大型ピロプラズマ(Babesia ovata)実験感染牛における臨床および臨床病理学的研究

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Bovine Babesiosis in Japan: Clinical and Clinico-pathological Studies on Cattle Experimentally Infected with Babesia ovata

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Abstract. The pathogenicity of Babesia ovata was studies clinically, hematologically and blood-chemically in splenectomized and non-splenectomized cattle experimentally infected with this parasite. In six splenectomized cattle, the parasite multiplied markedly in the blood stream soon after inoculation. An elevated rectal temperature, anorexia, severe anemia, icterus and hemoglobinuria were observed clinically. Two of six cattle died. Hematologically, a marked decrease was observed in erythrocyte count, packed cell volume, hemoglobin level, leukocyte count and platelet count, as parasitemia increased in severity. Conjugated and unconjugated bilirubin, glutamic oxalacetic transaminase, blood urea nitrogen and uric acid levels were elevated, but total serum protein and blood glucose levels declined during the hemolytic phase. On urinalysis, hemoglobinuria, urobilinuria and proteinuria were present at the same time. It is considered that these changes have close relation to the multiplication of parasites in the blood stream and that the higher the intensity of parasitemia, the severer clinical symptoms are. Parasitemia was very mild in six non-splenectomized cattle in which anemia was also mild. Consequently, clinical, hematological and blood-chemical changes were relatively mild in these cattle. Therefore, it is considered that the pathogenicity of Babesia ovata was characterized not only by anemia, icterus and hemoglobinuria, but also by hepatic and renal dysfunction when parasites multiplied markedly in the blood stream due to such immuno-suppressive factors as splenectomy.

Bovine babesiosis in Japan, except Okinawa Prefecture, is transmitted by the larva of *Haemaphysalis longicornis* [9]. On the basis of studies on vector and pathogenicity, Ishihara [8] reported that the *Babesia* species found in Japanese cattle was different from *B. bigemina*. Fujinaga et al. [6] revealed serologically that the former *Babesia* species was distinguished from *B. major*, *B. bigemina* and *B. bovis* by the immunofluorescent technique. Minami and Ishihara [15] carried out studies to compare biological properties between the Japanese *Babesia* species and *B. major*, *B. bigemina*, *B. bovis* or *B. divergens*, and designated the first one

that had been usually called "the Japanese *Babesia* species" *Babesia ovata* as a new species.

Most grazing cattle infected with *B. ovata* are co-infected with *Theileria sergenti*, since *H. longicornis* which transmits *B. ovata* also transmits *T. sergenti* [9]. Therefore, there has been no reported case of single infection with *B. ovata* in the fild. On the other hand, some papers dealing with the pathogenicity of *B. ovata* for cattle mentioned briefly clinical and hematological findings in cattle experimentally infected [8, 15].

This paper describes clinical, hematological and blood-chemical changes in 804

	Cow	Age		Inocul	ation		Period of
Group	No.	in months	Days after splenectomy	Level of syringe passages	Dose of parasitized erythrocytes	Route	observation (days)
_	1	6		13	1.0×10 ¹¹	IV	447
Non- splenectomízed	2	6		11	8.0 \times 10 ¹¹	IV	417
Non- ectomi	. 3	4	_	3	3.8 \times 108	SC	373
Nect Z	4	18	_	11	1.5×10^{9}	sc	130
pler	5	18	_	11	1.5×10^{9}	SC	90
v.	6	18	_	11	1.5×10^{9}	SC	158
	7	6	6	9	6.6×10 ⁹	SC	60
zed	8	7	357	12	1.5×10^{9}	- sc	42
Ë	9	6	0	11	5.0×10^{9}	SC	<i>7</i> 0
Splenectomized	10	8	107	9	2.0×10^{5}	SC	110
pler	11	6	6	10	1.0×10^{10}	SC	10*
2	12	17	357	12	1.5×10^{9}	\$C	14*

Table 1. Protocol on experimental Babesia ovata infection

splenectomized and non-splenectomized cattle experimentally infected with *B. ovata*.

Materials and Methods

Strain of *Babesia ovata*: The miyake strain of *Babesia ovata* was used. It was isolated in 1967 from grazing cattle in Miyake Island, Tokyo Prefecture, and maintained by syringe passage in cattle.

