Bovine Piroplasmosis in Japan

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In Japan the parasitism of *Theileria* on the cattle was discovered in an apparently healthy animal for the first time in 1905, and that of *Babesia* in a diseased animal in Kyushu district in 1909.[11] As mixed infection with *Babesia* and *Theileria* was common in Japan, babesiosis and theileriosis have collectively been called piroplasmosis. Before World War II, piroplasmosis had been considered to be due to *Babesia*. *Theileria*, a fellow of mixed infection, was altogether out of the question being regarded as a nonpathogenic organism.[11] After the war, systematic studies, however, have been carried out on the theileriosis, because it occurred in connection with malnutrition of cattle in various places throughout this country. And many facts have been made clear about this disease.[6,7] Studies have also been made on the fellows of mixed infection of this disease, *Babesia*, *Anaplasma*, *Eperythrozoon*[4] and *Grahamella*, etc., as to the species, pathogenicity, interrelation among them, and so on, elucidating step by step the anemic diseases of pastured cattle. The infectivity of *Theileria* was so high that 80-100 per cent of cattle were infected with it and about 20 per cent of the infected animals manifested the symptoms of theileriosis in many stock-farms.[3,5] It was thought that *Babesia* and *Anaplasma*, etc., were involved in the cause of death.

Species of *Theileria*

Theileria in Japan has been considered, without detailed investigations to be *Th. mutans* or its relative,[2,3,6,8,10,11] though there were some different opinions on its identification. The author and his co-workers studied its pathogenicity[6] and tick vector,[7] and discovered that the Japanese species was stronger in pathoenicity than *Th. mutans*. They also compared *Theileria* sp. in Japan (Japanese strain) with two other strains. African and Australian, by artificial infection of Holstein-Friesian calves of Japanese birth with those strains. The African strain was *Th. mutans* obtained through the kindness of Dr. W.O. Neitz, and the Australian strain was *Theileria* sp. which had been maintained by passage through the blood. Its origin was one of the cattle in quarantine for import from Australia.

The Japanese strain of *Theileria* was carried by the ticks of both parthenogenetic and bisexual generations of *Haemaphysalis neumanni* in the way of stage to stage, but not transmitted to the larva of the next yenyration. Neither *H. neumanni* nor *Boophilus microplus* was the vector of the Australian strain. The results of experiments on the tick vector of Piroplasma are summarized in Fig. 2.

Koch bodies could not be detected in the lymphatic gland of splenectomized calves after their infection with each of the three strains of *Theileria*. In case of artificial infection by the tick, remittent fever of 41°-42°C continued for several days till the time just before the appearance of protozoa in the circulating blood. The normal temperature of the body was recovered, when the protozoa appeared. The infected animal, however, became feverish again, when the protozoa increased in number in the blood. In case splenectomized calves were infected with the Australian or Japanese strain, the protozoa was parasitic in 40-70 per cent of the red blood corpuscles. The parasitic rate, however, never rose above 20 per cent when

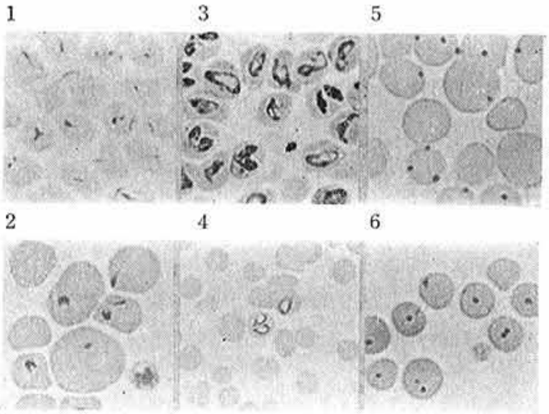


Fig. 1. Theileria, Babesia and Anaplasma in blood smears stained with Giemsa solution in calves.
1. *Theileria* sp. (Japanese strain), splenectomized calf, × 2,000.
2. *Theileria* sp. (Japanese strain), × 3,000.
3. *Babesia* sp. (Japanese strain), splenectomized calf, × 2,000.
4. *Babesia* sp. (Japanese strain), × 750.
5. *Anaplasma marginale* (Okinawan strain), Splenectomized calf, × 3,000.
6. *Anaplasma centrale* (Japanese strain), splenectomized calf, × 2,000.

they were infected with the African strain. The protozoa had a complicated structure, but there was no morphological difference among the three strains in case a small number of the organisms were found in the blood. The protozoa was always observed in the circulating blood of calves in case they were infected with the Japanese strain of *Theileria*, while it was difficult to detect the organism in the cattle tolerant of the African strain. Such a tolerant animal manifested symptoms characteristic of the Japanese strain after cross infection with it as shown in Fig. 2 and thereafter the protozoa could be detected in the circulating blood.

