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A PRELIMINARY REPORT ON AN EXPERIMENT ON
THE TRANSMISSION OF EQUINE INFECTIOUS
ANEMIA VIRUS TO SHEEP

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INTRODUCTION

The transmission of the virus of equine infectious anemia into experimental animals other than solipeds seems to be one of the most difficult problems. During the past 50 years, many workers have carried out experiments on such transmission into numerous animal species, however, as TAJIMA stated in his review, almost all the results obtained showed negative.

Pertaining to the susceptibility of sheep, the Japanese Commission, THEILER et al. and BALOZET reported that the blood of sheep inoculated with the infected horse serum sometimes contains the virus. However, most of the research workers could not prove this fact. Anyway, it seems to be very necessary to reexamine carefully the transmission experiments into sheep in order to arrive at some conclusion regarding this problem. Accordingly the authors carried out the following experiments.

MATERIALS AND METHODS

For these studies, virus serum was furnished by Dr. ISHII of the Government Experimental Station for Animal Hygiene in Tokyo. The sera were collected from 3 infected horses in the febrile stage by him and were pooled at our laboratory. The serum was inoculated subcutaneously in 60 ml amount into each of 5 lambs (corriedale or merino) numbered 1, 2, 3, 4 and 5 that were 6 to 7 months of age and weighed 20 to 30 kg. Thirty ml of citrated blood was withdrawn from

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each lamb respectively on the 1st, 2nd, 3rd, 5th, 7th, 10th, 14th, 21st and 28th days after inoculation. These blood samples were pooled in 3 lots and injected into 3 normal horses numbered 1, 3 and 5 as follows:

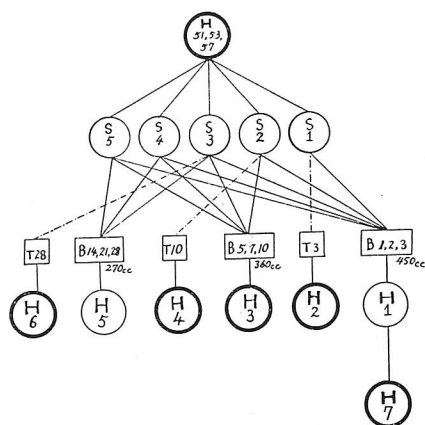
1. Lot 1 (B_1 , B_2 , B_3) that was collected from 5 lambs (Nos. 1~5) on the 1st, 2nd and 3rd days after inoculation, was introduced subcutaneously into an old (over 10 years of age) horse No. 1.

2. Lot 2 (B_5 , B_7 , B_{10}) from 4 lambs (Nos. 2~5) on the 5th, 7th and 10th days to horse No. 3 (foal).

3. Lot 3 (B_{14} , B_{21} , B_{28}) from 3 lambs (Nos. 3~5) on the 14th, 21st and 28th days to horse No. 5 (foal).

Furthermore, tissue emulsions were prepared from lambs Nos. 1~3 respectively killed on the 3rd, 10th and 28th days subsequent to the inoculation of virulent serum. The emulsion consists of 360 ml normal saline and 120 g organ tissues made up from a 15 g piece each of spleen, liver, kidney, lung, brain, testicle, bone-marrow and lymph nodes from each animal. After having been filtered twice through gauze the emulsions were injected subcutaneously into 3 normal horses numbered 2, 4 and 6 as follows:

CHART 1. Outline of Experiment



H...Horse. S...Sheep.
B...Blood. T...Tissue Emulsion.

1. Horse No. 2 (foal) with 400 ml of emulsion from lamb No. 1 (T_3) slaughtered on the 3rd day after inoculation.

2. Horse No. 4 (foal) with the same dosis of emulsion from lamb No. 2 (T_{10}) sacrificed on the 10th day.

3. Horse No. 6 (foal) with emulsion from lamb No. 3 (T_{28}) killed on the 28th day.

These experimental horses including the later mentioned No. 7 were assured clinically, hematologically and biopsically not infected with infectious anemia prior to the experiment. Experimental lambs were under observation for 3 to 77 days and horses for 25 to 136 days after injection. Temperatures were taken twice daily and hematological examinations were carried out at least once weekly. Biopsies (liver, spleen or lymph node) were also performed in horses when considered necessary.

At the end of the observation periods each animal was destroyed and subjected to post-mortem, histopathological and bacteriological examinations.

