FURTHER REMARKS ON THE PRODUCTION OF A MALARIAL FORM OF SOUTH AFRICAN HORSE-SICKNESS.

(Plate I. and Nine Temperature Charts.)

By ALEXANDER EDINGTON, M.D., F.R.S.E.,

Director of the Colonial Bacteriological Institute, Grahamstown, Cape Colony.

During my earlier experiments in connection with Horse-sickness I showed that donkeys could be inoculated with the virulent blood of horses dying with the disease without being seriously affected thereby[1].

Also the remarkable fact was demonstrated that the blood of such donkeys, drawn about the tenth or eleventh day subsequent to inoculation, was capable of setting up, in clean horses into which it was injected, a peculiar fever. This fever differed from the rapid sustained pyrexia of ordinary horse-sickness in having definitely marked remissions and intermissions, and in being, in such cases, usually non-fatal.

At this period—after several years' close and continued examination of the blood of affected horses—I had left off systematic examination of the blood, but in the blood of two horses in which this form of fever had been produced, I found one or two of the blood corpuscles to be infected with a parasite having some resemblance to that of Texas Fever, and I was led to think that the febrile attack had lowered the animal's resistance, thus permitting it to acquire an infection of Texas Fever to which horses are ordinarily insusceptible.

Nevertheless, I was never satisfied with this explanation, while the peculiar type of fever, so different from that found in horse-sickness, eventually induced me to make further enquiries.

It has been already shown in the former communication that when

[1] *Proc. Royal Society.*

https://doi.org/10.1017/S0022172400002047 Published online by Cambridge University Press

a number of donkeys are infected with equal doses of virulent blood, the blood of one may kill a clean horse, that of another may induce a milder form of fever with remissions and intermissions which may extend over many weeks, while the blood of another may show no effect at all when inoculated into a horse. Further, it was noted that the behaviour of the temperature in inoculated donkeys gave little or no indication as to the power of infection which the blood might possess. Two hypotheses might be advanced for this; firstly, a different susceptibility of the horses which, as a matter of fact, I have found to be correct by inoculating a number of clean animals from one donkey. The second hypothesis would be to credit individual donkeys with different degrees of susceptibility and to admit that the more susceptible animal would furnish the more virulent blood. This also has been proved to hold good. A striking corollary to this is shown in the first experiment of Colonel Joshua Nunn, A.V.D., F.R.C.V.S., who on beginning his investigations at Natal in 1888 inoculated three mules with the blood of a dead one. Two of these animals showed no results, and while the third one died, he was led to conclude that death must have been due to natural infection and that the disease was not communicable. I was for a long time unable to explain Colonel Nunn's failure to infect the other two mules, but experience has shown me, while horses are highly susceptible and donkeys refractory, that mules occupy an intermediate position, and as the refractory donkey lowers the virulence of horse-sickness passed through it, even for the susceptible horse, it has to be admitted that the mule may be expected to lower the virulence for one of its own kind.

I therefore determined upon using animals, for the production of infective blood, whose refractory condition should be more or less uniform. To this end I made use of a number of "salted horses," *i.e.* horses which had passed through the virulent stage of horse-sickness and thereafter been reinoculated at recurring periods with progressively increasing doses of virulent blood, of which the maximum was never greater than 500 c.c.

The "salted" horses which I used were each inoculated once a month during the previous six months with 10 c.c. of virulent blood. Ten days after the last injection they were bled and the blood was used to inoculate clean horses by subcutaneous injection with 20 c.c.

These experiments were not made *en masse* but extended over a long period of time, from the 14th of November, 1901, to April, 1902.

The following table sets forth briefly the results obtained.

Inoculation	Horse	Salted horse from which blood was used	Date	Result
1st.	No. 1.	A	November 14.	Severe fever.
	No. 2.	A	,,	,,
2nd.	No. 1.	B	December 29.	Little or no fever.
	No. 2.	C	,,	,,
1st.	No. 3.	B	November 14.	Slight fever.
	No. 4.	B	,,	Severe fever.
2nd.	No. 3.	Removed for other experiments.		
	No. 4.	D	December 29.	Slight fever.
1st.	No. 5.	C	December 4.	Severe fever.
2nd.	No. 5.	C	,, 29.	Slight fever.
1st.	No. 6.	D	December 4.	Severe fever.
2nd.	No. 6.	C	,, 29.	Slight fever.
1st.	No. 7.	A	December 6.	Slight fever.
2nd.	No. 7.	D	,, 29.	More severe fever.
1st.	No. 8.	B	December 6.	Severe fever.
2nd.	No. 8.	C	,, 29.	More severe fever.
1st.	No. 9.	A	December 19.	Mild fever.
2nd.	No. 9.	A	January 19.	Severe fever.
1st.	No. 10.	B	December 29.	Mild fever.
2nd.	No. 10.	A	January 19.	Slight attacks at intervals, culminating in a severe one at the 70th day, from which animal died of typical horse-sickness.
1st.	No. 11.	B	December 29.	Severe fever.
2nd.	No. 11.	A	January 19.	Mild fever.
1st.	No. 12.	B	December 29.	Mild fever.
2nd.	No. 12.	A	January 19.	,,
1st.	No. 13.	B	December 29.	Severe fever.
2nd.	No. 13.	A	January 19.	Very severe fever.
1st.	No. 14.	C	December 29.	Mild fever.
2nd.	No. 14.	A	January 19.	Severe fever.
1st.	No. 15.	C	December 29.	Very severe fever.
2nd.	No. 15.	A	January 19.	Severe fever.
1st.	No. 16.	C	December 29.	Fairly severe fever.
2nd.	No. 16.	A	January 19.	Severe fever.
1st.	No. 17.	C	December 29.	Very severe fever.
2nd.	No. 17.	A	January 19.	,,
1st.	No. 18.	D	December 29.	Very severe fever, ending in death.
1st.	No. 19.	D	December 29.	Very severe fever.
2nd.	No. 19.	A	January 19.	,,
1st.	No. 20.	D	December 29.	Mild fever.
2nd.	No. 20.	A	January 19.	,,
1st.	No. 21.	E	January 31.	Mild fever.
2nd.	No. 21.	F	February 24.	,,

https://doi.org/10.1017/S0022172400002047 Published online by Cambridge University Press

These twenty-one animals were obtained from the military authorities and were believed to have been recently imported. Of the two last, however, one (a stallion) was found to have been bred in the colony, while No. 20 had been certainly in the country for some time and in use during the war.

Five additional animals were subsequently obtained in the Albany district and experimented upon in the same manner.

During the periods shown, "control" animals were kept in the same stables, their stalls being frequently changed for those previously filled by animals suffering from the fever.

As a result nearly all the inoculated animals developed the fever, but those obtained locally showed it least of all.

During the febrile periods well-defined malarial parasites were found within the red blood corpuscles. In many cases the infected corpuscles were exceedingly numerous, but in others, with less fever, they occurred in much smaller numbers.