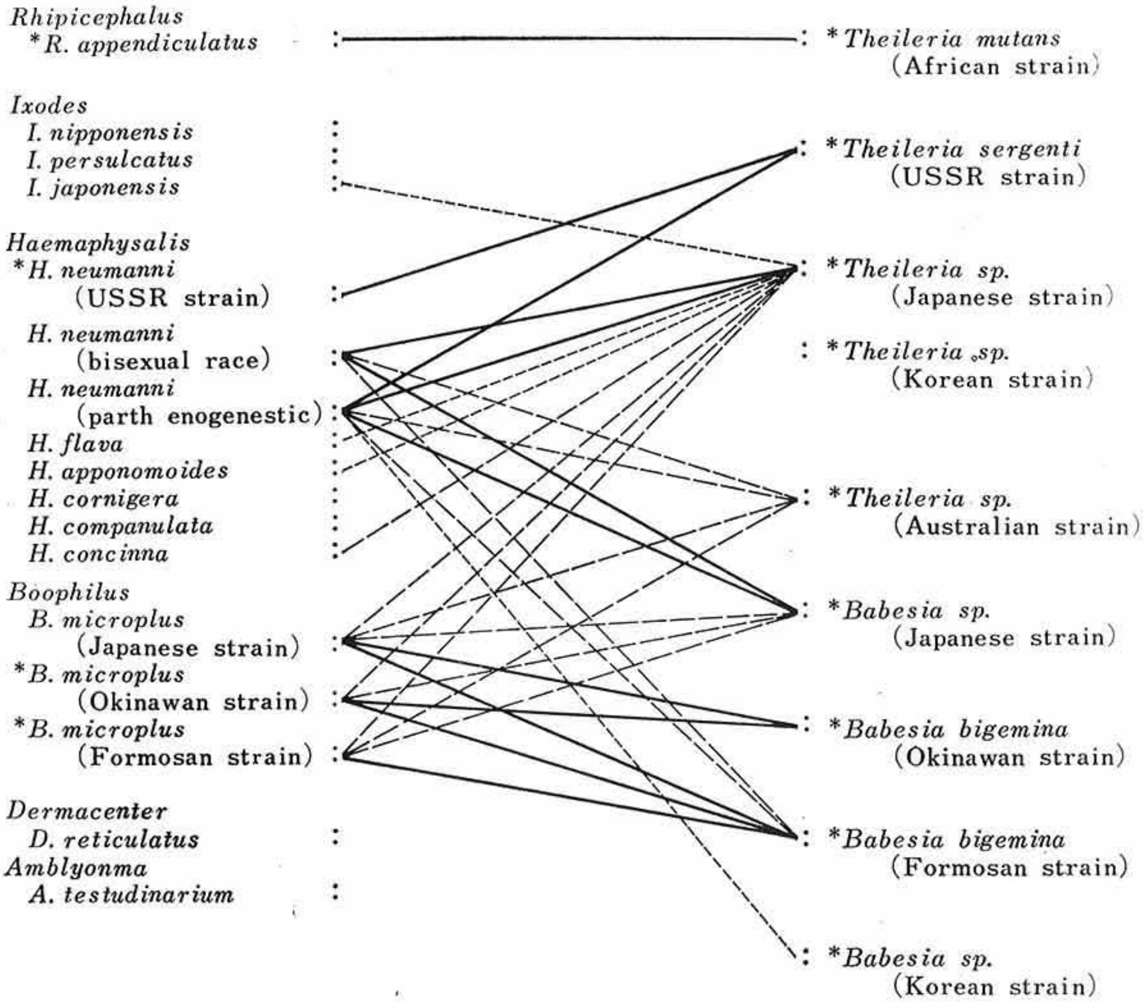
The African strain did not show any change in pathogenicity after seven passage generations through calves during seven years, and was the weakest in this character among the three strains (Table 1). The Australian strain manifested the symptoms in splenectomized calves as the Japanese strain did, but nearly no symptoms in non-splenectomized animals. The Japanese strain manifested the symptoms slightly even in non-splenectomized calves, though icterus was slight and hemoglobinuria has never been observed even in seriously attacked splenectomized animals. However, there were some cases of death due to icterus, when the animals were infected artificially, or naturally in the field, with *Babesia* and *Anaplasma*, etc., in addition to *Theileria*. It was thought that the difference of protozoa in virulence was due to the difference of multiplication among the strains.