EXPERIMENTAL RESULTS

Lambs

Lambs with the exception of No. 1 which was sacrificed on the 3rd day developed some temperature reactions over 40 °C. Moreover, erythrocyte decrease

TABLE 1. Blood Picture of Experimental Lambs Before (Upper Column) and After (Lower Column) Inoculation

LAMB NUMB.	ERYTHROCYTE (Million)		LEUCOCYTE (Thousand)		HEMOGLOBIN (Sahli-%)		DIFFERENTIAL COUNT OF LEUCOCYTE							
	m.	M.	m.	M.	m.	M.	N. (%)		Ly. (%)		Mon. (%)		Eos. (%)	
							m.	M.	m.	M.	m.	M.	m.	M.
1	8.19	9.50	5.8	8.6	58	92	20.0	36.0	61.0	70.0	1.5	5.5	0.5	5.5
	5.46		7.0		71		17.0		82.5		0.5		0	
2	7.21	9.12	4.7	13.2	45	64	32.5	53.0	41.5	60.5	1.0	7.0	0.5	2.0
	5.37	6.51	9.8	13.3	58	72	23.0	54.5	41.5	74.5	1.5	4.0	0	1.0
3	7.39	10.07	5.3	8.8	41	77	21.5	50.0	43.0	72.5	3.0	6.5	1.0	3.5
	4.30	7.57	3.8	8.3	50	74	34.0	55.0	36.0	66.0	4.0	8.0	0	1.0
4	6.52	7.60	4.6	11.0	47	75	30.0	43.5	51.0	57.5	1.5	6.5	2.5	6.0
	3.30	8.01	2.1	7.2	38	71	31.0	48.0	49.0	68.5	0	3.5	0	2.5
5	7.36	13.24	4.6	13.4	59	74	22.0	54.5	40.0	72.5	1.5	8.0	0	6.0
	3.30	8.70	4.6	8.7	37	77	33.0	47.5	44.0	61.5	0.5	8.0	0	4.0
6*	5.40	10.25	3.8	17.9	45	77	30.5	56.0	38.0	67.5	0	5.5	0	2.5

Notes: * No. 6 is control (not inoculated) animal.
m ... minimum value, M ... maximum value.

of 2~4 millions followed the virus exposure in all lambs (Chart 2~6). However, non-treated control lamb numbered 6 also developed some temperature reactions (Chart 7) and the diminution of erythrocytes during the same period (Table 1). Aside from the febrile reactions or the erythrocyte reductions no other clinical symptoms were revealed within 3~77 days after inoculation. Therefore, it seems to remain obscure whether the above-mentioned changes are due to infectious anemia virus or to some other causes.

Lambs Nos. 1, 2, 4 and 5 showed no histopathological alterations in the tissues indicative of infectious anemia. In lamb No. 3 some histopathological reactions resembling the changes in chronic infectious anemia horse are observed. Bacteriological examinations resulted negative so far as common aerobes were concerned.

Horses

Horse No. 1 (Chart 8) which

CHART 2. Lamb No. 1 CHART 3. Lamb No. 2

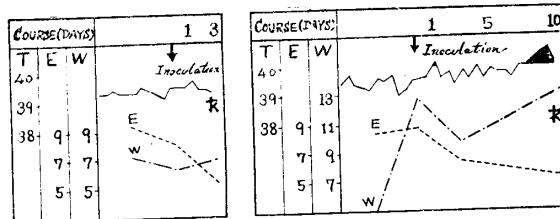
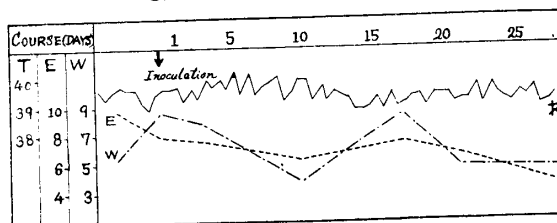


CHART 4. Lamb No. 3



T: Temperature (C), E: Erythrocyte (Million),
W: White blood cell (Thousand).