In several cases the fever gradually evinced a more malignant type ending in death. Of two such animals, the one dying on the 39th day after inoculation and the other on the 75th day, blood was drawn previous to death. 10 c.c. of each was injected subcutaneously into a clean horse on April 1st, 1902, which died of typical horse-sickness on April 8th.

During the periods when the disease in these two former horses was assuming a virulent form there were no cases of virulent horse-sickness in the Institute, and it has to be also remembered that the disease is not actively contagious. Even a dose of half an ounce of fluid blood given by the mouth may fail to infect, although a quantity as large as half a pint is generally successful in producing the disease. The incubation period of virulent horse-sickness after subcutaneous inoculation is from 8 to 10 days. The maximum, in my experience during ten years' investigation, has been about 14 days. In the case of the two animals referred to, which died at periods respectively of 39 and 75 days after inoculation, I have already shown that they suffered at frequent intervals from attacks of fever which remitted and intermitted, and the only explanation of their deaths is to admit that the mild and modified fever gradually attained virulence. Such a phenomenon is already well known in malarial fever occurring in man.

I thought proper to denote this form of the disease by the name of the "malarial form of horse-sickness."

Subsequent to this it was suggested that my experiments had been

accidentally contaminated with a disease which is known to occur in horses in South Africa, that is commonly called Biliary Fever.

In answer to such a criticism it has to be stated that no one has yet succeeded in transferring biliary fever from an infected horse to a healthy one in South Africa, in fact that those who have attempted to do so have failed. Under such circumstances it cannot but be surprising that I should succeed again and again in experiments extending over many months, if the condition which I produced had actually been biliary fever.

Biliary fever in the horse is not a disease limited to Africa, indeed it is well known in India. In 1887, Colonel Joshua Nunn, F.R.C.V.S., of the Army Veterinary Department, who had been trained under M. Pasteur, was selected by the Director-General of the Army Veterinary Department, "because of his special fitness for the task, and particularly because of his experience in dealing with destructive horse and cattle diseases in the North-West Provinces of India, where he had gained an excellent reputation," to proceed to S. Africa in order to investigate horse-sickness.

In the valuable report[1], which he made after spending two years in South Africa, he describes the pulmonary form of horse-sickness known as Dunpaardziekte, the Dikkop form, and one which he and also Major Rutherford, A.V.D., describe as the "bilious form of horse-sickness."

It would therefore seem that Colonel Nunn found a disease here which was not, according to his ideas, biliary fever but a variety of horse-sickness.

The results of my post-mortem examinations are in close agreement with the detailed account furnished by Colonel Nunn. I am therefore of opinion that this excellent and careful observer was correct in describing another form of horse-sickness, and it is very probable, while *true* biliary fever exists in this country, that the condition which I have succeeded in producing artificially is neither more nor less than the form of horse-sickness described by Colonel Nunn as the "bilious form."

With a view, however, to meeting criticism by better means than argument, I decided to undertake a critical experiment or series of experiments.

To this end, the stables, which have brick floors cemented on a concrete basis, stone and brick walls, wooden fittings and wooden ceilings, were emptied and kept thus for some weeks. A 1 % solution

[1] Report of the Principal Veterinary Surgeon to the Forces to General Viscount Wolseley, Adjutant-General, 27th October, 1888.

of caustic soda was then applied freely to every part including the ceilings with a powerful metal spray. Several days were permitted to elapse, when the whole was again sprayed in the same manner with 1 % of formaline. After some days workmen were put in who went over all the brick and stone work filling up any cracks with cement, and all wood-work was also overhauled. Finally the walls and wood-work were repainted.

By the kind assistance of the military authorities, I was now enabled, through the Honourable the Colonial Secretary, to procure ten clean newly imported horses. These were put in trucks at Port Elizabeth, sent to Grahamstown station, from whence they were led directly to the stables. After being placed in the stables, more than a fortnight was permitted to elapse before any experiments were begun. Meanwhile the temperature of each animal was taken five times a day. The most rigorous care was exercised in the case of each animal to exclude any vitiation of the experiment. Each head-stall was thoroughly disinfected; each animal was closely clipped. The food was dry forage which, being cut and stored, was delivered in a special bucket into each manger. Each animal possessed its own water bucket, while the water was taken from that supplied by the town. One horse had to be discarded as being unmanageable.

Examination of the blood.

Each animal, including those used to produce infection, was examined on the average every second day. The want of sufficient staff made it impossible for me to make a daily examination. The blood was drawn from the ear, which was previously shorn and washed with lysol. The cover-glasses were primarily cleaned and kept till required in absolute alcohol. The films were made with strips of papier Joseph and were then fixed with alcohol and ether. Staining was made by the Romanowsky method. Examination was made with Zeiss apochromatic 2 mm. homogeneous immersion objective and compensating oculars 6 and 8.

The examination was made on a Zeiss stand having a mechanical stage fitted with verniers, and the whole cover was systematically examined. Where few parasites occurred more than an hour was required to examine the slide properly. The position on the slide of all parasites found was recorded in my journal and the slides carefully stored. The advantage of this method lies in the fact that the parasites of any case can be found at any time in a few seconds.

EXPERIMENT 1. Two horses, Nos. 27 and 28, were each inoculated on December 4th, 1902, by subcutaneous injection with the blood of "salted" horse *B* which had been reinoculated once 10 days previously with 10 c.c. of virulent blood.

Note. This "salted" horse had not been interfered with since the preceding experiments of the former year. In the former experiments the "salted" horses had been regularly reinoculated each month previous to being used in the experiments.

Horse 27 (Chart I). The temperature rose suddenly eight days after inoculation to 105·8° Fahr. and continued to be high with remissions and some intermissions during thirteen days, after which slighter attacks were noted.

Nine days after inoculation, being the day after the first onset of fever, a few parasites were found in the blood, mostly of the pear-shaped form. On December 15th they were still very few, on the 21st more numerous, while on the 22nd a typical rosette was seen. The term rosette is not quite satisfactory since, as a rule, it consists of four segments arranged as a cross, but in many cases in the form of the imprint of a crow's foot. I think the term "*quadret*" would be more accurate. On the 24th, 26th, 29th and 30th they were again few in number but showed an increase on January 1st. On January 2nd the horse was again inoculated from the same "salted" horse which had not been meanwhile reinoculated. This second inoculation did not produce any severe fever.

On January 4th a "quadret" was again seen together with some large more or less circular parasites. Some corpuscles contained two such parasites. On the 6th and 10th they were few in number but showed an increase on January 12th. On the 14th and 17th they were again few and none were seen after this date until February 9th when a quadret was seen. No more were found after this date.

Horse 28 (Chart II). On the evening of December 11th, being seven days after its inoculation, the temperature rose to 101·6° Fahr., reaching 103·8° on the following day, and fever with remissions occurred during the following seven days. The maximum was 107°.