Experimental animals: All the animals used were Holstein-Friesian cows four to 18 months of age demonstrated to be free from blood parasites and clinically normal and healthy.

Inoculations: The experimental cows were divided into two groups, one of six non-splenectomized (intact) cows and the other of six splenectomized cows. Cows 1 and 2 of the intact group were injected intravenously with living parasitized erythrocytes which had been collected from other cows experimentally infected with *B. ovata*. Cows 3 to 12 were injected subcutaneously in the neck region with cryopreserved parasitized erythrocytes. The protocol on each cow of the two groups is shown in Table 1.

Clinical observation: The rectal temperature was measured daily in the morning and afternoon before experimental inoculation and thereafter. Vigor, appetite, visible mucous membranes and other signs were observed clinically every morning.

Urinalysis: Urinary color gross inspection) and the chemical composition of urine (by the rapid dip technique; Uro-labstic, Miles-Sankyo Co., Ltd., Tokyo) were examined at one to seven days' intervals before infection and thereafter.

Hematological examination: Blood was collected from cows by puncture at the jugular vein at one to seven days' intervals before inoculation and thereafter. Total erythrocyte, leukocyte and platelet counts, packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by the electronic counting method with microcell counter, hemoglobin counter, MCVhemato counter and platelet counter (Toa Medical Electronic Co., Ltd., Tokyo). Blood smears were stained with Gimsa stain and examined microscopically for the differential leukocyte count, the figure of erythrocytes and the presence of Babesia parasites. Neutrophil and lymphocyte counts were determined from the total and differential leukocyte counts in the blood smear. The degree of parasitemia was expressed as a percentage of parasitized erythrocytes in the blood smear. Osmotic fragility of red blood cells was indicated by maximum and minimum resistances to hypotonic saline buffered solution.

Blood-chemical examination: Serum samples were obtained from blood collected from cows 4 to 6 of the intact group and cows 7, 8, 11, and 12 of the splenectomized group before infection and thereafter at one to seven days' intervals for biochemical analysis. Total serum protein (TP) amount (by the refractometric method), protein fractions, albumin-globulin ratio (Λ/G , electro-

^{*} Died in the acute hemolytic stage after infection.

Group		Prin	nary par	asitemia	Minimum	Minimum	Rectal te	emperature		Symp	tom*	
	Cow No.	Prepa- tent period	Dura- tion	Maximum parasite- mia	RBC $(\times 10^6/$	WBC $(\times 10^3/$	Duration above 39.5°C	Maximum	Listless-	Anorexia		mucous brane
		(days)		(%)	mm³)	mm ⁸)	(days)	(°C)	11633		Anemia	Icterus
	1	1	4	0.8	6.05	4.8	4	41.5	++	#	++	+
Non- splenectomized	2	1	4	4.0	5.61	5.4	4	40.9	#	#	#	+
Non-	3	12	12	0.01	6.43	7.3	9	40.6	+	+	+	_
ž ti	4	7	4	0.1	5.66	4.0	3	40.5	+	+	+	_
ple	5	7	8	0.1	6.78	5.3	2	39.9	_	_	_	_
s	6	7	. 6	0.1	5.89	7.1	4	40.5	土	_	_	-
	7	9	10	13.1	2.16	4.5	4	41.4	##	+++	##	##
pez	8	9	5	5.9	3.49	5.5	4	41.8	++	++	++-	#
Ë	9	7	12	6.7	4.29	5.3	2	41.7	#	++	#	#
Splenectomized	10	7	6	5.7	2.43	4.3	5	41.5	##	##	#	#
len	11	6	5	32.4	3.56	8.5	3	40.9	##	##	##	##
γŞ	12	9	9	22.6	3.88	3.1	4	42.1	##-	##	##	##

Table 2. Parasitemial responses, minimum number of erythrocytes (RBC) and leukocytes (WBC), and clinical findings in the acute hemolytic stage of experimental Babesia ovata infection

phorogram), and bilirubin level (modified Michölson method, Bilirubin Kit N; Nippon-Shoji Co., Ltd., Osaka) were determined. Blood glucose, glutamic oxalacetic transaminase (GOT), blood urea nitrogen (BUN), and uric acid were determined by the Unitest system (Unimeter 300; Bio-Dinamics Inc., Indianapolis, Ind.).