From the above-mentioned facts, it was considered that the Japanese strain was different from *Th. mutans*, and resembled *Theileria sergenti* Yakimoff and Dekhtereff, mentioned in the 1930 literature. The author received *Th. sergenti* being carried by the tick (*H. neumanni*) from the USSR, and after comparing it with the Japanese strain in 1967, obtained the result that they resembled each other in many biological characters (unpublished).

Species of *Babesia*

Till recently, it has been thought that *Babesia* in Formosa, Okinawa, Korea and Japan are only different strains of the same species, *Babesia. bigemina*, respectively, being transmitted by the tick of *Boophilus*.[5] And it was also reported that there was a developmental stage showing a *Babesia* type in the life cycle of *Theileria*. The author and his co-workers found that *Babesia* in Kyushu could not be carried by *Boop. microplus*, and have reexamined *Babesia* in Japan since 1957 paying their attention further to the fact that mixed infection with *Theileria* and *Babesia* occurred in the area where *Boophilus* was never distributed.

The author succeeded in passage of isolated *Babesia* through blood by application of pamaquine, a protozoacide, though it had been said that *Babesia* could not be isolated from *Theileria* by the passage through blood.[11] He also proved experimentally that *Babesia* in Japan was transmitted by *H. neumanni* to the larva of the next generation and the larva thus became the vector of the protozoa. As the larvae and adults of this tick were vectors of *Theileria*, the reason of mixed infection with both kinds of protozoa also became clear. It was also shown that there was an inhibition phenomenon between *Babesia* and *Theileria*, and multiplication of the former was inhibited by the latter. This phenomenon explained the reason why the detection rate of *Babesia* was low and babesiosis was correspond-



* Species are not present in Japan.

——— : Ticks are vector of piroplasma.

– – – – : Ticks are not vector of piroplasma.

- - - - - - - : Ticks may not be vector of piroplasma. in author's experiments.

No line : Not yet examined.

Fig. 2. Vectors in Theileriosis and Babesiosis

Table 1. Clinical manifestations and changes of peripheral blood in calves infected with Theileria.

			Splenectomized calf			Non-splenectomized calf		
Strain			Japanese strain	Australian strain	African strain	Japanese strain	Australian strain	African strain
No. of examined calf			6	3	8	21	4	4
Theileria organisms	Koch body		–	–	–	–	–	–
	Incubation period, in days (average)	by tick	10–21 (13.5)		35–38 (36.5)	10–36 (13.0)		22
		by blood		11–14 (12.5)	19–65 (43.0)		4–20 (17.2)	39–49 (45.6)
	No. of organisms, Max. per 1,000 RBC (average)		246–708 (446.5)	145–238 (186.0)	1–52 (13.0)	1–218 (63.5)	8–45 (17.2)	0.1–1 (0.3)
	Disappearance from blood		–	–	±	–	±	+
Fever (temp. C, Min. average)	Before the appearance of organisms		++++ (40.2)	?	++++ (40.8)	++++ (40.6)	?	++++ (40.7)
	After the appearance of organisms		++++ (40.7)	++++ (40.7)	+ (39.6)	+++ (40.0)	+++ (40.0)	+ (39.7)
Anemia (No. of RBC., in million, Min., average)			++++ (1.9)	+++ (1.0)	+ (6.2)	+++ (4.4)	++ (5.5)	+ (6.9)
Anorexia			++	++	–	+	–	–
Hemoglobinuria			–	–	–	–	–	–
Icterus			–	–	–	–	–	–

Table 2. Interference between Babesia and Theileria

		Maximum No. of Babesia (per 1 mm³)						Appearance of Babesia in peripheral blood (days)			
		10^1	10^2	10^3	10^4	10^5	10^6	1–3	4–10	11–20	21–
Splenectomized cattle (Cases)	Babesia alone	–	–	2	9	8	1	–	1	1	18
	Babesia, Theileria	1–	–	1	4	1	–	1	1	1	4
Nonsplenectomized cattle (Cases)	Babesia alone	4	13	5	5	2	–	7	13	7	2
	Babesia, Theileria	19	3	1	–	–	–	17	6	–	–

Table 3. Clinical manifestations and changes of peripheral blood in cattle infected with Babesia.