On the day of the first indication of fever no parasites were found, but on January 13th, which coincided with the first remission, a few pear-shaped parasites were seen. They were still few on the 15th, 16th, 17th, and 26th of December, and still nearly always pear-shaped. On December 28th a quadret was seen, and on the 30th the parasites found were nearly all spherical, some being about half the size of a red blood corpuscle.

On January 2nd the horse was reinoculated from the same "salted" horse, but here also no severe fever followed.

A few parasites were subsequently found on January 19th, February 9th, and finally on March 7th, when only one or two were found in a whole slide after a prolonged period.

EXPERIMENT 2. *Horse* 29 (Chart III) was inoculated subcutaneously on January 14th with 20 c.c. of the blood of "salted" horse *B*, which had been reinoculated 51 days previously.

Eight days after inoculation the temperature rose suddenly to 104° Fahr. and

fever continued during twelve days, being of the remittent and intermittent type; the maximum being 107·2°.

On the day subsequent to the first occurrence of fever, being January 23rd, a few parasites were found. Three days later a quadret was seen, but also some large spherical forms. On the 28th some spherical forms were seen, while on February 2nd numerous parasites, spherical, pear-shaped, and irregular types, were seen. A few were seen on the 18th, 21st, 24th and 26th of February and on March 5th and April 4th.

EXPERIMENT 3. *To determine whether the blood of an animal suffering from fever, induced by the injection of the blood from a "salted" and reinoculated horse, can induce fever in a clean animal:—*

Horse No. 27 was bled on January 14th and with 20 c.c. of this blood *Horse* No. 30 (Chart IV) was immediately inoculated by subcutaneous injection.

Eight days after this inoculation the temperature rose to 103·2°. Fever continued with remissions and intermissions during thirteen days, the maximum being 106·8°.

On January 23rd, being the day subsequent to the first elevation of temperature, a few parasites were found. On the 26th they were numerous, after which they disappeared. On February 2nd they appeared in considerable numbers, while coincidentally a very sharp rise of temperature occurred. On February 13th they were still few, as also on the 21st, 24th and 28th days of the same month.

EXPERIMENT 4. To show

(1) *That infection remains in a salted horse for at least a year.*

(2) *That if such horse's blood produces infection in a clean horse, this infection protects against further infection with the same salted horse's blood, even when the latter has been reinoculated with virulent blood.*

On February 23rd "salted" horse *A*, which had not been reinoculated during the past year, was bled. 20 c.c. of this blood was injected into clean *Horse* No. 31 (Chart V) on the same date. Nine days later the temperature began to rise in the early morning and attained 104·6°, as the highest point, that evening. Thereafter the fever continued with remissions and intermissions during eight days. On March 13th the clean horse was reinoculated with the blood of the same "salted" horse, which had, meanwhile, been reinoculated with virulent blood ten days previously. No rise in the temperature occurred which could be considered to be due to this inoculation.

At later dates the horse was inoculated on two occasions with the blood of goats that had been infected ten days previously with virulent horse-sickness blood.

In this case parasites were found on March 7th, being four days after the first rise of temperature, and were fairly numerous. On the 9th they were fewer in numbers. A few were found on March 23rd and also on the 4th and 14th of April.

EXPERIMENT 5. To show

(1) *That the blood of an unsalted horse, although it has been frequently inoculated with salted blood, only conveys a weak infection to a clean horse.*

(2) *That the blood of a goat, infected with horse-sickness blood, can accelerate and intensify that infection.*

A stallion which was inoculated in the former experiments, on several occasions, with salted blood without, however, showing any marked fever, was bled on March 4th. It had not, therefore, received any inoculation during the past year.

Twenty c.c. of its blood were inoculated into clean *Horse* No. 34 (Chart VI) on the same date, March 4th.

During twelve days following no marked rise of temperature occurred, although on the 8th, 9th and 10th days slight rises occurred of which the maximum was 101·8°. No parasites meanwhile were found. On March 16th it was inoculated with 10 c.c. of glycerinated blood derived from a goat which had been inoculated, ten days previously, with 10 c.c. of virulent horse-sickness blood. The temperature rose immediately to 103·2° and a few spherical parasites were found in the blood. During the following day the temperature rose to 106·4° and fell during the night. A second inoculation from a goat with fresh blood was made on the 25th, and on the 27th and 31st of March and 1st of April well-marked rises occurred.

Parasites, which were never very numerous, were seen on the 16th, 18th, 23rd and 27th of March and on the 4th and 14th of April.

EXPERIMENT 6. *To show that the blood of a clean goat, infected ten days previously with virulent horse-sickness, can, even sometimes when glycerinated, convey infection to a clean horse.*

In a previous communication to the Royal Society I showed, when the blood of infected donkeys was preserved, that great differences were found in that taken from different animals. In cattle and goats I have found similar variations in virulence. This fact has to be borne in mind where experiments have to be carried out with blood taken from the donkey, the ox, or the goat.

On February 13th, clean *Horse* No. 32 (Chart VII) was inoculated by subcutaneous injection with 10 c.c. of an equal admixture of blood and glycerine dilution. This dilution consists of equal volumes of water and glycerine containing 1 per 1,000 of pure phenol.

The blood was derived from a goat which was bled ten days after inoculation with 10 c.c. of virulent horse-sickness blood. After mixing the blood with the preservative dilution it was kept at ordinary temperature for two days.

A rise of temperature, slight in degree, occurred on the following day and during six days some fever occurred, the maximum being on the third day, 102·4°.

On the 16th a few parasites were seen, one being a very characteristic "quadret." On the 18th a few were also seen, but from this date onward no more were detected.

EXPERIMENT 7. To show

(1) *That the blood of a clean goat does not convey infection,—*

but (2) *That, after its inoculation with virulent horse-sickness, it does convey infection.*

On March 5th, clean *Horse* No. 35 (Chart VIII) was inoculated with 20 c.c. of fresh blood taken from the jugular vein of a clean goat.

During the subsequent twelve days no fever occurred.

Meanwhile this goat had been inoculated with virulent blood and ten days later was bled. The blood was immediately glycerinated and, after 24 hours, 10 c.c. was injected into the same horse. A sharp rise of temperature occurred during the following day, but it is very probable that it was due simply to irritation in the area of the injection. Nine days later the same goat was bled and 10 c.c. of the fresh blood was injected into the horse. Eleven days later the temperature rose slightly, was high the following day and more or less fever persisted during fourteen days.

On April 6th, being the day subsequent to the first real attack of fever, parasites were found in the blood. They were also found on the 7th, 8th, 11th and 15th of the same month.

EXPERIMENT 8. *To show that the blood of a goat infected with virulent horse-sickness fails to infect a clean horse which has already been successfully infected with the blood of a "salted" horse.*

On February 14th, clean *Horse* No. 33 (Chart IX) was inoculated with 20 c.c. of the blood of "salted" horse *B* (which had been reinoculated 82 days previously. See also Horse No. 29).

Six days later the temperature began to rise and fairly severe fever persisted, with remissions and intermissions, during the subsequent ten days.

