ON A HITHERTO UNREPORTED PHENOMENON SUGGESTING POSSIBILITY OF MULTIPLICATION OF EQUINE INFECTIOUS ANEMIA VIRUS IN LAMB BODY

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In the previous papers[1,2], the present authors supposed that equine infectious anemia (E. I. A.) virus may multiply in sheep body, from the following facts: 1) The virus appeared in peripheral blood of lamb during a definte period subsequent to the subcutaneous inoculation of E. I. A. virus; 2) the virus was demonstrated from the organs at least on the 77th day, so far as traced, after the subcutaneous introduction of this virus into the animals; 3) sometimes, histopathological alterations analogous to those of chronic E. I. A. in the horse were recognized in the experimental lambs.

Recently, the authors have made effort to find any additional available evidence indicating the propagation of E. I. A. virus in sheep body. The present report deals with a never previously described phenomenon, viz., contradiction in the relation between the antigenic components of equine serum (equine serum components) in peripheral blood of the experiment lambs and the appearance of E. I. A. virus therein, which seems to be pertinent to the writers' above-noted object.

MATERIALS AND METHODS

Virus strains in the experiments are "Goshun" and "Ayame". The former is the same strain as used in the experiment reported in 1954[1] and the latter the same as in that of 1956[3]. The seed virus was refreshed by horse inoculation just before the application to the experimental lambs. The animals, Merino breed lambs, weighing 23~29 kg, were aged 139~199 days when they received the virus. They were bled from jugular vein on the 1st, 2nd, 3rd, 5th, 7th, 10th, 14th, 21st and 28th days subsequent to subcutaneous inoculation of 60 ml of virulent horse serum. Some amount of serum was separated from a part of each blood sample and used for the titration (by means of precipitation test) of the equine serum components in peripheral vein blood. Concentrations of equine serum components were titrated at 37°C for 30~60 minutes. Precipitin titre of the antiserum in these experiments was 10,240. Another part of the blood sample, about 20 ml, was citrated and stored for various (21~220) days at about −40°C. These blood samples were grouped in

TABLE. *Grouping of the Blood Samples of Experimental Lambs*

GROUP OF EXPER.	VIRUS STRAIN USED	LAMB NO.	DAY OF BLEEDING AFTER THE INOCULATION OF E. I. A. HORSE SERUM								
			1	2	3	5	7	10	14	21	28
1st	Ayame (A3)	19	A	A	B	B	C	C	·	·	·
		20	D	E	F	G	G	H	H	J	J
		21	D	E	F	G	G	H	H	J	J
		22	D	E	F	G	G	H	H	J	J
		23	D	E	F	G	G	H	H	I*	·
2nd	Goshun (G12)	25	K	K	K	L	L	L	M	M	M
		26	K	K	K	L	L	L	N	N	N
		27	K	K	K	L	L	L	N	N	N
3rd	Ayame (A7)	28	O	O	O	P	P	P	Q	Q	Q
		29	O	O	O	P	P	P	Q	Q	Q
		30	O	O	O	P	P	P	Q	Q	Q

Remarks: · indicates blood samples not examined.
* Lot I consists of samples taken on the 20th, 21st and 22nd days.

16 (A~Q) lots as indicated in the table.

Each lot of the blood was injected subcutaneously into a horse certified free from E. I. A. by clinical, hematological and biopsic examinations prior to the injection.

Observations on the experimental lambs and horses were carried out in the same way as described in the previous report[1].

EXPERIMENTAL RESULTS

Lambs

Clinical findings

Among the first group of lambs (Nos. 19~23) that received the virulent serum A3 (Ayame

CHART 1. *Fever Curve of Lamb No. 19*

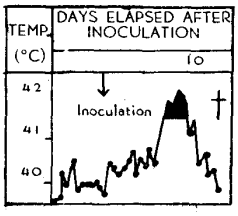


CHART 2. *Fever Curve of Lamb No. 23*

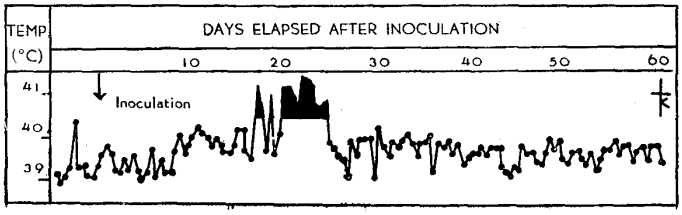


CHART 3. *Fever Curve of Lamb No. 25*

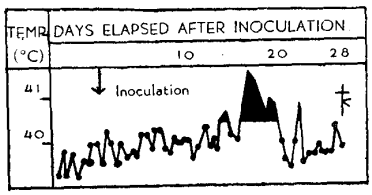
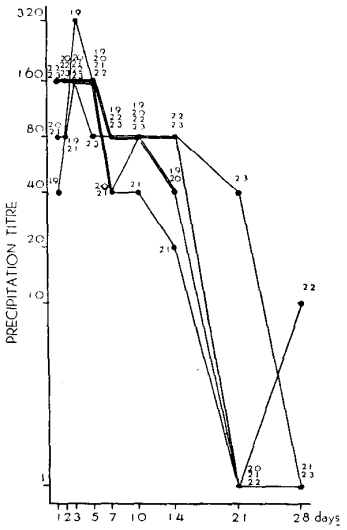