	Babesia sp. (Japanese strain)									*B. bigemina* (Okinawan strain, Formosan strain)								
	Cattle No.	Incubation period (Days) by tick	Incubation period (Days) by blood	Babesia in blood*	Anemia**	Leukopenia	Fever	Other symptoms***	Remarks	Cattle No.	Incubation period (Days) by tick	Incubation period (Days) by blood	Babesia in blood*	Anemia**	Leucopenia	Fever	Other symptoms***	Remarks
Cows	210		8	++	+	+++	+++	–		236			++++	++++	++++	++++	++++	
	211		6	++	++	++++	+	+		242		4	+++	++++	++++	++++	+++	
	213		11	+	+++	+++	+++	–		285	18		++	+++	++++	++++	+	
	237		10	++	+++	++	+	+										
Calves	176		9	++	++	–	–	–		214		4	+++	++	+	+	–	
	182		4	++	+	–	–	–		225		4	++	++++	++++	++	–	
	188		20	+	++++	+	++++	–		232		6	++++	+++	+++	++++	+	
	189		11	++	++	–	–	–		230	17		++	++++	++	++++	–	
	193		12	+	+++	++	++	–		231	14		++++	+++	+++	++	+	
	198		7	+	++	–	++++	–		233	16		+++	++++	++	+++	–	
	207		6	+	+++	++++	–	–		244	20		++	++++	+++	+++	–	
	212		6	+	++	–	–	–		245	16		+++	++++	+	++++	+	
	229		10	++	+	–	++	–		247	14		++	+++	+++	++	–	
										248	14		++	+++	++++	++	–	
										251	17		++	++++	++	++++	–	
Splenectomized calves	171		13	+++	+++	+++	+	++		194		10	++++	++++	+++	++++	+	
	185		7	++++	++++	++	+++	++++		219		4	++++	++++	+++	++++	++++	Died
	191		9	+++	++++	+	++++	+		226		4	++++	++++	+	++++	++++	
	195		5	++++	++++	–	++++	++++	Died	240	17		++++	++++	+	++++	+++	
	201	10		+++	++++	++	++++	++		267		6	++++	++++	+++	++++	++++	
	202	16		++++	+++	++	+++	++		278	16		++++	++++	+++	++++	++++	Died
	203	13		++++	++++	+	++++	++										
	204		10	+++	++++	++++	++++	–										
	227		10	+++	++++	++++	++++	–										
	239		6	++++	++++	–	++++	++										
	241		10	++++	++++	++	++++	++++	Died									

* Babesia in blood
- ++++ more than 10^5 in the number of parasitic RBC.
- +++ more than 10^4 in the number of parasitic RBC.
- ++ more than 10^2 in the number of parasitic RBC.
- + less than 10^2 in the number of parasitic RBC.

** Anemia (leucopenia, fever and other symptoms)
- ++++ very severe degree, +++ severe degree
- ++ moderate degree, + slight degree

*** Other symptoms involve icterus, anorexia, dyspnoea, jaundice, lie down and milk yield.

Table 4. Cross immunization experiment

Findings after primary inoculation				Findings after secondary inoculation			
				Japanese strain		Formosan strain	
Strain	Cattle No.	Babesia in blood	Clinical changes	Babesia in blood	Clinical changes	Babesia in blood	Clinical changes
Babesia sp. (Japanese strain)	210	++	+++	−	−		
	211	++	+++	−	−		
	213	+	+++	−	+		
	176	++	+	−	−		
	188	+	+++	−	−		
	189	++	+	±	−		
	212	+	+			++	++
	228	++	+			+++	+++
	237	++	++			+++	++
B. bigemina (Formosan strain)	225	++	++++	++	+++		
	231	++	+++	−	++		
	233	++++	+++	±	+	±	−
	242	+++	++++	++	+		
	212	+++	++			−	−
	214	+++	+			+	±
	230	++	++++			±	−
	232	++++	++++			±	−
	236	++++	++++			+	−

Comment : Signs of degree (++++−+) are the same as that in Table 3.

ingly slight in pastured cattle (Table 2). It, however, is certain that mixed infection with *Babesia* and *Anaplasma*, etc., induces theileriosis and raises the death rate of cattle in the field. This is shown by the high mortality of animals artificially infected with a mixture of the three kinds of protozoa.