Results

1. Changes of parasitemia

The parasitemial response of each cow is shown in Table 2. Parasitemia was higher in level in the two cows injected intravenously with a large quantity of living parasites than in four cows of the intact group infected subcutaneously. There was an apparent difference in the maximum level of parasitemia between the splenectomized and the intact group, except the two cows infected intravenously of the former group. In the splenectomized group, this level was markedly elevated, and cows 11 and 12 died of severe acute anemia.

After the disappearance of primary parasitemia, the relapse of parasitemia was observed in intact cows 1 to 3 and splenectomized cows 7 to 10, but the maximum level

of parasitemia was constantly below 0.01%. Such recrudesence of parasitemia was always noticed within about 60 days after initial infection. It disappeared thereafter and was seen again at the end of the observation period.

2. Clinical findings

Table 2 shows clinical findings in the acute hemolytic stage after inoculation. There was invariably a rise in rectal temperature (above 39.5°C) in the primary parasitemia after initial infection, even when the maximum level of parasitemia was below 0.1%. Some cows showed continuous fever which lasted for a short time (2 to 5 days). Cow 3 manifested continuous fever lasting for a relatively long time (9 days) and characterized with recurrence. The duration of fever was always shorter than that of primary parasitemia in every cow. The rectal temperature rapidly fell to a normal level, as parasitemia declined in severity. The paler the mucous membrane, the severer the symptoms. In accordance with the decrease in PCV, the mucous membrane changed in color from pale to

^{*} Severity of symptoms. -: Normal, $\pm:$ very mild, +: mild, +: relatively severe, +: severe.

Table 3.	Urinalytic findings in the acute hemolytic stage of experimental
	Babesia ovata infection

Group		Urinalysis						
	Cow	Gross		Che	emical compo	sition		
Group	No.	Reddish ¹⁾ urine	Occult ²⁾ blood test	Ketone ³⁾ bodies	Glucose ⁴⁾	Protein ⁵⁾	Urobili- ⁶⁾ nogen	
75	1	_	NT	NT	NT	NT	NT	
Non- splenectomized	2	_	NT	NT	NT .	NT	NT	
Non- sctomi	3	_	NT	NT	NT	NT	NT	
Z	4	_	+	_	_	30	1	
ple	5		+	_	_	100	1	
v	6	_	<u> </u>	<u> </u>	_	100	0.1	
70	7	##	+++	_	-	300	8	
Splenectomized	8	##	+++	_	_	300	8	
e ot	9	##	##	_	_	300	4	
nec	10	+++	NT	NT	NT	NT	NT	
ple	11	+++	## .	_		30	1	
S	12	##	##	_	_	100	1	

NT: Not tested.

Degree of chemical composition.

white. Animals suffered from acute hemolytic anemia followed by icterus, which disappeared rapidly when PCV started to recover. Icterus was not often observed in rapid, mild anemia or slow, relatively severe anemia.

Other clinical signs manifested than those indicated in Table 2 are as followed. As anemia developed, pulse and respiratory rate were accelerated, and rough coat and epiphora observed. Dehydration caused the eyes to sink in the sockets. Diminution of rumination, splitting of heart sound, irregularity of pulse, and constipation frequently followed by diarrher were exhibited. In the splenectomized group the cows manifested severe signs, including hypesthesia and collapse, and cows 11 and 12 died after showing dyspnea and rapidly dropping rectal temperature.

On the other hand, clinical symptoms

were very mild in four cows in which the maximum level of parasitemia was very low, except cows 1 and 2 of the intact group.

3. Urinalysis

Urinalytic findings in the acute hemolytic stage are shown in Table 3. Reddish brown urine was seen in the splenectomized cows suffering from severe anemia. A positive reaction to the occult blood test was slight in cows 4 and 5 which voided normal urine. The occult blood test was positive in cows before and after voiding reddish urine. Urinary protein and urobilinogen appeared with an increase in severity of anemia, and urobilinogen disappeared relatively fast. Proteinuria gradually disappeared with a decrease in severity of anemia. No glucose or ketone bodies were present in any urine sample tested.

4. Hematological findings

Minimum erythrocyte and leukocyte

¹⁾ Negative (-), Reddish (+), Reddish brown (##).

