Bulletin No. 138 - The Experimental Transmission of Swamp Fever or Infectious Anemia by Means of Secretions

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The Experimental Transmission of Swamp Fever or Infectious Anemia by Means of Secretions

JOHN W. SCOTT

INTRODUCTION

In considering the natural transmission of any infectious disease, it is highly desirable to know the channels through which the causal organism is eliminated from the body of the diseased animal. It is likewise important to know the methods by which the organism is transferred from diseased to well animals. In a previous bulletin from this station, No. 133 (1922), the insect theory of transmission and the urine contamination theory of the transmission of infectious anemia or swamp fever, of horses, have been discussed in some detail. It was shown by the experiments described in that bulletin that, under certain conditions, this disease may be readily transmitted from diseased to well horses by means of certain biting insects. Other evidence was also adduced to indicate that such biting insects furnished at least one of the means for the natural transmission of swamp fever. While not denying the possibility of the natural transmission of infectious anemia by means of food or drink contaminated with infective urine, it was shown, I believe, that this method of infection was not probable under our experimental conditions, and could not explain all cases. It will be the purpose of this bulletin to describe some experiments which appear to be related to other possible means of transmitting the disease.

The secretions of horses that find a way to the external surface, and that in this sense may be called excretions, include part of the feces from the alimentary canal, urine from the genitourinary tract, saliva from the mouth, nasal secretions from the nose, eye secretions from the eye, milk from the mammary glands, and the sweat or perspiration through the skin. It is quite common for some of these secretions to contain the agents of disease, for example, the feces, the urine, the nasal secretions; and perhaps no secretion is always free from disease germs. In
studying infectious anemia of horses, different workers have paid some attention to all of the excretions mentioned above, but in this bulletin particular attention will be paid to the nasal and the eye secretions.

Vallée and Carré, 1905, first demonstrated that infectious anemia of horses may be transmitted by feeding urine. This has been confirmed by Schlathölter, (1910-11), Van Es, Harris and Schalk (1911), the Japanese Commission (1914), and Theiler and Kehoe, (1915). Van Es, Harris and Schalk, the Japanese Commission, and Theiler and Kehoe, have also transmitted the disease by the subcutaneous inoculation of urine. From these researches it appears that the virus of swamp fever is commonly present in urine.

In 1908 Ostertag failed to transmit the disease by means of saliva. While this appears to have been the only experiment tried with saliva, many investigators have reported failure to get transmission where both well and diseased horses used the same watering troughs and feed boxes, and where there was an excellent chance for contamination with saliva.

Van Es, Harris and Schalk, 1911, failed to transmit swamp fever by the subcutaneous inoculation of extract of feces, though they tried the experiment on three different horses and used as high as 4000 cc. of extract. The Japanese Commission also failed to get transmission with filtered extract of feces. The same Commission likewise failed to find the virus in sweat. This Commission succeeded in infecting three horses by injecting from 30 to 100 cc. of milk from infected mares, but such milk, fed every morning to healthy stallions and colts, gave a negative result. Lührs, 1919, states that both the milk and the eye secretion are infectious, and he transmitted the disease by introducing infectious material under the eye-lid.

The present writer, 1920, stated that the virus of swamp fever had been demonstrated in the nasal secretion, and this bulletin will describe some experiments on which that statement was based. There will also be included in this bulletin a description of an attempt to transmit infectious anemia by means of eye-secretion, the effects of the inoculation of tabanid extract as
related to transmission, a description of two cases that were apparently of spontaneous origin, and some observations with the offspring of affected mares. The different results obtained in transmission by blood inoculation will be deferred until a later bulletin on the blood. A discussion of the probable relation between the excretions and natural transmission will be deferred until after a description of the experiments.

1. TRANSMISSION BY MEANS OF THE NASAL SECRETION

It is generally agreed that swamp fever, or infectious anemia, is due to a filterable, probably ultravisible, virus which so far has proved refractory to cultural and staining methods. It is true that some Japanese authors have claimed that the disease is due to a certain spirochaete, but so far this work lacks confirmation.

In some of the diseases produced by filterable viruses, it is well known that the virus or casual agent occurs in the nasal secretion. The question very naturally arises, does the virus of swamp fever occur in the nasal secretion of horses infected with the disease? If so, we may have here a possible means of natural transmission. The idea involved in the above question furnished the point of departure for the series of experiments that will now be described.