The Japanese species maintained by us could not be carried by *Boop. microplus*, while *Boop. microplus* collected in Formosa, Okinawa and Kyushu were the vector of *B. bigemina* from Formosa and Okinawa, and *H. neumanni* was not its vector.

The Japanese species cannot be morphologically discriminated from *B. bigemina*. The former was a little weaker in pathogenicity, but their difference was not essential. Both of them manifested the symptoms in adult cattle and splenectomized calves of the first infection. The symptoms were manifested when the number of protozoa was more than 10^3 per mm^3 of the blood. And hemoglobinuria was mostly observed, when the number was over 10^4 per mm^3 (Table 3).

There are different opinions on the identification of protozoan species by cross immunization and by their vectors.[12,13] It was shown by the author's experiment that cross immunization was not effected between *B. bigemina* and the Japanese species. And the cattle immunized from the former manifested the symptoms characteristic of the Japanese species after the infection with it, though the manifestation was somewhat slight (Table 4). In case of reinfection with the same species of protozoa, nearly no symptom was observed irrespective of the appearance of organism in the blood. The author is planning to make identification of the Japanese species after completing its serological investigations. All the *Babesia* which were brought in our passage from Japanese cattle have been thought to belong to the same species. It, however, is probable that *B. bigemina* and *B. argentia*, etc., distribute to some extent in Japan, because this country has been importing cattle for a long time. But it seems that the distribution is narrow and the contamination with them is slight, if ever.

Protozoacide

Since 1948 when the effect of pamaquin, a chemical of 8-aminoquinoline compounds, on the erythrocytic stage of *Theileria* in Japan was reported,[6] pamaquin and primaquin have been in wide use. In general, these chemicals must be used continuously to be effective. Pamaquine oil, which contains 20 per cent of olive oil, however, has an immediate effect at the first intramuscular injection of 1-2 ml dose. Occurrence of the strains of protozoa resistant to chemicals has come into question recently. Injection of diazoaminedibenzamidine, a babesiacide, at a large dose (6-9 mg per kg of body weight) is efficacious against the strain resistant to 8-aminoquinoline, though it is slow in its effect. 4-aminoquinoline compounds, pyrimethamine and some antibiotics, as aureomycin, are effective to some extent for preventing infection with *Theileria*, but they are not in practical use. Pamaquine oil also had nearly no effect on the tropical theileriosis caused by *Th. annulata*, though tests were carried out at the request of the author in Iran (unpublished).

There are reports that trypaflavin works well on babesiosis in Okinawa and also ichthargan on piroplasmosis. These chemicals, however, are only efficacious against *Babesia*, and not against *Theileria* at all. Symptomatic treatments are necessary for curing piroplasmosis, and transfusion of blood is effective for malignant anemia of cattle. [15]

Prevention

BHC and low poisonous organophosphorous chemicals are in use to rid the pasture land of ticks. For this purpose dusting of chemicals from helicopters and fumigation are practiced experimentally. It, however, is difficult technically as well as commercially to exterminate ticks in a wide area of meadow. The direct sprinkling of chemicals on the body of cattle is effective to kill the tick of *Boophilus* which, live all their lives on a single individual and are referred to as one-host ticks, but is less effective to exterminate the other kinds of ticks which feed on another animal every time and are referred to as three-host ticks.

Plunge dip is not practiced, as animals of a herd are small in number at present in Japan.

When the use of pasture land is suspended for 1-2 years, ticks carrying *Theileria* move to some other domestic animals other than cattle and wild animals, as mice and hares, etc., and are parasitic on them, not being prevented from developing and propagating. Adult ticks thus lay eggs and larvae develop from the eggs. *H. neumanni* does not transmit *Theileria* to the next generation through the egg, so examination of the newly hatched larvae for detection of the protozoa always gives negative results. The ticks, even if they inhabit the land after the suspension of pasture, thus cannot be the origin of infection as far as *Theileria* is concerned. According to the experiment carried out in a pasture land which had been contaminated with *Theileria* before the suspension, the cattle pastured after it could be prevented from infection with the protozoa, while all the animals pastured in the adjacent lot were infected with the organism.