CHART 5. Lamb No. 4

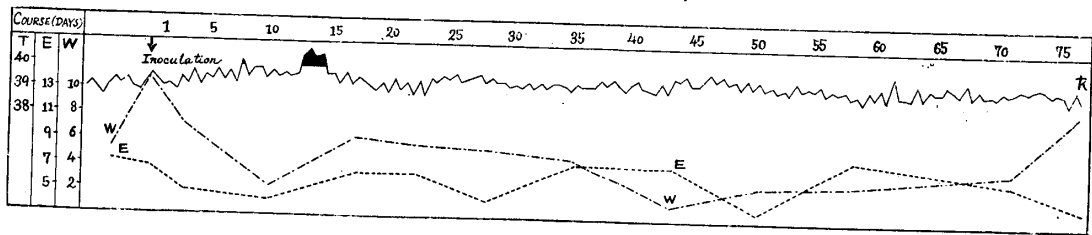


CHART 6. Lamb No. 5

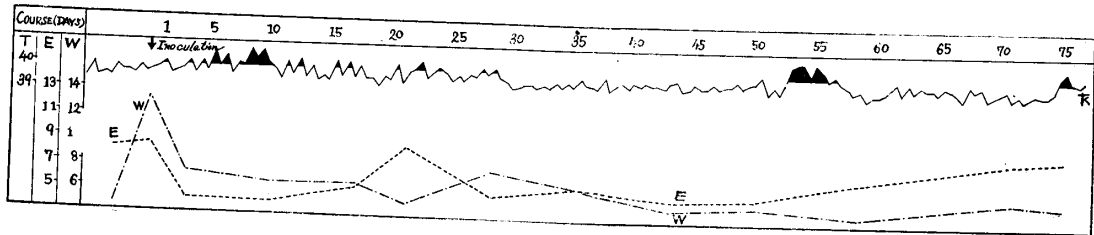
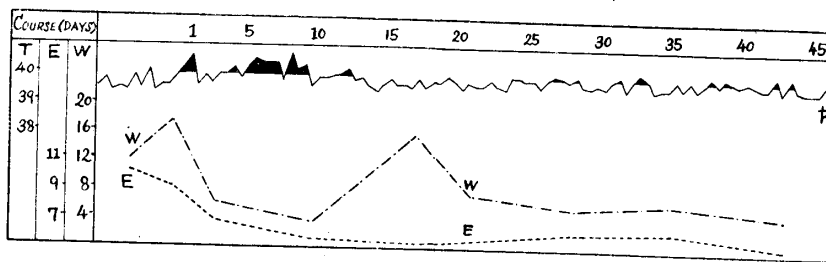
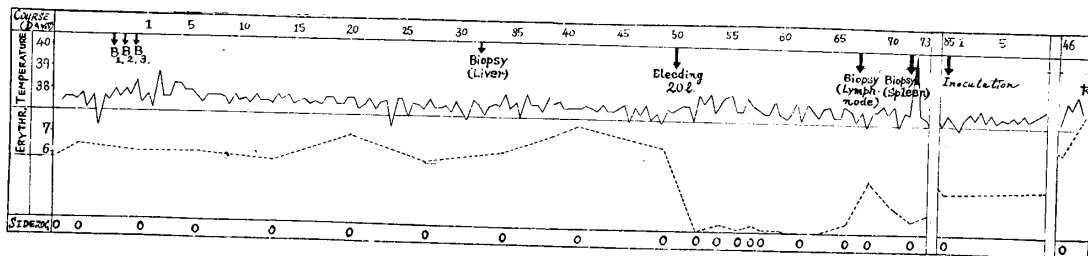


CHART 7. Lamb No. 6 (Control)



received lot 1, i. e. the samples of lamb bloods collected on the 1st, 2nd and 3rd days after injection, remained well until the 50th day of the test. Then it was bled 20l from jugular vein to provoke the possible inapparent infection, but without success. The animal was exposed to the virus of known virulency on the 85th day but ran normally for 50 days thereafter. On the other hand, 300 ml of defibrinated blood was withdrawn from No. 1 on each the 27th and 34th days after the exposure to the known virus and that quantity was injected intravenously into an old (over 10 years of age) horse No. 7 (Chart 9). This horse

CHART 8. Horse No. 1



Note: Figures in siderocyte column indicate the number of this cell in 10,000 leucocytes.