Experiment 1. On July 1, 1919, a locoed, black gelding, horse number 61, was obtained from a ranch on Dale Creek. So far as could be learned, swamp fever had never been present on this ranch. This horse was rather nervous and erratic, as is frequently the case in locoed animals, and his temperature was somewhat variable, averaging about 101° F. He was in fairly good flesh but was not in a vigorous condition. On July 12 the left nasal cavity of horse No. 31, a typical, long-standing chronic case, described in Bulletins 121 and 133, was washed out with a warm normal saline solution. Washing was accomplished by means of a douching tube which was inserted through the nostril up into the nasal cavity for a distance of about eight inches. By this method the spray reached the upper levels of the left nasal cavity, and the solution flowed down and trickled out of the cor-
responding nostril. About 110 cc. of nasal washings were caught in this manner. Eleven cubic centimeters of the solution were then injected by means of a hypodermic syringe under the skin of the left breast of horse 61. No noticeable toxic effects were observed. The next day there was a local swelling at the point where the injection had been made, but this swelling disappeared before July 14th; the temperature remained normal.

On July 16th the left nasal cavity of horse 31 was again washed out with normal saline and 15 cubic centimeters of the washings were injected subcutaneously into horse 61. At the close of the injection No. 61 appeared decidedly uneasy, stopped eating, and walked about. However, at the end of five minutes he appeared normal again. On July 21st, 10 cc. of nasal washings obtained in the same manner from the same source was injected subcutaneously into horse No. 61. No serious effects were observed. On July 30th No. 61 was again inoculated subcutaneously with about 10 cc. of nasal washings obtained in the same manner as before. No. 61 had never been active and on this date there appeared to be some pus present above his left eye where the skin had been severely bruised; there was also a soft lump on his left front leg near the region where one of the inoculations had been made. One other inoculation of 10 cc. of nasal washings from horse 31 was made on August 13th; the same procedure was used as before.

As seen from the preceding description this horse was a poor subject for experiment, and it is not surprising that the results were somewhat inconclusive. An inspection of his temperature record (Plate 1) shows that he was perhaps not in a normal condition even at the beginning of our observation. Between July 1st and August 7th, his temperature varied erratically from 100 to 102° F. A more definite fever reaction occurred on August 8th, 102.4° F; on August 9th, there was a subnormal drop to 97.3°, followed quickly by recovery to normal on August 10th. Another two-day fever exacerbation began on August 21st, which reached 102.6° on August 22d. This was followed by a rapid decline; between the afternoon of August 27th and the morning of the 28th his temperature fell from 100° to 96° F. and he died.
a few hours later. There was a rapid emaciation during the last few weeks and he grew very weak toward the end. A post-mortem report of the veterinarian diagnosed his death as due to “auto-intoxication, accompanying extreme emaciation.”

Thinking that No. 61 may have been infected with swamp fever, 20 cc. of his blood serum was saved and injected subcutaneously into horse No. 64 on August 29th. Horse No. 64 was a wild, nervous, excitable young stallion that was brought in from the range on July 19th. Temperatures of 103° F. were recorded on September 21st and 25th, but the temperature of this horse, normally rather high, varied between such extremes, both before and after the inoculation, that the above records may or may not have been significant. When No. 64 was killed on November 25th, he was rather thin and run down. Examination showed the presence of bots (Gastrophilus) in the stomach, some catarrhal enteritis in part of the small intestine which contained four large Ascaris, and large numbers of smaller roundworms, including three or more species, in the colon and coecum. When horses are highly parasitized, as in this animal, we expect a variable temperature and frequently more or less pronounced fever reactions, accompanied by emaciation. Consequently, if horse 64 was infected with swamp fever the symptoms were not sufficiently pronounced to diagnose that disease.

Judging from subsequent results, I am inclined to believe that horse 61 contracted swamp fever as the result of inoculations of the nasal secretion from horse 31. There were some symptoms of the disease in both 61 and 64, such as fever reactions and progressive emaciation, but so many complications were involved that no satisfactory conclusion was possible. The experiment is described here on account of its relation to Experiment 2. Incidentally, it shows the difficulties that may arise in studying swamp fever unless one uses normal animals for experimental purposes.