CHART 4. *Rise and Fall of Concentrations (Precipitation Titres) of Normal Equine Serum Components in Peripheral Blood of Experimental Lambs Nos. 19~23*



No. 3), No. 19 (Chart 1) showed a fever of 40.5~42°C on the 6th~10th days after the administration of the virus and died on the 13th day. Lamb No. 23 (Chart 2) developed some temperature reaction with a peak of 41.5°C for about one week (from the 18th to 25th days) and ran normal up to the 60th day when it was sacrificed and autopsied. However, Nos. 20~22 remained normal for 28~260 days with exception of a transient elevation of body temperature.

In the second group of animals (Nos. 25~27) receiving the virus serum G12 (Goshun No. 12), No. 25 (Chart 3) developed a fever of 40~41.6°C beginning on the 14th day; it was sacrificed on the 28th after the virus administration. No. 26 which was kept under observation for 80 days revealed no definite febrile signs nor did No. 27 for an observation period of 180 days.

CHART 5. *Rise and Fall of Concentrations (Precipitation Titres) of Normal Equine Serum Components in Peripheral Blood of Experimental Lambs Nos. 25~27*

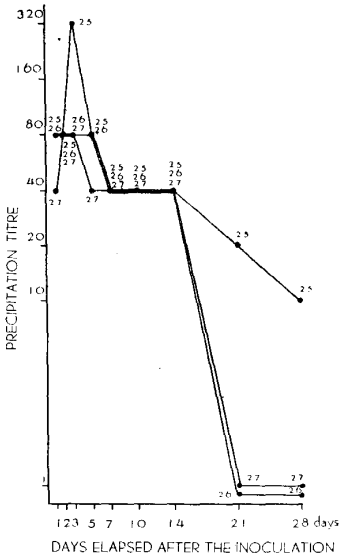
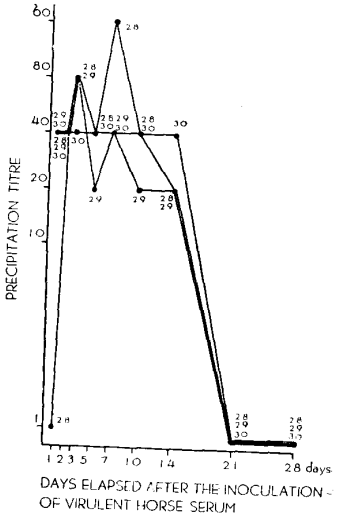


CHART 6. *Rise and Fall of Concentrations (Precipitation Titres) of Normal Equine Serum Components in Peripheral Blood of Experimental Lambs Nos. 28~30*



Each animal of the third group (Nos. 28~30) inoculated with the virus A7 (Ayame No. 7) generally ran normal for 28, 80 and 260 days respectively.

Rise and fall of equine serum components in peripheral blood

Concentrations (precipitinogen titres) of the equine serum components in peripheral blood of the lambs on the days when the blood samples composing each lot were collected were as follows:

First group (Chart 4)

Lot A: 40 and 80
Lot B: 320 and 160
Lot C: 80
Lot D: 80 and 160
Lot E: 80 and 160
Lot F: 160
Lot G: 40 and 80
Lot H: 20, 40 and 80
Lot I: Not titrated, 40 and not titrated
Lot J: 1

Second group (Chart 5)

Lot K: 40 and 80
Lot L: 40 and 80
Lot M: 40, 20 and 10
Lot N: 40, 1 and 0

Third group (Chart 6)

Lot O: 1, 40 and 80
Lot P: 20, 40 and 80
Lot Q: 20, 40 and 0

Histopathological and bacteriological findings

In No. 23 which was autopsied on the 28th day, some suspicious histological changes suggesting E. I. A. infection were recognized. However, the authors failed to find any pathognomonic findings indicative of infection with E. I. A. in other lambs autosied between the 13th~260th days of experiment.

Significant microorganisms were detected in no cases so far as common aerobes are concerned.

