tbody>
</table>

* Per 10,000 leukocytes.
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Fig. 1. Anisocytosis  
Case No. 8. Giemsa stain. x 200.

Fig. 2. Erythrophage (arrow)  
Case No. 1. Giemsa stain. x 200.

Fig. 3. Sideroleukocyte (arrow)  
Case No. 3. Prussian blue and carbol-fuchsin stain. x 200.

Fig. 4. Changes in blood findings  
(Case No. 3)

Fig. 5. Changes in blood findings  
(Case No. 4)

noted in all the cases (Fig. 2), or sometimes in 6 cases and frequently in 4 cases. They seemed to consist of neutrophils and monocytes.

Icterus index: In all the cases, the icterus index was very high. It ranged from 45 to 200 units and was 99.2 units on the average.

Sideroleukocytes: Sideroleukocytes were observed in the blood of all the cases, though they varied considerably in number (Fig. 3). In case No. 8, they were the most abundant, numbering 80 per ten thousand leukocytes. In case No. 10, they were the fewest, numbering 0.5 per ten thousand leukocytes.

Leukocyte count: The total leukocyte
increase in count was a little higher in 4 cases (No. 1, 4, 6 and 8), however, in other 6 cases, they were within normal range.

Differential leukocyte count: In all cases, neutrophils were the most predominant in percentage. In two cases (Nos. 3 and 10), a clear nuclear shift to the left was observed. Eosinophils were absent or decreased in percentage.

All the diseased foals were examined for blood characters until their clinical recovery. Especially in two cases (Nos. 3 and 4), changes in blood components were followed up longer than in any other case.

Figs. 4 and 5 show changes in packed cell volume, icterus index, minimum resistance of red blood cell fragility, and sideroleukocyte count in these two cases. It was recognized in these figures that a recovery of packed cell volume took place within about 40 days, normalization of minimum resistance of red cell fragility within about 60 days, reduction of icterus index to the normal value within about 30 days, and disappearance of sideroleukocytes within 60 days after the onset of disease in both cases.

4. Immunohematological findings

In all the 6 diseased cases, the hemolytic test was strongly positive. On the other hand, it was negative in all the 5 controls.

The agglutination test was negative in all the 6 diseased cases and the 5 controls.

In the direct Coombs' test, the red blood cells of all the 10 affected foals were agglutinated strongly.

5. Electron microscopy of blood cells

The red blood cells in the peripheral blood contained almost normal cytoplas-
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Figs. 8. and 9. Two neutrophils phagocytizing red blood cells (R)
Case No. 1. x 25,000.

mic substances lined by cell membranes in the fine structure. Even in these cells, however, most of the cell membranes were destroyed to such extent as to show irregular lines. In addition, there were fine flocculent substances attached to the outer surface of the cell membranes (Fig. 6). Some of these red blood cells were destroyed so severely that they lost the cytoplasmic substances to have only a fine cell membrane or rough globular substances lining the cell membrane (Fig. 7).

Electron microscopy frequently revealed the presence of neutrophilic leukocytes phagocytizing red blood cells. These phagocytized cells in the cytoplasm were digested. Their cytoplasmic substances became granular in the fine structure (Figs. 8 and 9).

Discussion

On the basis of the results obtained from the present studies, it can be said with safety that the ten clinical cases studied were surely affected with hemolytic icterus of foals. That is, in the clinical observation of the diseased foals, the mode of onset of disease and clinical signs were perfectly identical with those of hemolytic icterus of foals reported already.

Hematological examination revealed a decrease in erythrocytes and packed cell volume and an increase in icterus index. These findings agreed with those reported by some previous workers.
In addition, the reduction in resistance of red blood cells in the descending series of saline solutions and the appearance of erythrophages and sideroleukocytes were noticed in all the diseased foals. It was already reported that in experimental immunohemolytic anemia, erythrophages appeared and the osmotic fragility of red blood cells was reduced by the sensitization of antibodies. Since the present cases were accompanied by such findings as these, they may have been affected with an immunohemolytic disease.

In Japan, the appearance of sideroleukocytes in the equine peripheral blood is checked routinely as a key for the clinical diagnosis of equine infectious anemia. When a thick blood smear film fixed in methanol contained one or more sideroleukocytes per ten thousand leukocytes in the peripheral blood, the respective horse is destroyed as a case of equine infectious anemia in accordance with the Law for the Prevention of Equine Infectious Anemia.

Blood smear films prepared from the present cases contained sideroleukocytes, though they had been fixed in formaldehyde gas. Accordingly it may be possible to say that the foals affected with the disease in question were on the verge of being condemned due to a wrong diagnosis of infectious anemia. Especially, if the foals had been checked earlier after birth, they would have been in danger of being destroyed under an erroneous diagnosis.

In the immunohematological examination, all the 6 cases examined were strongly positive for the hemolytic test, but were all negative for the agglutination test. Furthermore, all the 10 diseased foals were positive for the direct Coombs' test. These findings seem to indicate that both complete and incomplete antibodies were related to the clinical occurrence of the disease in question.

Electron microscopy clarified that the cytoplasm and cell membrane had been destroyed slightly or almost perfectly in red blood cells of the diseased foals. This destruction may have been caused by the sensitization of red blood cell antigens transmitted from the dam through milk. Furthermore, in the cytoplasm of neutrophils, phagocytized and degenerated red blood cells were frequently observed.

From these findings, it is considered that erythrophagocytosis might play an important part in the formation of sideroleukocytes in the disease.

The present studies lead to a conclusion that anemia, high icterus index, reduction of red blood cell fragility, appearance of sideroleukocytes, and positive reaction to the hemolytic test and the direct Coombs' test are useful hematological findings for the diagnosis of hemolytic icterus of foals.

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References

Studies on Hemolytic Icterus of Foals


子馬の溶血性黄疸に関する臨床的ならびに血液学的研究

赤木三夫*・呼田 寛**・小林好作***・前出吉光*

北海道日高地方の10例のサラブレッド種の新生児に
みられた溶血性黄疸について検索した。その結果、発
症状況および臨床症状は、従来諸外国で報告されてい
る溶血性黄疸のそれと全く同様であった。血液では、すでに知られている貧血および黄疸
の所見のほか、赤血球貧血細胞および担鉄細胞の出現,
ならびに赤血球抵抗の減弱化が全例で観察された。直
接グロムス試験は全例で陽性性であり、また溶血反応
も検査した5例では、いずれも陽性であった。顕微
学的観察で、赤血球の破壊像および好中球の赤血球背食
像を明らかにした。

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