Experiment 2. About 110 cc. of the nasal washings that were caught as they trickled out of the nostril of No. 31 on July 12, 1919, were sprayed by means of a douche high in the left nasal cavity of horse No. 62. It was thought that if the virus of
swamp fever was present in the nasal cavities of horse 31, it might in this manner be transferred to the mucous membrane of the nasal cavities of horse 62 and thereby produce the disease. Horse 62 was a healthy gelding in good flesh, and, like No. 61, was kept isolated from the horses infected with swamp fever.

On July 16th, about 250 cc. of the normal saline nasal washings from horse 31 were used as a douche, in the manner described above, in the left nasal cavity of horse 62. Similar treatments, using the same horses, were repeated on July 21st, July 30th, and August 13th. It should be noted that in Experiments 1 and 2 portions of the same nasal washings were used. The result in Experiment 2, however, was positive and much more definite.

The first temperature observation on horse 62 was made on the afternoon of July 3d, shortly after this horse had been brought in some twenty miles from a ranch. This explains the rather high initial temperature, 101.2°, shown in Plate II. For the succeeding three weeks his temperature was quite uniform, averaging about 100.1°. On July 25th, thirteen days after No. 62 had received the first nasal douche and nine days after he had received the second douche, his temperature rose to 101.3°. His temperature was normal again on July 26th, but on the following day it suddenly rose to 102.5°. By ten o'clock on the morning of July 28th, the temperature had dropped to 97.4°, but by 7 p.m. on the same day it had recovered to 99.5°. See Plate II for this and subsequent temperature ranges. The slight rise on September 4th was due to colic from which he soon recovered.

By September 1, it was noted that No. 62 was losing flesh. His general appearance on September 29th is shown in Figure 1. Considering these symptoms, including the fever temperatures, it was believed that No. 62 was infected with swamp fever. To make sure of this conclusion the following test was made:

On October 1st, horse No. 65, which had been under observation since the third of August, was inoculated subcutaneously with 25 cc. of fresh blood from No. 62. Two mild fever reactions followed on October 6th and October 16th-17th. Again on October 28th horse 65 was inoculated subcutaneously from
No. 62, this time with 40 cc. of fresh blood. A mild reaction occurred on November 7th, followed by more severe fever reactions on November 18-19, 102.0°, December 2-5, 104.0°, December 18-20, 105.0°, and on January 4-8, 1920, 103.7°. Additional fever reactions of this horse are shown in Plate III. It might be added here that on January 17, 1920, 36 cc. of blood, drawn from horse 65, was inoculated subcutaneously in horse No. 54. Horse 54 had been used in other experiments, one of which will be described later in this bulletin; he was a very hardy animal, and probably had already been infected with the virus though he had been highly resistant to it. However, a new series of reactions began on the thirteenth day after his inoculation with the blood of No. 65. How severe these reactions were is shown in Plate IV.
From the results obtained in Experiments 1 and 2 it was believed that the virus of infectious anemia or swamp fever is, or at least may be, at times present in the nasal secretion of horses infected with the disease. It is true that the nasal washings which were used as a douche in horse 62 were allowed to fall to the ground, and it is possible that both 61 and 62, kept in the same lot, may have picked up some stray bits of hay that had become contaminated with these washings. While this latter possibility is not probable, the virus was in either event derived from the nasal secretion. The results of the two experiments were encouraging and it was decided to repeat them under more rigid control.

 Experiment 3. A big, healthy bay gelding, pronounced by the veterinarian to be free from disease, was bought on March 22, 1920. He was number 67 in our series and was isolated throughout the experiment. On April 27th one of the nasal cavities of horse 31 was washed out with normal saline, and after passing through filter paper 10 cc. of these nasal washings were injected subcutaneously into horse 67. Again on April 30, 15 cc. of nasal washings, from the same source and prepared in the same way, was inoculated subcutaneously into No. 67. The first fever reaction, 103.7°, occurred on May 3d, just six days after the first inoculation. Other reactions occurred: May 16th, 102.4°, May 23d, 105.0°, June 7th, 105.5°, and at later dates. (See Plate V). There also developed other symptoms characteristic of some cases of infectious anemia. Perhaps it should be mentioned that a local infection developed as the result of the last mentioned inoculation. By May 4th the shoulder was considerably swollen in the region of and below the point of injection. On May 6th pus was present; the shoulder was opened and continued discharging pus up to May 19th, but was apparently well before the severe fever reaction of May 23d. An inspection of Plate V shows that No. 67 had several other severe attacks of fever. It is possible, though not probable, that the fever reactions which occurred on May 3d and May 16th may have been due to the local infection, but the later fever attacks cannot be accounted for in this way. Since we were unable to find any
other satisfactory explanation of the later fever attacks, and since there was no other way in which to explain the infection of horse 67, it was concluded that the source of the virus by which this horse became infected with swamp fever was the nasal secretion of horse 31.