In some parts of this country, immunization by artificial infection is practiced for the cattle to be pastured seasonally in such contaminated land as the infection rate of *Theileria* is 100 per cent and high in the incidence and death rates. There are two ways for the immunization of cattle. One is artifical infection of cattle reared in stables by inoculating blood of already infected animals into them, and the other is natural infection of the animals by a short time pasture in the preceding autumn. The latter seems to be higher in preventive effect, because the animals will have an opportunity to be infected with viruses and other parasites and immunized against them. In an area of mixed infection with *Babesia* and *Anaplasma*, etc., in addition to *Theileria*, the effect of artificial infection is also increased by simultaneous immunization against them.

For immunization of cattle against theileriosis, it is necessary that the protozoa increases in number to a certain extent in the body of the cattle after the infection. Infection alone is insufficient for immunization. The blood necessary for systematic practice of artificial infection is stored at $-80°C$ after freezing by the simple quick freezing method of Ishihara and supplied in case of need. The hematozoon can be stored by the author's method for 3 years. It, however, is desirable to make studies of more advanced immunological preventive methods such as immunization with X-rayed living protozoa or dead one.

The protozoa of *Theileria* can be detected by examination of blood smear, but this method is hardly applied to demonstrate *Babesia*. When young calves are infected with *Babesia*, they become tolerant of it before the breeder is aware. To detect *Babesia* in such tolerant cattle, it is recommended to inoculate their blood, bone-marrow fluid and homogenate of spleen into splenectomized calves. The detection rate can be raised by injection of pamaquin which inhibits multiplication of *Theileria*, when the tolerant animals are also infected with this organism.

Serological reaction is not yet adopted to make a diagnosis of piroplasmosis of cattle at present in Japan, though complement fixation of piroplasmosis was reported in the horse in 1945.[1] There are reports on the increase of 7s γ-globulin in the serum of cattle artificially infected with *Theileria*[9] and on the precipitation test against this protozoa in gel.[14] Serological diagnosis is essential for classification of protozoa, detection of tolerant cattle and examination of protozoa in the body of ticks inhabiting the pasture land, so it is desired to make studies of complement fixation test, precipitation test and fluorescence antibody inhibition test, etc.