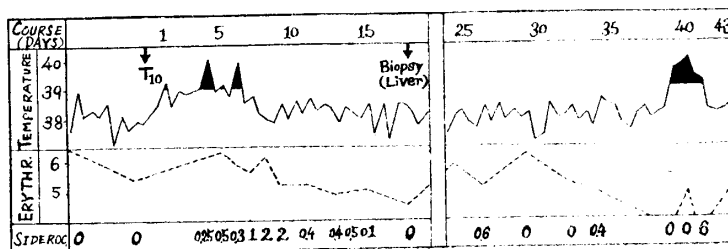
TABLE 2. *Blood Picture of Experimental Horses Before (Upper Column) and After (Lower Column) Inoculation*

HORSE NUMB.	ERYTHROCYTE (Million)		LEUCOCYTE (Thousand)		HEMOGLOBIN (Sahli-%)		DIFFERENTIAL COUNT OF LEUCOCYTE								SIDER. NO. IN 10,000 LEUCOCYTES	REMARKS
	m.	M.	m.	M.	m.	M.	N. (%)		Ly. (%)		Mon. (%)		Eos. (%)			
							m.	M.	m.	M.	m.	M.	m.	M.		
1	5.92	6.43	8.2	9.6	61	63	39.5	53.5	40.0	49.5	3.0	6.5	3.0	4.0	0	After Bleeding (20 l) After Virus Exposure
	5.96	7.93	3.0	9.0	67	110	40.0	67.0	26.0	49.5	2.0	8.0	1.0	7.5	0	
	2.98	8.72	2.4	8.4	36	67	45.5	83.5	13.0	50.0	1.0	8.0	0.5	2.5	0	
	4.96	8.53	5.4	9.8	50	90	41.0	71.5	25.0	54.0	0.5	4.0	1.0	8.0	0	
7	6.26	8.07	4.0	7.0	88	115	50.5	60.0	34.0	42.5	1.5	2.5	4.0	4.0	0	0.4
	6.20	7.71	5.8	8.4	72	110	52.0	77.0	21.0	42.0	1.0	6.0	0.5	4.0	0	
2	6.22	6.96	9.2	11.9	49	60	46.5	59.5	30.0	45.0	6.0	6.5	2.5	4.0	0	33.0
	3.63	5.50	3.7	9.8	41	77	28.5	68.5	28.5	67.0	1.0	13.0	0	4.5	0	
3	5.58	6.25	8.5	10.8	60	68	60.0	60.5	27.0	34.0	1.5	10.0	3.0	4.5	0	3.0
	4.05	7.22	3.0	7.7	47	75	50.5	86.0	11.0	42.0	3.0	8.0	0	5.0	0	
4	4.59	6.61	7.8	13.6	60	62	68.5	74.0	14.0	22.0	5.5	7.0	1.5	6.0	0	6.0
	3.45	6.47	3.6	15.0	45	80	33.5	74.0	22.0	53.5	1.0	11.5	1.0	8.0	0	
5	9.08	12.23	6.2	7.2	84	87	52.5	67.0	27.5	46.5	0.5	5.0	0	2.0	0	4.0 After Virus Exposure
	6.00	8.34	6.0	13.6	60	82	37.5	63.0	33.0	59.0	1.0	7.5	0	3.5	0	
	4.55	6.25	4.9	12.0	58	72	35.0	65.0	33.0	63.5	0	3.5	0	3.0	0	
6	8.64	9.73	9.6	12.0	75	78	22.5	36.0	62.5	76.0	0	1.5	0	1.0	0	0
	5.25	10.47	6.2	12.4	45	80	9.0	54.0	42.0	89.5	0	6.5	0	2.5	0	

Notes: m ... Minimum value, M ... Maximum value.

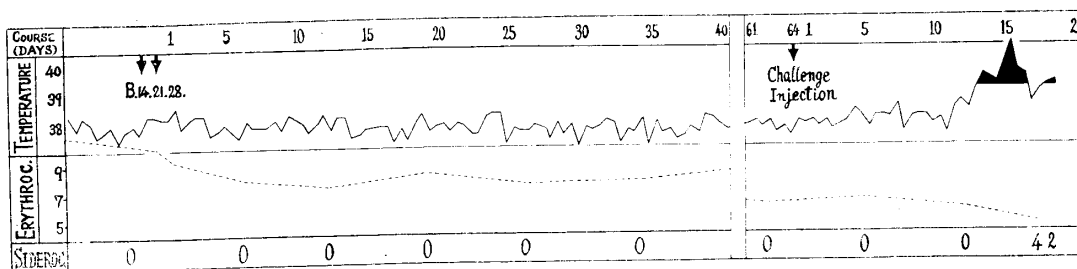
Horse No. 4 (Chart 12) had two fever reactions of 40°C and 39.9°C on the 4th and 6th days respectively after the administration of the emulsion from lamb No. 2 (Chart 3). In the febrile stage, an anemic discolouration on mucous membranes appeared and jaundice followed. Erythrocyte count reduced from 5.5 millions to 4.5 millions and 3.5 millions at the second febrile attack with a peak of 40°C which occurred on the 40th day. Siderocytes were also confirmed on the days succeeding the febrile attacks. The post-mortem alterations in the animal were identical with those of infectious anemia.