On the 14th and 25th of March and 2nd of April it was inoculated with infected goat's blood without any definite result. The inoculation on March 25th was with the same fresh blood as was used in the preceding experiment.

Parasites were fairly numerous on February 24th, when a "quadret" was also seen. There were but few present on the 26th. A few were also seen on the 2nd and 7th of March.

Summary.

The conclusions I seek to draw from this part of my investigations are:—

1. That a malarial form of horse-sickness probably occurs naturally.

2. That when animals which are either naturally relatively susceptible, such as the donkey, ox, and goat, or animals which have acquired protection, *e.g.* "salted" horses, are inoculated with virulent horse-sickness blood, their blood conveys a modified infection of horse-sickness which is malarial in type.

3. That this malarial form is associated with the presence of parasites within the red corpuscles of the blood of the infected animal.

4. That the blood of "salted" horses which have been previously regularly inoculated during several months is of a dangerous order of virulence. *Vide* former experiments.

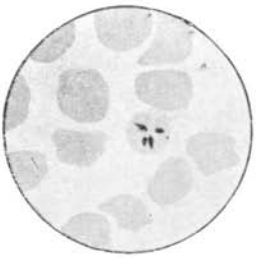


Fig. 1.

Fig. 2.

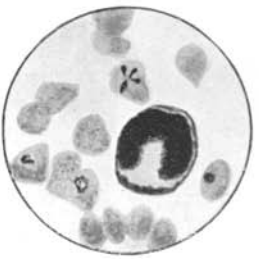


Fig. 3.

Fig. 4.

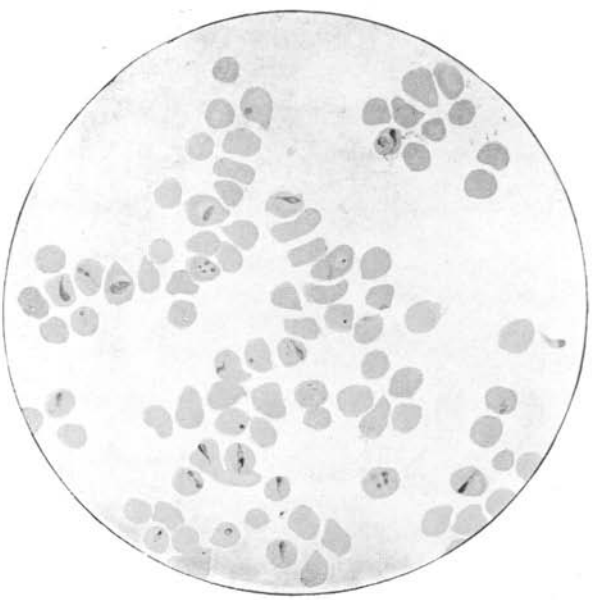


Fig. 5.

https://doi.org/10.1017/S0022172400002047 Published online by Cambridge University Press

5. That the blood of "salted" horses which have only been once inoculated after several months' rest conveys a fairly severe fever.

6. That the blood of "salted" horses, which have not been inoculated for even a year, can still convey infection.

7. That "unsalted" horses which have been inoculated a year previously with "salted" blood convey only a weak infection.

8. That a severe attack of malarial horse-sickness gives a considerable degree of protection against subsequent inoculation with "salted" blood.

9. That when regularly inoculated "salted" horses are used to produce blood giving severe fever, such fever may become so severe as to cause death with symptoms of horse-sickness.

10. That the blood of the latter animals which die can produce virulent horse-sickness in clean horses, if used in a considerable dose, *i.e.* 20 c.c.

DESCRIPTION OF PLATE I.

Fig. 1. A field from the blood of *Horse* No. 32. The careful examination of a whole coverglass only revealed two parasites, of which one is seen in the plate. Subsequent to this the febrile condition came to an end and the parasites totally disappeared.

Fig. 2. A field from the blood of *Horse* No. 27. Only a very few parasites were found at this date, and in this case also the appearance of a "quadret" with very few parasites of other form was followed by the disappearance of fever and parasites during a considerable time.

Figs. 3 and 4. Fields from the blood of *Horse* No. 29 seen on the same day. In this case a "quadret" is also seen but also many other forms, and this condition was followed by a sharp attack of fever and the appearance of numerous parasites.

The above cases were all mild in type.

Fig. 5. A field from the blood of *Horse* No. 16 of the former experiments. This case was extremely severe in type, and the animal died on the day following that on which this field was observed.

Note. Photographs of water-colour figures. In Figs. 1 and 5 the corpuscles are pink, the bodies dark-blue. In Figs. 2, 3, and 4 the corpuscles are violet, the bodies dark-blue or (Fig. 4) violet-blue. The figures show no indication of chromatin staining.—*Ed.*

CHART I. *To show that the blood of a salted horse, which had been reinoculated* 10 *days previously with virulent horse-sickness, can set up infection in a clean horse.*

Disease. **Horse-Sickness.** *Horse No.* **27.**

Infecting Material. 20 *c.c. subcutaneously of blood of salted horse B* 4. XII. 02 (*inoculated* 10 *days previously with* 10 *c.c. preserved virulent blood*).

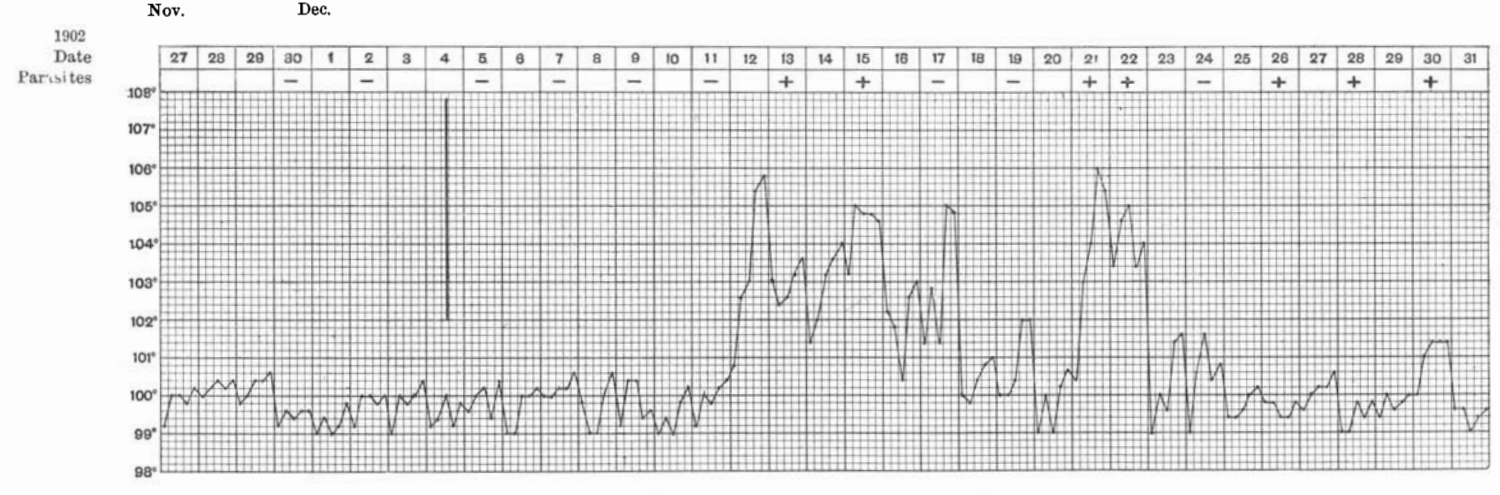


CHART II. *To show the same condition as in preceding No.* 27.