²⁾ Negative (-), Small (+), Middle (+), Large quantity (+).

 $^{^{3)}}$ Negative (-); $^{4)}$ Negative (-); $^{5)}$ mg/dl.

⁶⁾ Ehrlich units/dl.

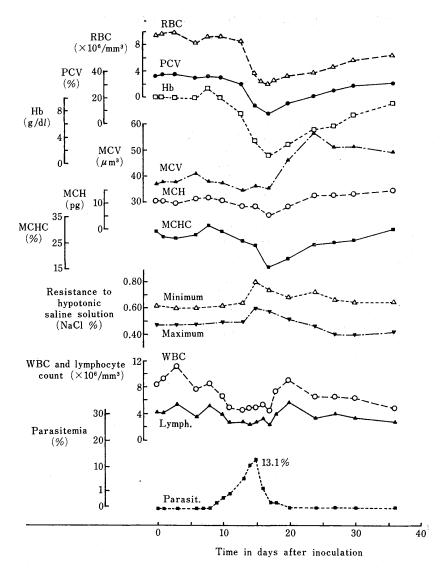


Fig. 1. Changes in hematological findings after experimental Babesia ovata infection in splenectomized cow 7.

For keys see Table 4.

counts in the acute hemolytic stage of primary parasitemia are shown in Table 2. Changes in cow 7 were illustrated in Fig. 1. Results obtained from seven cows in the acute hemolytic stage are shown in Table 4. The minimum erythrocyte count was inversely proportional to the maximum level of parasitemia. Parasites could barely be

detected in blood smears for a few days after parasitemia reached a maximum level. At that time, the total erythrocyte count, PCV and Hb level all declined greatly. In cow 7, MCV level rose conspicuously to $56~\mu \text{m}^3$ on the 24th day and fell gradually to $48~\mu \text{m}^3$ on the 36th day after inoculation. Changes in MCV level in any other animal

Table 4.	Hematological findings in the actue hemolytic stage in seven
	cows experimentally infected with Babesia ovata

Group	Non-	splenecto	mized	Splenectomized					
Cow No.	4	5	. 6	7	8	11	12		
Days after inoculation	10	12	10	17	13	10	13		
RBC ($\times 10^6/\text{mm}^3$)	5.66	6.78	5. 97	2.16	3.88	3.56	3.88		
PCV (%)	26.9	29.3	24.8	7.7	16.6	11.0	16.4		
Hb (g/d l)	8.8	9.9	8.5	1.2	5.5	2.0	5.2		
MCV (μm ³)	47	43	41	36	42	31	42		
MCH (pg)	15.5	14.6	14.7	5.6	7.1	5.6	13.4		
MCHC (%)	32.7	33.8	34.3	15.6	24.1	18.2	31.7		
Resistance to Minimum	0.72	0.68	0.64	0.74	0.76	0.80	0.80		
hypotonic saline solution (NaCl %)	0.54	0.54	0.50	0.58	0.60	0.64	0.64		
Appearance of immature erythrocytes	+	+	+	#	++	+	+		
WBC ($\times 10^3/\text{mm}^3$)	4.0	7.2	9.3	4.5	5.5	8.9	8.2		
Neutrophil ($\times 10^8/\text{mm}^3$)	0.6	1.9	2.3	1.9	0.9	3.7	4.3		
Lympocyte ($\times 10^3/\text{mm}^3$)	3.3	5.5	6.7	2.3	4. 2	4.5	2.5		
Platelet ($\times 10^4/\text{mm}^3$)	10.9	14.3	10.2	NT	15.3	NT	18.9		

RBC : Total erythrocyte count.

MCV: Mean corpuscular volume.

WBC: Total leukocyte count.

MCH: Mean corpuscular hemoglobin.

PCV : Packed cell volume.

MCHC: Mean corpuscular hemoglobin concentration.

Hb : Hemoglobin.

+ : Anisocytosis.

#: Anisocytosis and the appearance of a few other immature cells.

resembled those in cow 7. MCH level declined or remained stable in the acute hemolytic stage, and then increased slightly. MCHC level fell in the acute anemic phase, and thereafter recovered gradually.

Both minimum and maximum resistances of red blood cells to hypotonic saline buffered solution rose rapidly in the acute hemolytic stage, and thereafter fell gradually. In hemolytic crisis, hemoglobinemia occurred in all the splenectomized cows.