CHART 12. Horse No. 4

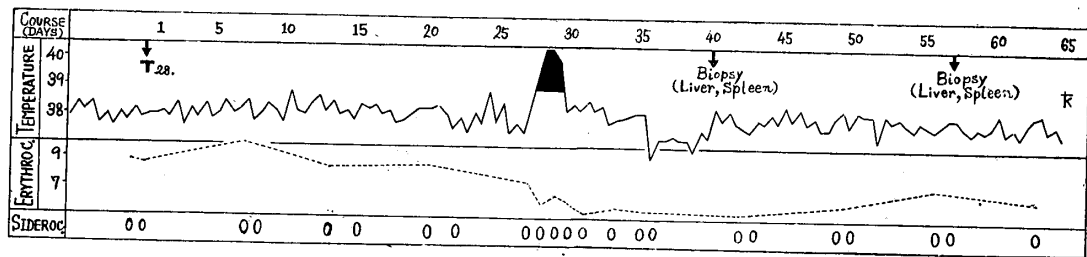


Horse No. 5 (Chart 13) ran healthy for 64 days subsequent to the injection with blood lot 3. On the 64th day it received intravenously the certified anemia virus forwarded from Dr. ISHII. The horse revealed the typical signs of infectious anemia such as rise of temperature, erythrocyte reduction and appearance of siderocytes in blood during the 13th~22nd days after the exposure to virus. Thus it was proved that the lambs' blood of Nos. 3~5 collected on the 14th, 21st and 28th days subsequent to the inoculation of anemia virus was free from the virus.

CHART 13. Horse No. 5



Horse No. 6 (Chart 14) developed a temperature reaction with a maximum of 40.5°C on the 28th~30th days after the administration of organ emulsion from lamb No. 3 (Chart 4). The mucous membranes changed to pale and later icteric; erythrocytes reduced in number from 9 millions to 5.5 millions or less. Biopsic findings in liver and spleen were not indicative of infectious anemia nor were siderocytes detected in circulating blood. Nevertheless, in addition to the fact that the clinical and hematological findings seemed certainly to indicate infection, histopathological (post-mortem) changes in lymph nodes proved the chronic type of infectious anemia.

CHART 14. *Horse No. 6*

SUMMARY AND CONSIDERATION

1. Almost all lambs revealed some temperature reactions and reduction in number of erythrocytes subsequent to subcutaneous inoculation with equine infectious anemia virus. However, it remained uncertain whether the anemia virus was actually responsible for the above-mentioned alterations.

2. Histopathological changes of lamb No. 3 which was destroyed on the 28th day after the exposure to virus seem to be analogical with those of a chronic case of equine infectious anemia. On the other hand, the lambs Nos. 1, 2, 4 and 5 sacrificed on the 3rd, 10th and 77th days developed no histopathological changes indicative of this disease.

3. Existence of infectious anemia virus was confirmed in the blood sample that was collected from lambs Nos. 2~5 on the 5th, 7th and 10th days. However, the virulency of the blood removed from lambs Nos. 1~5 on the 1st, 2nd and 3rd days was not proven with certainty. Later, the blood taken from lambs Nos. 3~5 on the 14th, 21st and 28th days was proved free from the anemia virus.

4. Each of the organ emulsions from lambs Nos. 1~3 that were slaughtered on the 3rd, 10th and 28th days, respectively, after the inoculation contained the virus.

From the above described experimental results, it may be considered that the infectious anemia virus is able to invade into the blood stream and also to persist in the organs of lambs at least for about one month after the subcutaneous inoculation with the virus.

The histopathological changes observed in an experimental lamb may suggest the propagation of this virus in lamb tissues.

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