Disease. **Horse-Sickness.** *Horse No.* **28.**

Infecting Material. 20 *c.c. blood of salted horse B injected subcutaneously on* 4. XII. 02 (*Horse B was inoculated ten days previously with* 10 *c.c. virulent blood*).

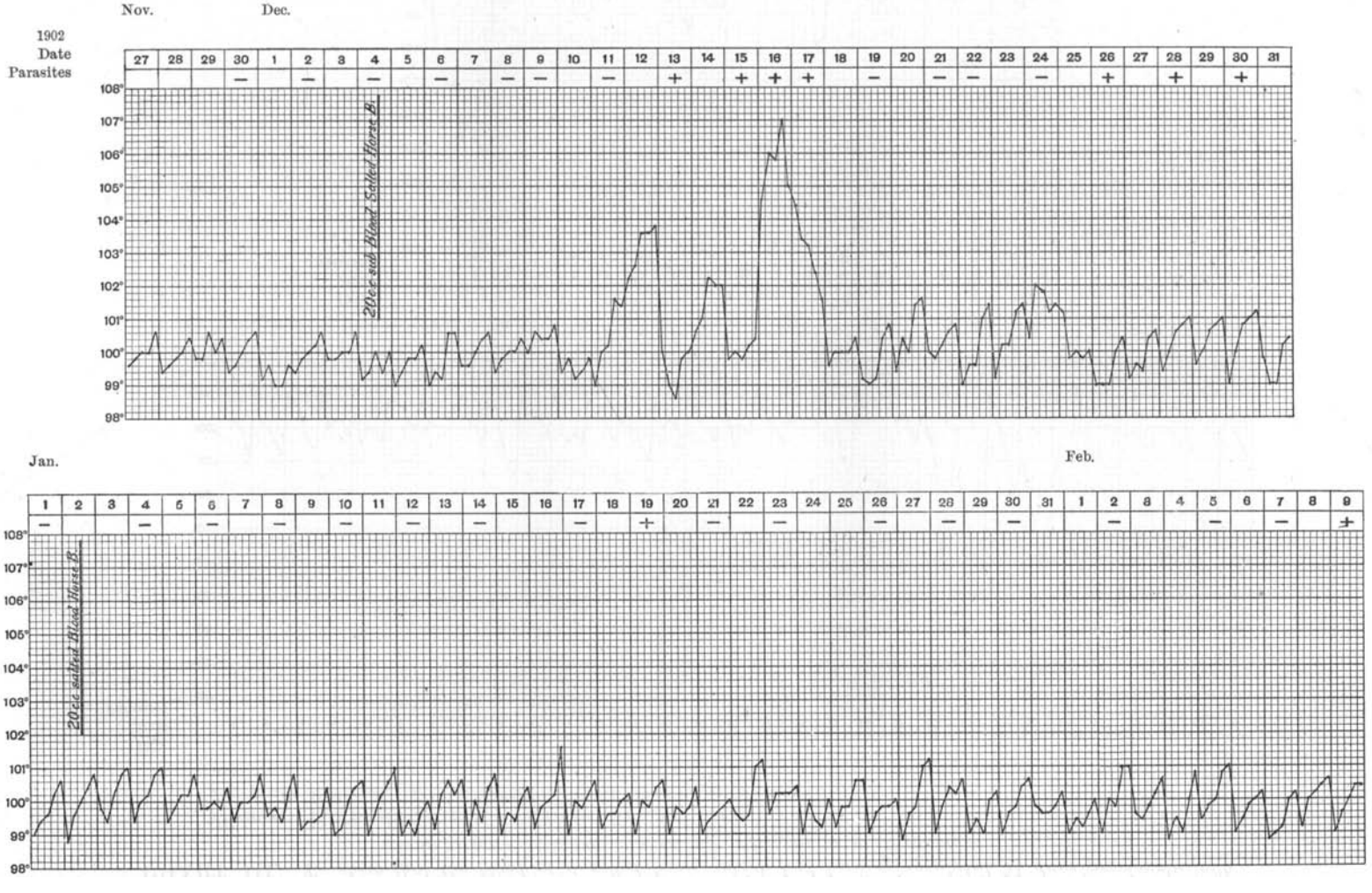


CHART III. *To show that the blood of a salted horse, which had been reinoculated 51 days previously with virulent horse-sickness, can set up infection in a clean horse.*