Morphological observation of erythrocytes, revealed anisocytosis, erythroblasts, basophilic stippling and polychromasia of erythrocytes, and Howell-Jolly bodies or Cabot's ring bodies in the recovering stage after the severe acute hemolytic stage was over. Only anisocytosis was observed in two dead animals and the cows of the intact group in which anemia was mild. Hematological changes were mild, however, in

the intact cows which showed a low level of parasitemia.

Leukocyte counts fell to about one half of pre-infection levels at the appearance of parasitemia in all the experimental animals. The leukocyte count increased in cow 11 and returned to the pre-infection level in cow 12 immediately before death. In the other 10 cows, thereafter, it rose gradually in accordance with the disappearance of parasitemia. This leukopenia was accompanied with a decrease in neutrophil and lymphocyte counts. The total platelet count fell gradually to about one half in the presence of parasitemia in all the cows of the two groups measured, and then turned to the pre-infection level.

5. Blood-chemical findings

Blood-chemical changes in cow 7 are demonstrated in Fig. 2. Results obtained from seven cows are indicated in Table 5.

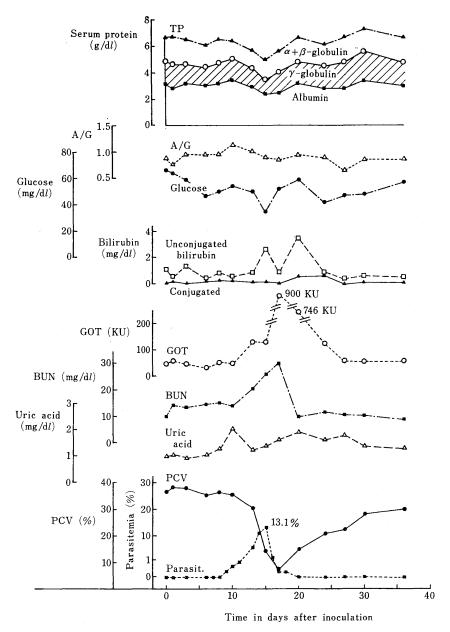


Fig. 2. Changes in blood-chemical findings after experimental Babesia ovata infection in splenectomized cow 7.

For keys see Table 5.

A marked decline in TP level was observed in hemolytic crisis in these cows. The level, thereafter, gradually rose to the pre-infection level until the 30th to 40th day after inoculation. The gamma-globulin level also fell in parallel to the TP level. Thereafter, it showed a tendency to be slightly higher than the pre-infection level. The 810

Table 5.	Blood-chemical	findings	in	the	acute	hemolytic	stage	in	seven	cows
	experimentally	infected	١w	ith I	Babesi	a ovata				

Group	Non-	splenecto	mized	Splenectomized				
Cow No.	4	5	6	7	8	11	12	
Days after inoculation	10	12	10	17	13	10	13	
TP (g/d $m{l}$)	5.3	6.2	5.9	5.6	6.2	6.4	7.0	
Albumin (g/d $m{l}$)	2.7	3.0	2.1	2.4	3.0	2.8	3.4	
γ -globulin (g/d l)	1.2	1.3	1.8	1.6	1.5	1.3	1.5	
A/G	1.04	0.94	0.55	0.77	0.94	0.80	0.94	
Glucose (mg/d l)	72	70	65	52	43	5	5	
Bilirubin (mg/d $m{l}$)								
Unconjugated	1.33	1.10	1.98	0.76	3.20	10.04	8.90	
Conjugated	0.07	0.14	0.12	0.08	0.78	1.52	1.49	
GOT (KU)	87	106	106	900	220	195	203	
BUN (mg/d $m{l}$)	11.3	11.6	6.5	29.8	22.8	27.3	19.6	
Uric acid (mg/d \emph{l})	1.08	1.08	1.20	1.62	1.43	4.30	4.71	

TP: Total serum protein.

GOT: Glutamic oxalacetic transaminase.

A/G: Albumin-globulin ratio.