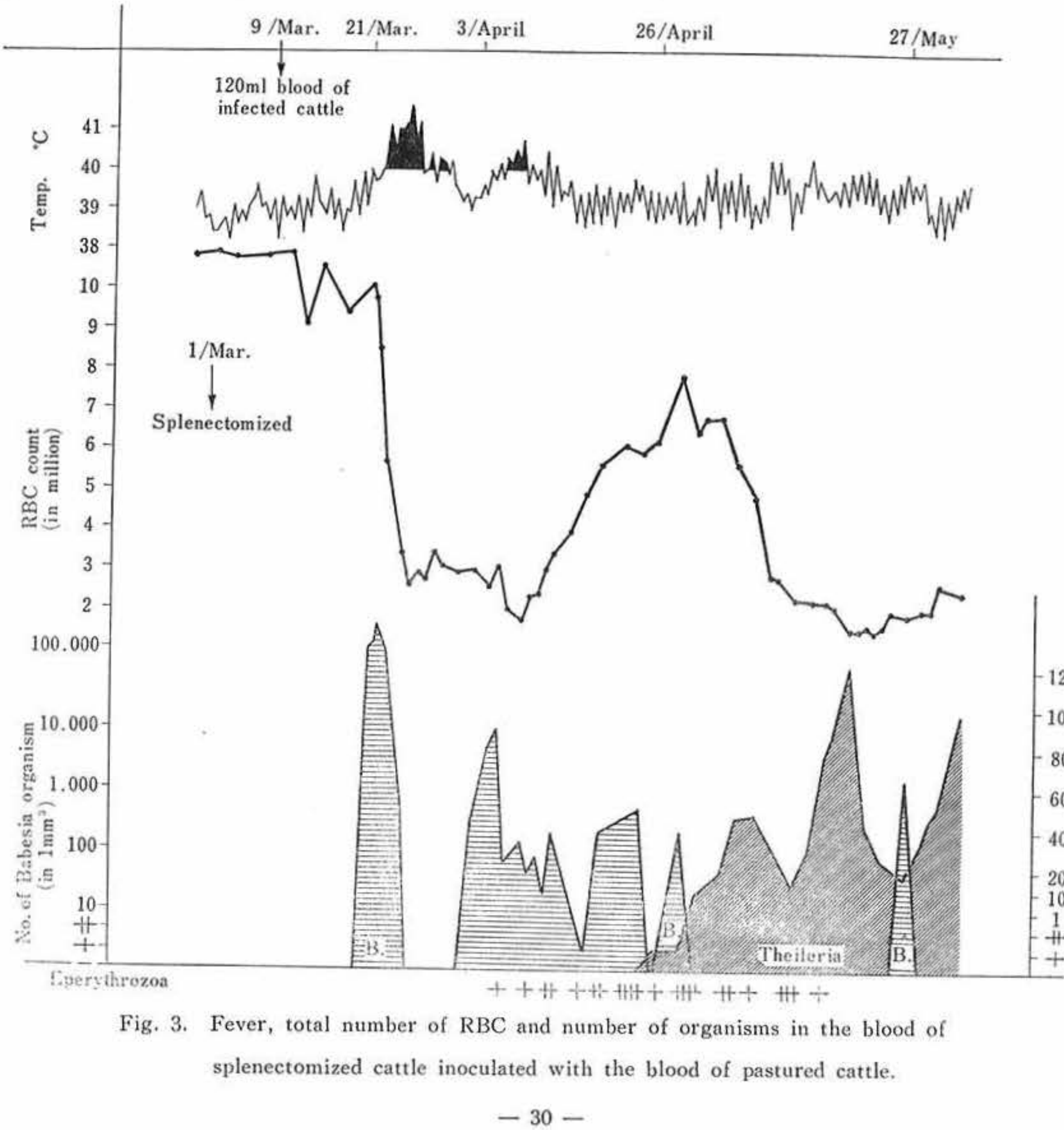


Fig. 3. Fever, total number of RBC and number of organisms in the blood of splenectomized cattle inoculated with the blood of pastured cattle.

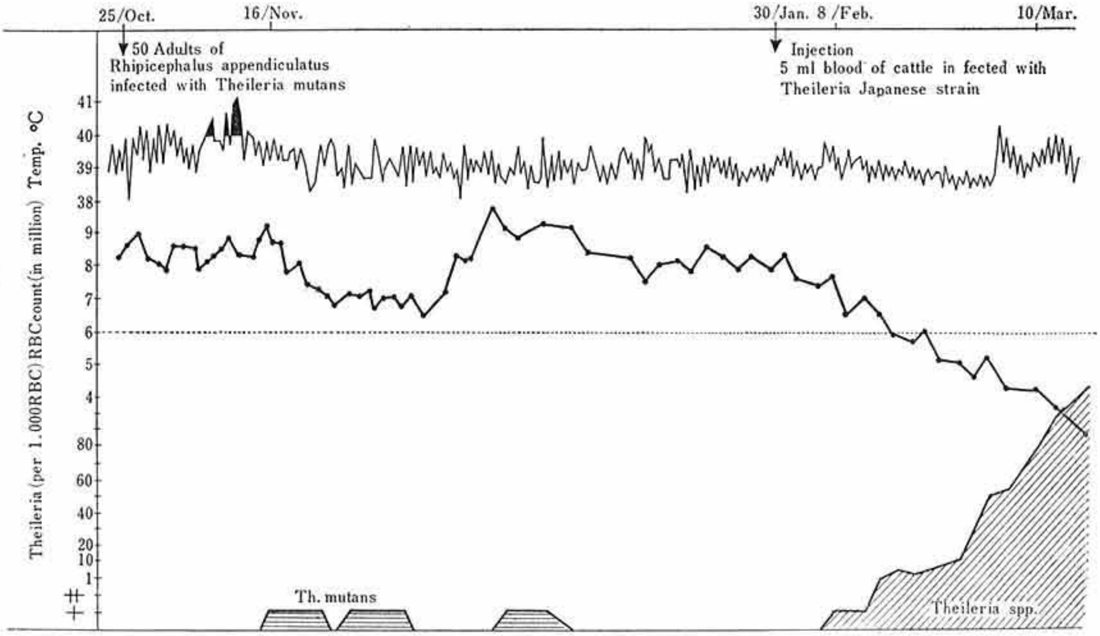


Fig. 4. Cross immunization experiment between *Theileria mutans* and *Theileria* sp. (Japanese strain)

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