Disease. **Horse-Sickness.** *Horse No.* **29.**

Infecting Material. 20 *c.c. subcutaneously blood of salted horse B.*

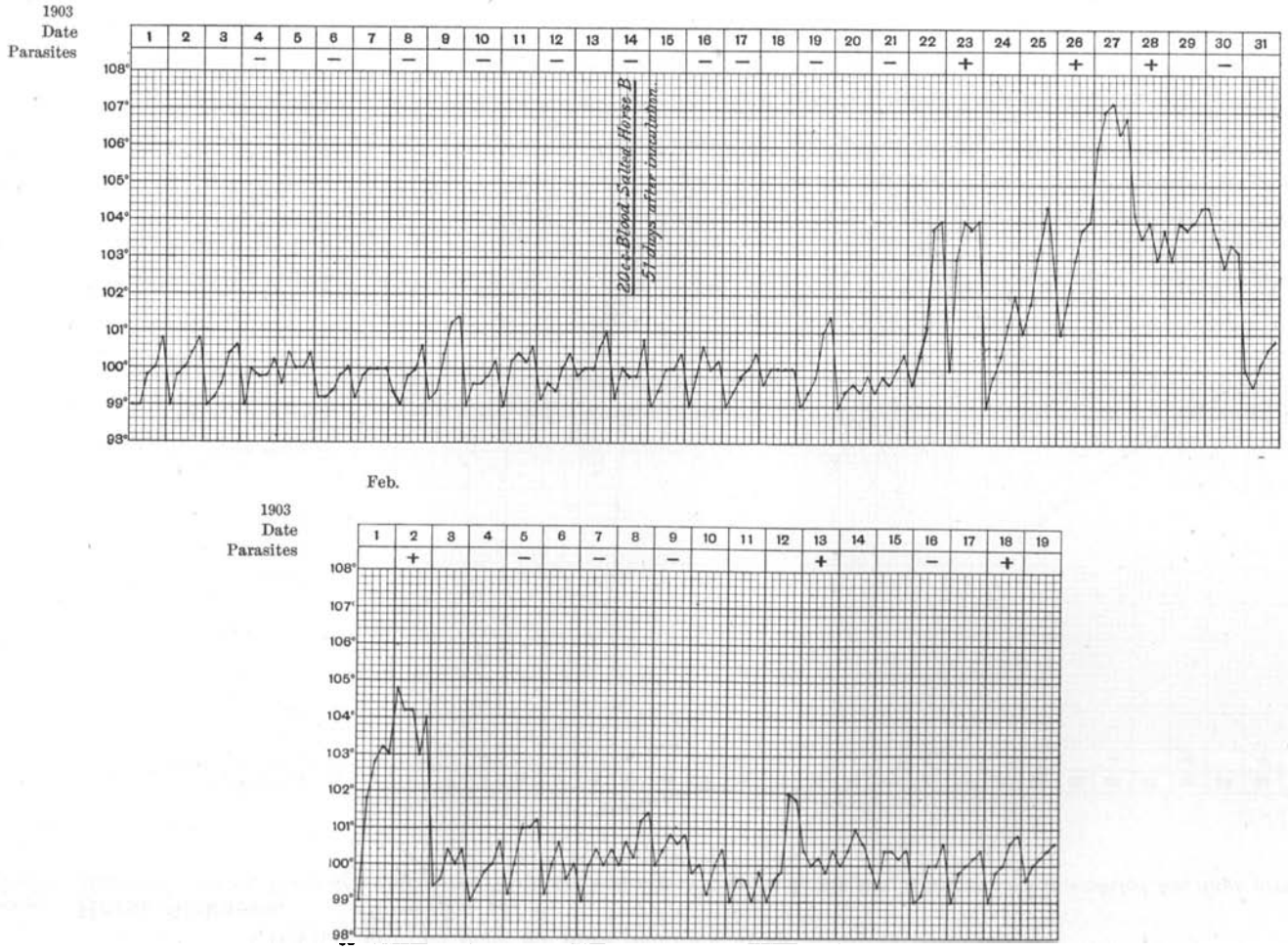


CHART IV. *To show that the blood of a horse, suffering from malarial horse-sickness, can induce the same fever in a clean horse.*

Disease. **Horse-Sickness.** *Horse No.* **30.** *Infecting Material.* 20 *c.c. subcutaneously of the blood of Horse* 27 *which was suffering from fever induced by injection with "salted" blood.*

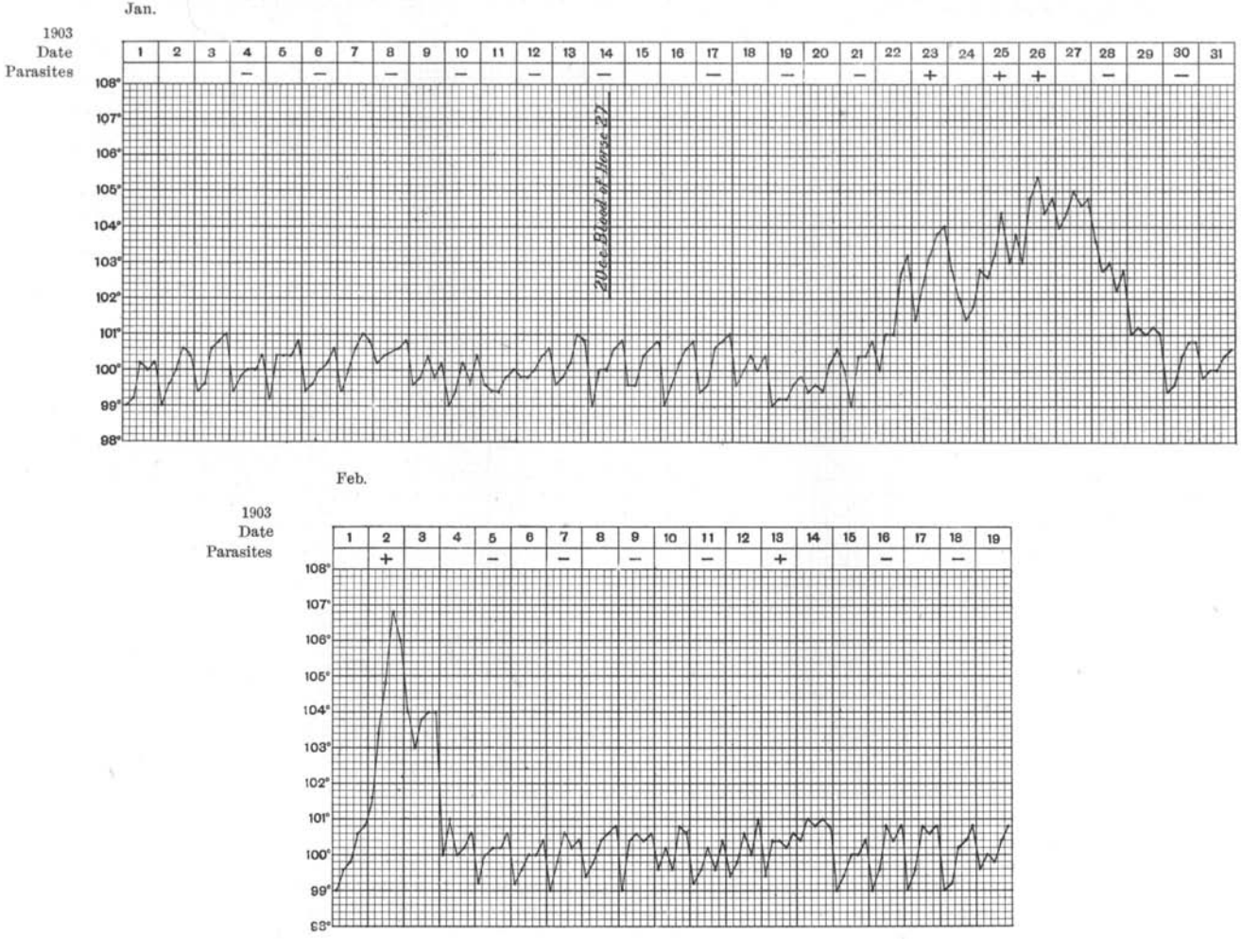


CHART V. *To show:* (1) *that infection remains in a "salted" horse for at least a year;* (2) *that if such a horse's blood produces infection in a clean horse, this infection protects against further infection with the same salted horse's blood, even when the latter has been reinoculated with virulent blood.*