BUN: Blood urea nitrogen.

albumin-globulin ratio fell gradually following the slight rise of gamma-globulin level. The glucose level decreased in acute hemolytic crisis, and to 5 mg/dl or below in cows 11 and 12 before death. A marked rise in the unconjugated bilirubin level occurred in the hemolytic phase. Afterward, the level fell to the pre-infection value, as the PCV increased to reach about 20%. The conjugated bilirubin level rose after the crisis of anemia. A conspicuous rise in GOT level occurred in the hemolytic phase. In the hemolytic stage the BUN level was higher than the pre-infection level, or below 10 mg/dl. The increased uric acid level remained for a relatively long period.

On the contrary, only small changes in blood-chemical properties were observed in cows 4 to 6 in which anemia was very mild. Accordingly, a slight increase was observed in unconjugated bilirubin and GOT levels in the mild hemolytic stage. No changes were seen in conjugated bilirubin, glucose, BUN, or uric acid level.

Discussion

There is a great variation in the clinical sign of babesiosis for the pathogenicity of *Babesia* and the susceptibility of the host [12]. The pathogenicity of *B. ovata* was interpreted to be milder than that of *B. bovis* and *B. bigemina*, but severer than that of *B. major* [15].

Clinical impairment by *B. ovata* infection was considered parallel to developing anemia which was attributable to the destraction of red blood cells, as evidence by the results of this experiment. As the maximum level of parasitemia rose, anemia became severe with the PCV level decreasing. Consequently, the extent of clinical symptoms appeared to be agreeable with the multiplication of parasites in the blood stream.

When calves were exposed experimentally to infestation with ticks, *B. ovata* parasites were detected in blood smears after a prepatent period of nine to sixteen days [8, 15]. It was considered that the prepatent period after infection with blood parasites could

be shortened in compliance with the dose level or the route of inoculation, as expressed by the results in Table 2.

Maximum parasitemia was higher in level in the splenectomized group than in the intact group experimentally infected with B. ovata, similar to the results with B. bovis and B. bigemina infections [18]. Anemia and clinical symptoms, therefore, were severer in the splenectomized group. While the cows were recovering from anemia, the PCV level rose to the pre-infection value faster than the elevating speed of erythrocyte count, which was also known clearly by a marked rise in MCV level. From these changes in MCV, MCH, and MCHC levels, anemia caused by B. ovata infection was normochromic or hypochromic and normocyte in the acute hemolytic stage, and was normochromic and macrocytic after the erythrocyte count started to increase. Wright [21] also reported that normochromic and normocytic anemia was observed at first and followed by macrocytosis. An increase in myelopoietic function may be evidenced by the appearance of immature erythrocytes and a rise in MCV level after the hemolytic phase.

Marked leukopenia was observed during parasitemia in *B. ovata* infection [8] and *B. bigemina* [8, 10]. In the present studies, leukopenia was caused during parasitemia because both neutrophil and lymphocyte counts declined to about one half. Leukocytosis which had been seen in *B. bovis* [21], *B. bigemina* [16, 21], and *B. canis* [5, 19] infections was not clearly exhibited in the present studies, following a similar pattern in *B. bigemina* infection [10].

As described by Wright [22] in *B. bige-mina* and *B. bovis* infections, an increase was noticed in the osmotic fragility of red blood cells in experimental *B. ovata* infection. Since hemoglobinuria and hemo-

globinemia appeared, and the maximum and minimum resistances to the hypotonic buffered saline solution of red blood cells increased in the crisis of hemolytic anemia, it was certain that red blood cells had been broken down in the blood stream. Wright [21] presumed that hemolysis might have mostly resulted from an increase in fragility of blood cells due to antigenic stimulation of both existing cells and hemopoietic centers in bovine babesiosis. The mechanism of hemolysis in bovine babesiosis will be elucidated by further work on the alteration of erythrocytes by the presence of parasites in erythrocytes and antigenic alteration of red cell membranes.