Horses

Horse No. 21 which received lot I (mixed blood collected from a lamb, No. 23, of the first group on the 20th~22nd days when the animal was febrile) was sacrificed on account of an accident on the 16th day after the application of the lamb blood. Therefore, a mixed organ emulsion of the horse was transferred to other E. I. A.-free horse, No. 20, which had manifested no signs of the disease for about 60 days after the injection with lot J and had been certified free from E. I. A. by biopsy several days before the administration of lot I. This animal developed symptoms of E. I. A. on the 35th day after the superinjection. On the other hand, other horses (Nos. 12~16, 18 and 19) revealed neither clinical nor histopathological (by liver puncture) evidences of E. I. A. infection for two months following the introduction of lots A~H. However, they responded to test inoculation with the challenge virus with typical signs of E. I. A. infection.

Horse No. 23 developed typical symptoms of E. I. A. on the 20th day after the injection with lot K (mixed blood collected from the second group of lambs on the 1st, 2nd and 3rd days). However, No. 26 remained well until the 60th after the receipt of lot L and its biopsic findings indicated E. I. A.-free. The animal, thereafter, was proved to be susceptible to the virus. Horse No. 28 ran normally for about two months following the injection of lot M including the blood sample collected from lamb No. 25 in the febrile stage and was normal on biopsic examination. However, it revealed typical symptoms of E. I. A. on the

20th day subsequent to the administration of lot N (mixed blood of lambs Nos. 26 and 27 taken on the 14th, 21st and 28th days) and post-mortem investigations of this horse confirmed the infection.

Horse No. 24 remained apparently healthy for 72 days after the introduction of lof O (mixed blood from the third group taken on the 1st, 2nd and 3rd days). However, its post-mortem examination performed on the 72nd day proved E. I. A. infection. Another horse, No. 29, which received lot Q (mixed blood from lambs, No. 28～30 of this group on the 14th, 21st and 28th days) manifested typical symptoms of E. I. A. on the 18th day and histopathological findings of the disease were made thereafter. Prior to the administration of lot Q, the animal had been injected with lot P and had run healthy for about two months. Moreover, it was proved E.I.A.-free by histopathological examinations of liver punctate at the end of that observation period.

Discussion

In the first group of lambs, the present authors demonstrated E. I. A. virus in the peripheral blood mixture (lot I) taken from lamb No 23 on the 20th, 21st and 22nd days after the subcutaneous inoculation of the virulent horse serum. However, they failed to detect the virus in other blood samples (lots A～H) that contained larger quantities of the equine serum components than lot I. It is interesting that the virus appeared in the peripheral blood of a lamb in its febrile stage, but not in that of other stages in spite of the contents of a larger quantity of the equine serum components.

In the second group, E. I. A. virus was demonstrated from two lots (K and N) out of four (K～N). One (lot K) of the former was a mixed sample bled from three lambs on the 1st, 2nd and 3rd days subsequent to the inoculation of E.I.A. virus. The other, lot N, consisted of blood collected from two lambs on the 14th, 21st and 28th days. Comparing the blood level of equine serum components of lot L with that of lot N, it is unquestionable that the quantity of equine serum components of the former was larger than that of the latter. In this group, there was failure to detect the virus in blood sample at the febrile stage (No. 25-lot M).

In the third group, two lots (O and Q) out of three (O～Q) were virulent for horses. Disregarding lot O, the quantity of equine serum components of lot P was larger than that of lot Q without doubt.

Thus, E. I. A. virus appeared both in the peripheral blood of the lambs at the periods when the blood contains a large quantity of equine serum components and in the blood including a small quantity of the components. Besides, it is very noteworthy that the blood samples are virulent at the period when they possess comparatively smaller quantity of the components and that the samples with larger amount of the equine serum components are avirulent in the same

group of lambs. These facts seem to indicate the high probability of propagation of E.I.A. virus in lamb body.

SUMMARY

Through the present three series of experiments the authors found a hitherto unknown phenomenon that seems to ensure the possibility of propagation of equine infectious anemia virus in lamb body. That is:

1. In one lamb of the first group, E.I.A. virus appeared in a lot of peripheral blood samples collected on the 20th, 21st and 22nd days after subcutaneous inoculation of the virus. This lot contained a smaller quantity of the equine serum components than other lots from the same lamb taken on the 1st, 2nd, 3rd, 5th, 7th, 10th and 14th days.

2. The virus was detected both in lot K which was collected from three lambs of the second group on the 1st, 2nd and 3rd days subsequent to introduction of the virus and in lot N from two lambs out of three on the 14th, 21st and 28th days. However, attempts to demonstrate the virus in two other lots, L and M resulted in failure. Lot L consisted of nine samples of blood taken from three lambs on the 5th, 7th and 10th days and included a larger amount of the equine serum components than did lot N.

3. Moreover, two lots (O and Q) out of three of the third group were virulent for horses. The quantity of the equine serum components in virus-negative lot P was larger undoubtedly than that of virus-positive lot Q.

4. In lamb No. 23, the time of viremia coincided with the febrile period of the animal.

5. The authors recognized some histopathological findings suggestive of E.I.A. infection in one lamb out of 11.

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