Disease. **Horse-Sickness.** *Horse No.* **31.**

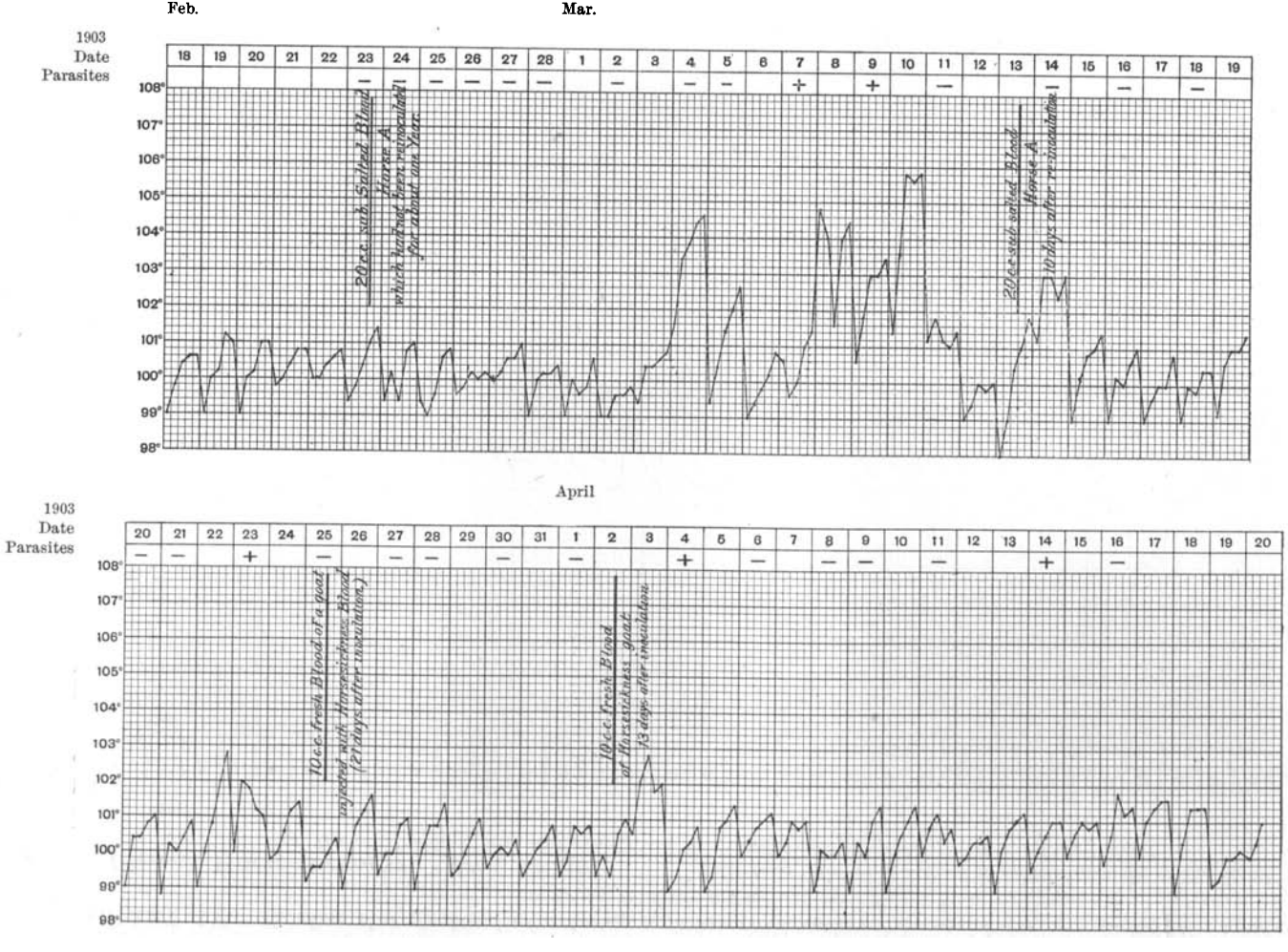
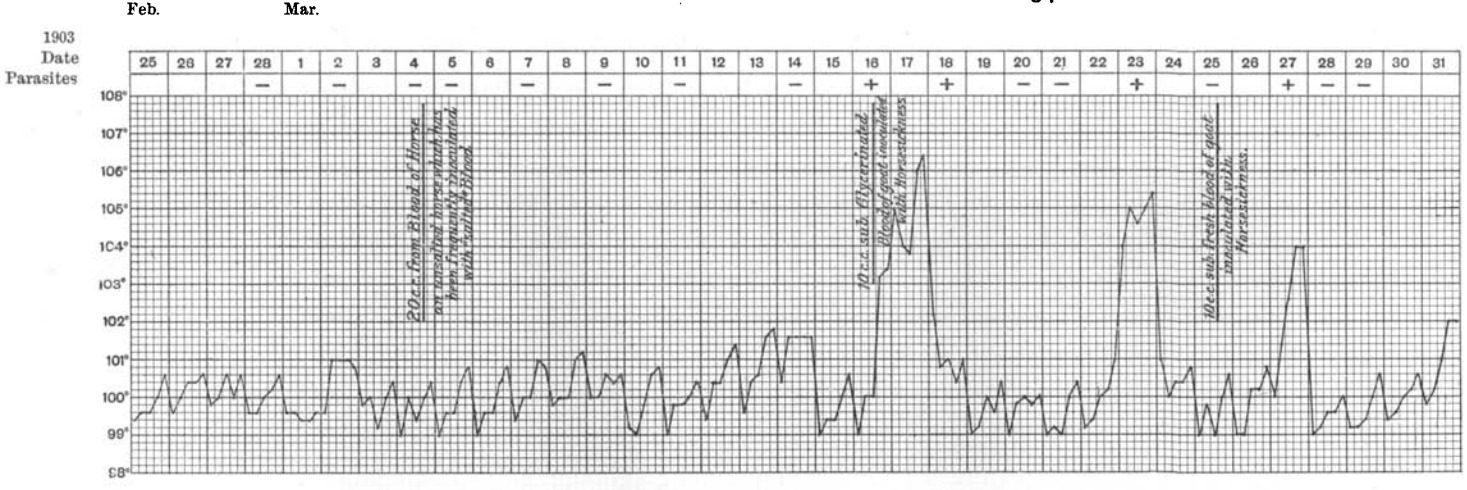


CHART VI. *To show that:* (1) *the blood of an unsalted horse, although it has been frequently inoculated with salted blood, only conveys a weak infection to a clean horse;* (2) *the blood of a goat, infected with horse-sickness, can accelerate and intensify that infection.*

Disease. **Horse-Sickness.** *Horse No.* **34.**



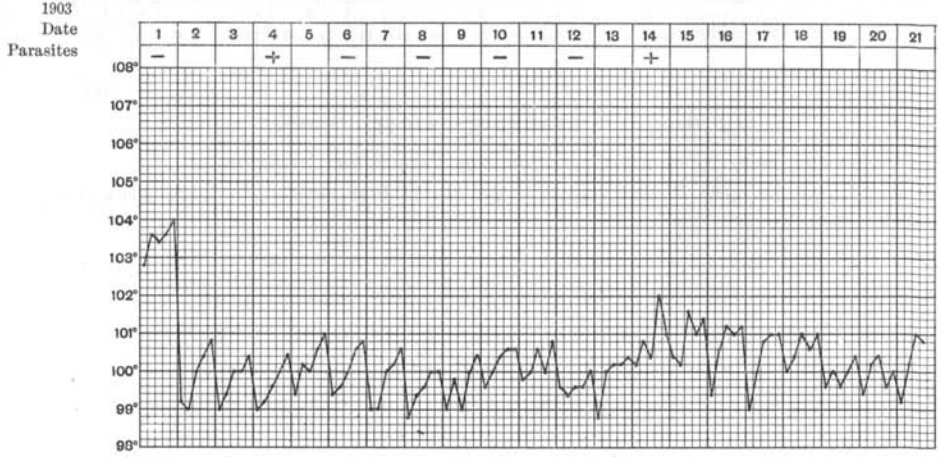


CHART VII. *To show that the blood of a clean goat, infected with virulent horse-sickness, can convey the malarial form to a clean horse.*