From the results of urinalysis and bloodchemical examination, the hepatic dysfunction was indicated by a decrease in TP level and a marked increase in GOT, conjugated bilirubin, BUN, uric acid and urinary urobilinogen levels, and the renal dysfunction by proteinuria and an increase in BUN and uric acid levels, as compared with the pre-infection values. GOT and BUN levels were reported to increase in B. bigemina and B. bovis infections [20], and the BUN level was observed to increase in B. canis infection [14]. Urobilinuria and a rise in unconjugated bilirubin level indicated not only hepatic damage, but also hemolytic anemia [4]. The same can be said about the elevated GOT level, because this enzyme exists not only in the liver, but also in the heart, kidney and skeletal muscle [2]. An increase in BUN level dose not necessarily indicate the hepatic dysfunction. Malherby [13] reported that the elevated level of bromsulphalein retention was affected by a hepatic lesion in dogs infected with B. canis. Annable and Ward [1] revealed that immune complex-induced nephritis was associated with glomerular deposits of IgG and the third component

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of complement in B. rodhaini infection.

Hypoglycemia was also observed in severe cases of B. bigemina infection [16]. A decrease in liver glycogen level was reported in B. canis infection [7, 11] and acute malaria [3]. Hypoglycemia in severe B. ovata infection may result from the depression of glycogenesis in the liver, judging from the result that the liver glycogen level appeared to be influenced by the intensity of cellular degeneration in the liver [20]. On the other hand, hypoglycemia was caused by the consumption of glucose which was important for Babesia as an energy source [17] by a marked multiplication of parasites and by a metabolic increase as a result of pyrexia.

Therefore, in intact cows, the maximum level of parasitemia was very low and followed by mild anemia. Abnormalities in urinalysis and blood-chemical examination were hardly found, as compared with the above-mentioned findings on the splenectomized cows. Consequently, hepatic and renal dysfunction appeared to develop in accordance with anemia caused by an increase in intensity of parasitemia. The pathogenicity of *B. ovata* appeared to be essentially the same as that of *B. bigemina* [8, 10, 16, 21, 22], but was milder than the latter, as described by Minami and Ishihara [15].

From the present studies, it is considered that the pathogenicity of *B. ovata* was characterized not only by anemia, icterus and hemoglobinuria, but also by hepatic and renal dysfunctions, since parasites multiplied markedly in the blood stream due to the immuno-suppressive action [12] of splenectomy or others.

Further studies, including histo- and immuno-pathological research, on *Babesia* ovata infection are necessary to elucidate the mechanism of hepatic and renal damage

and destruction of red blood cells in the blood stream.

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References

- Annable, C. R., and Ward, P. A. (1974). Immunopathology of the renal complications of babesiosis. *J. Immunol.* 112, 1–8.
- [2] Boyd, J. W. (1962). The comparative activity of some enzymes in sheep, cattle and rats — Normal serum and tissue levels and changes during experimental liver necrosis. *Res. Vet.* Sci. 3, 256-268.
- [3] von Brand, T. (1973). Carbohydrates. III. Host-parasite relationships. In Biochemistry of Parasites, 2nd ed., Academic Press Inc., New York, 171-206.
- [4] Cornelius, C. E. (1970). Liver function. In Biochemistry of Domestic Animals, 2nd ed., Vol. 1, Kaneko, J. J. and Cornelius, C. E., editors, Academic Press Inc., New York, 161– 231.
- [5] Dorner, J. L. (1967). A hematologic study of babesiosis of the dog. Am. J. Vet. Clin. Pathol. 1, 67–75.
- [6] Fujinaga, T., Minami, T., and Ishihara, T. (1980). Serological relationship between a large Babesia found in Japanese cattle and Babesia major, B. bigemina and B. bovis. Res. Vet. Sci. 29, 230-234.
- [7] Gilles, H. M., Maegraith, B. G., and Andrews, W. H. H. (1953). The liver in *Babesia canis* infection. *Ann. Trop. Med. Parasitol.* 47, 426-430.
- [8] Ishihara, T. (1968). Bovine piroplasmosis in Japan. Jpn. Agric. Res. Q. 3, 23-31.
- [9] Ishihara, T., and Minami, T. (1978). Bovine theileriosis and babesiosis in Japan. In Protozoan Diseases, JGAPD publication No. 1, Proceedings of the First Japanese-German Cooperative Symposium on Protozoan Diseases, The Japanese-German Association on Protozoan Diseases, Tokyo, 201–209.
- [10] Löhr, K.-F., Otieno, P. S., Meyer, H., Higgs, J., and Ashford, W. A. (1977). Haematological reactions to experimental *Babesia bige*mina infection in splenectomised and nonsplenectomised cattle. *Zentralbl. Veterinaer*med., B 24, 508-516.

