

## 小型ピロプラズマ自然感染牛の末梢血液中の担鉄細胞

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## SIDEROLEUKOCYTES IN THE PERIPHERAL BLOOD OF COWS AFFECTED NATURALLY WITH PIROPLASMA OF THEILERIA TYPE

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Bovine piroplasmosis of *Theileria* type<sup>2)</sup> is the most popular anemic disease of cattle distributed widely in Japan<sup>1,5,17)</sup>. On the disease, a number of studies have been conducted from various aspects<sup>1,2,5,6,17,19,20)</sup>. There are no reports, however, on sideroleukocytes in the peripheral blood of cattle affected with the disease. Therefore, the present observation was carried out by the authors.

### MATERIALS AND METHODS

**Cows:** Two hundred and ten cows pasturing on two farms located in Shintoku in the southeastern area of Hokkaido were provided for the experiment during June and July in 1970. For the observation of sideroleukocytes, 38 clinically anemic and 10 clinically non-anemic cows were selected from them at random on the basis of anemic findings of the visible mucous membranes. They were divided into two groups, non-anemic and anemic. They were all Holstein-Friesian cows, and one and two years old. In all of them, piroplasmas of *Theileria* type were observed in red cells of blood films. A control group was set up with 20 Holstein-Friesian cows two to six years old reared on the Hokkaido University Farm. These cows were all clinically healthy and confirmed to be free from piroplasma of any type by the checking of blood films.

**Blood:** Blood samples were obtained by the puncture of the jugular vein and anticoagulated by addition of EDTA-2K.

**Check of piroplasma:** The numbers of red cells containing piroplasma of *Theileria* type per 1,000 red cells were counted on the blood films of each case stained by Giemsa stain.

**Determinations of hemoglobin (Hb.) and hematocrit (Hct.):** Hb. and Hct. were determined by the cyanmethemoglobin and the microhematocrit methods, respectively.

**Check of sideroleukocytes:** Thick smear films of leukocytes were prepared by the water method of ODAJIMA and SONODA<sup>10)</sup>. They were fixed with formaldehyde gas and stained by the Prussian blue method followed by carbolfuchsin staining. All over the surface of a thick smear film per each case was examined carefully. The numbers of cells containing bluish granules, cells with cytoplasm stained diffusely light blue, and cells with cytoplasm containing bluish granules and stained diffusely light blue were counted per ten thousand leukocytes in each thick film.

### RESULTS

The results obtained from this observation were summarized in a table. Sidero-

## \*Results of Observation

Group	No. of cases	Hemoglobin (g/dl)	Hematocrit (%)	Rate (%) of Red cells containing piroplasma of Theileria type	Sideroleukocytes	
					No. (%) of positive cases	No. (%) of negative cases
Anemic	38	4.1*	16.7*	60.7*	21 (55.3)	17 (44.7)
Non-anemic	10	10.1*	32.2*	3.8*	0 (0)	10 (100)
Control	20	11.2*	35.5*	0	0 (0)	20 (100)

\* Mean value.

leukocytes containing iron-positive substances were detected from 21 cows (55.3%) of the 38 of the anemic group. No sideroleukocytes were seen, however, in any cow of the other two groups. In the sideroleukocyte-positive cases of the anemic group, the number of sideroleukocytes per head ranged from 0.16 to 6.70 per 10 thousand leukocytes counted, or 1.72 on the average.

The sideroleukocytes were divided into three types according to the morphological feature of the iron-containing substances positive for the Prussian blue method. In the granular type, the cytoplasm contained one to several bluish granules of various size. In the diffuse type, the cytoplasm was stained diffusely light blue. In the mixed type, the cytoplasm contained bluish granules and stained diffusely light blue.

The rate of detection of sideroleukocytes was the highest (49.0%) in the diffuse type, the second highest (35.5%) in the granular type, and the lowest (15.5%) in the mixed type.

The values of Hb. and Hct. decreased more markedly in the anemic group than in any other group.

## DISCUSSION

ISHII et al.<sup>3)</sup> reported the appearance of sideroleukocytes in the blood of equine infectious anemia for the first time. Since then, a number of papers<sup>7,8,9,11~16,18)</sup> have been published to deal with sideroleukocytes in equine medicine.

However, as far as the authors know, there are no reports on sideroleukocytes in the peripheral blood of normal and diseased cattle. In the present observation, sideroleukocytes containing some substances which reacted positively to iron staining were present in the peripheral blood of 55.3% of the cows affected with the piroplasma of Theileria type. They could be divided into three types, granular, diffuse, and mixed.

In addition, it was seen that the mean values of Hb. and Hct. were lower and the mean rate of detection of red cells containing the piroplasma of Theileria type was higher in the anemic group in which sideroleukocytes were observed than in any of the other two groups in which no sideroleukocytes were observed at all.

In the field of equine medicine, studies on the nature of sideroleukocytes in the peripheral blood have been carried out by some workers<sup>4,11,15,16,18)</sup>. Although no definite conclusions have been drawn as yet, the following opinions have been proposed up to this time. Some author<sup>4)</sup> asserted that sideroleukocytes might be histiocytes which contained hemosiderin derived from the reticulo-endothelial system. Others<sup>11,15,16,18)</sup> stressed that sideroleukocytes might be composed of neutrophils, monocytes, and histiocytes all of which contained hemosiderin and ferritin.

In the present observation, the nature or the mechanism of generation of sideroleukocytes could not be clarified as yet. It was suggested, however, that the severities

of anemia and parasitization of the piroplasma of Theileria type might be related to the generation of sideroleukocytes in the infection of this organism.

#### SUMMARY

Sideroleukocytes in the peripheral blood of cows affected naturally with the piroplasma of Theileria type were studied. In 55.3% of the cows with clinical signs of anemia, the number of sideroleukocytes per ten thousand leukocytes ranged from 0.16 to 6.70, being 1.72 on the average. No sideroleukocytes, however, were observed in any cow affected with piroplasmosis and showing no clinical signs of anemia or in any control normal cow. The sideroleukocytes observed could be divided into three types, granular, diffuse, and mixed.

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## 小型ピロプラズマ自然感染牛の末梢血液 中の担鉄細胞

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臨床的に貧血症状を認め、かつ赤血球中に小型ピロプラズマを有する放牧牛38例、臨床的に貧血症状を認めないが、赤血球中に小型ピロプラズマを有する放牧牛10例、および臨床的に異常がなく、かつ小型ピロプラズマを全く認めない舎飼牛20例の3群について、担鉄細胞の検索を試みた。

その結果、貧血の症状を示し、かつ小型ピロプラズマを有する放牧牛群のみで担鉄細胞がみられ、その陽性率は55.3%であった。これらの担鉄細胞は、その形態から、顆粒型、瀰漫型および混合型の三型に分類された。

### EXPLANATION OF PLATE

Eight typical sideroleukocytes are shown in the plate. All of them were stained with Prussian blue first and then with carbolfuchsin.

Figs. 1 and 2. Sideroleukocytes of diffuse type.  $\times 2,000$ .

Fig. 3. A sideroleukocyte of granular type. Two clear granules are in the cell.  $\times 2,000$ .

Fig. 4. A sideroleukocyte of granular type. A big granule occupies almost the whole cell.  $\times 2,000$ .

Figs. 5 and 6. Sideroleukocytes of mixed type with granules contained in the bluish cytoplasm.  $\times 2,000$ .

Figs. 7 and 8. Sideroleukocytes of mixed type.  $\times 2,000$ .