Disease. **Horse-Sickness.** *Horse No.* **32.**

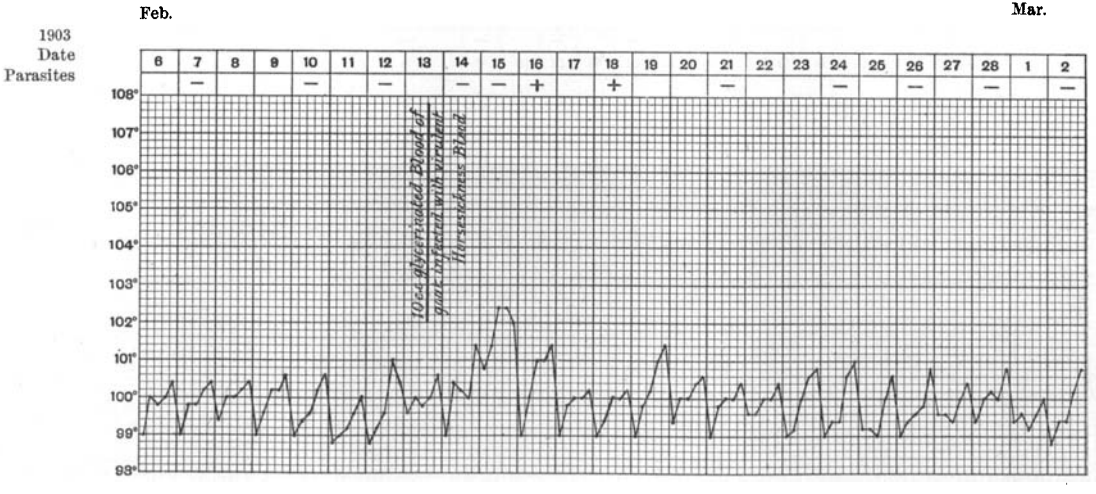


CHART VIII. *To show:* (1) *that the blood of a clean goat does not convey infection, but* (2) *that after its inoculation with virulent horse-sickness, it does convey infection.*

Disease. **Horse-Sickness.** *Horse No.* **35.**

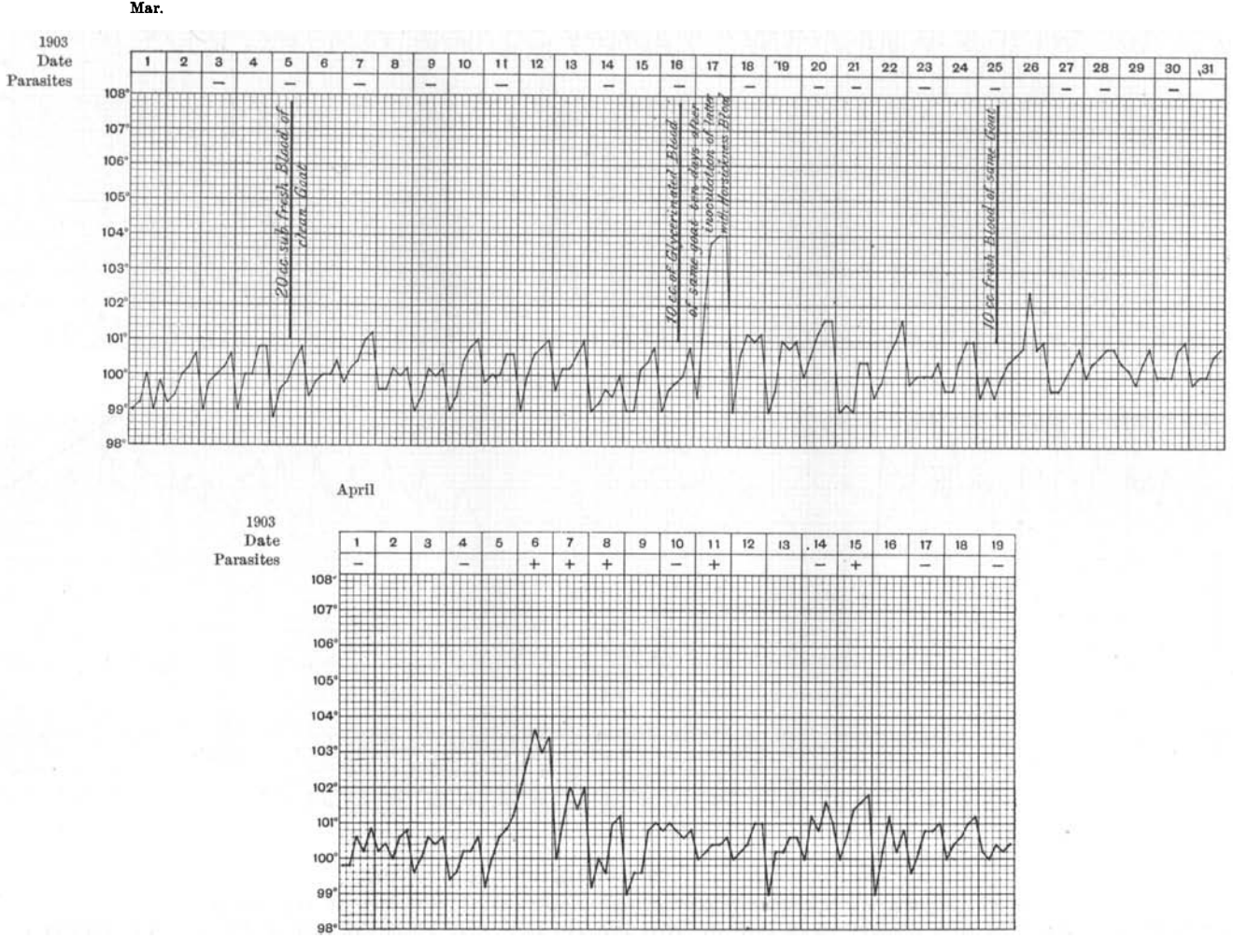


CHART IX. *To prove that the blood of a goat infected with virulent horse-sickness fails to infect a clean horse, which has already been successfully infected with the blood of a "salted" horse.*

Disease. **Horse-Sickness.** *Horse No.* 33.