- [11] Maegraith, B., Gilles, H. M., and Devakul, K. (1957). Pathological processes in Babesia canis infections. Z. Tropenmed. Parasitol. 8, 485-514.
- [12] Mahoney, D. F. (1977). Babesia of domestic animals. In Parasitic Protozoa. Vol. 4, Kreier, J. P., editor, Academic Press Inc., New York, 1-52.
- [13] Malherbe, W. D. (1965). Clinico-pathological studies of *Babesia canis* infection in dogs. I. The influence of the infection on bromsulphalein retention in the blood. J. S. Afr. Vet. Med. Assoc. 36, 25-30.
- [14] Malherbe, W. D. (1966). Clinico-pathological studies of *Babesia canis* infection in dogs. V.
 The influence of the infection on kidney function. J. S. Afr. Vet. Met. Assoc. 37, 261-264.
- [15] Minami, T., and Ishihara, T. (1980). Babesia ovata sp. n. isolated from cattle in Japan. Natl. Inst. Anim. Health Q. (Jpn.) 20, 101-113.
- [16] Pandy, N. N., and Mishra, S. S. (1977). Studies on the haematological changes and blood glucose level in *Babesia bigemina* infection in indigenous cow calves. *Indian Vet. J.* 54, 880– 883.
- [17] Rickard, M. D. (1969). Carbohydrate meta-

- bolism in *Babesia rodhaini*: Differences in the metabolism of normal and infected rat erythrocytes. *Exp. Parasitol.*, **25**, 16–31.
- [18] Ristic, M. (1970). Babesiosis and theileriosis. In Immunity to Parasitic Animals, Vol. 2, Jackson, G. J., Herman, R., and Singer, I., editors, Appleton Century Crofts, New York, 831–870.
- [19] Schalm, O. W., Jain, N. C., and Carroll, E. L. (1975). The erythrocyte in disease. *In Veterinary Hematology*, 3rd ed., Lea and Febiger, Philadelphia, 405–470.
- [20] Wright, I. G. (1972). Studies on the pathogenesis of Babesia argentina and Babesia bigemina infections in splenectomised calves. Z. Parasitenkd. 39, 85-102.
- [21] Wright, I. G. (1973). Observations on the haematology of experimentally induced Babesia argentina and B. bigemina infections in splenectomised calves. Res. Vet. Sci. 14, 29– 34.
- [22] Wright, I. G. (1973). Osmotic fragility of erythrocytes in acute *Babesia argentina* and *Babesia bigemina* infections in splenectomised *Bos taurus* calves. *Res. Vet. Sci.* 15, 299–305.

要 約

大型ピロプラズマ (Babesia ovata) 実験感染牛における臨床および臨床病理学的研究:藤永 徹 (農林水産省家畜衛生試験場,九州支場)——日本の牛から分離され,新種のバベシアとして報告された Babesia ovata (和名:大型ピロプラズマ) の病原性を検討するため,摘脾牛および非摘脾牛を用いて実験感染を行い,臨床および臨床病理学的に観察した結果,次の成績を得た.摘脾牛6頭では,接種後原虫は流血中で著明に増殖し,発熱,食欲および元気消失,貧血,黄疸および赤色尿の排出が認められ、2頭が死亡した.尿検査では,血色素尿,ウロビリン尿および蛋白尿の排出が認められた.血液学的には,原虫寄生率の上昇につれ,赤血球数,Ht および Hb 値の低下,白血球および血小板数の著明な減少が認められた.貧血極期には,赤血球浸透圧抵抗の著明な減弱が認められた.血液生化学的には,直接および間接ビリルビン,GOT,BUN および尿酸値の著増と血清蛋白質量および血糖値の低下が認められた.このような変化は原虫寄生率の上昇程度と密接な関連があり,最高寄生率の高いものほど重度であった.いっぽう,非摘脾牛6頭では,原虫の最高寄生率は低く,貧血は軽度であり,臨床,血液および血液生化学的所見の変化も軽度であった.以上のことから,B. ovata は牛に対して貧血,黄疸および血色素尿の排出だけではなく,摘脾あるいはその他の免疫抑制作用によって,流血中で原虫が著明に増殖した場合,肝・腎機能に障害を及ぼす病原性を有するものと考えられた.