Experiment 4. Horse No. 71, a black gelding, was bought on June 8th, 1920, and was kept in an isolated lot until June 23d, on which date he was placed in a box-stall, in a screened cage. On July 20th, while the temperature of horse 72, a severe chronic field case, stood at 104.6°, his nasal cavities were washed out with one pint of warm normal saline solution. These washings were then filtered through a Berkefeld filter and on the following day, July 21st, at 2:30 p. m., 15 cc. of the filtered nasal washings were injected subcutaneously into horse 71. The inoculation was done very carefully to prevent local infection. At 5 p. m. there was evidence of toxic effects; it was observed that some of the muscles in the hind legs of No. 71 were twitching; that he kept moving about uneasily with head drooping, and that his temperature was slightly above the normal average. No fever reaction resulted from this treatment within the usual period. On August 25th nasal washings were obtained from horse 67 while he had a temperature of 104.0°. Some of these washings were filtered as before through a Berkefeld filter and on August 27th were injected subcutaneously into No. 71, care being taken to avoid local infection. No evidence of swamp fever had developed by August 5, 1921. On this date horse 71 was inoculated subcutaneously with 12 cc. of blood from No. 31. Twelve days later the first fever reaction developed. The temperature record of this horse is shown in Plate VI.

The history of this case is somewhat mystifying. First, horse 71 was evidently susceptible to the virus of infectious anemia. Second, there was no local infection, thus indicating that the Berkefeld filter had excluded all suppurative bacteria. Third, it is probable that the virus was in the nasal secretions of horses 72 and 31 at the times when the douches were administered, for each had a high fever and a slight running at the nostrils. Fourth, if the virus of infectious anemia is capable of passing through a
Berkefeld or Chamberlain filter, as claimed by various workers on this disease and as indicated by one of our previous experiments, it is difficult to explain the failure to infect horse 71. In this connection it might be mentioned that we had failed to secure infection in one of our previous experiments in which blood from a known virulent source did not produce the disease after it had been passed through a Berkefeld filter. On several occasions, too, we have apparently failed to produce the disease even with the direct inoculation of blood from a known virulent source. At the present time we are unable to give a satisfactory explanation of the result of this experiment, though there are several possibilities.

Experiment 5. The object of this experiment was similar to that of Experiment 2, and the same general procedure was adopted. The nasal cavity of an infected horse was washed out with normal saline by means of a nasal douche. Then in turn by means of a douche these washings were sprayed high in the nostril of horse No. 69. Horse No. 69, a brown gelding, lame in one knee, was brought in on May 29, 1920, from a ranch on which there had been no previous history of swamp fever. He was pronounced sound by the veterinarian and was placed in a lot at some distance from other horses. On June 8th two other horses, from another ranch, Nos. 70 and 71, were placed in the same lot. These horses fought No. 69 away from his feed for some time. Perhaps this behavior accounts for the high temperature, 102.3°, recorded for No. 69 on June 11th, on which date they were particularly aggravating. On June 23d No. 69 was placed as the only occupant in a screened cage. On July 4th one pint of warm, sterile, normal saline was used as a douche in the left nostril of No. 31, and one-half pint of the washings was sprayed high in the left nostril of No. 69. At this date horse No. 69 was in poor flesh but had a good appetite. The experiment was repeated on July 8th, 13th, 16th, 20th, 23d, 27th, 30th, and on August 3d. No. 31 showed no indications of fever during this period, except on July 23d, when his temperature, 101.8°, was slightly above normal. On August 25th the experiment was repeated, using as the source of the virus horse No. 67, which had a fever of 104.0°,
Again, on August 31st, the experiment was repeated, this time using horse No. 72 as the source of the virus. No. 72 had just returned to normal temperature following a three-day fever period. No evidence of swamp fever developed in horse No. 69. However, he was not immune, for on July 2, 1921, he was inoculated subcutaneously with 18 cc. of blood from horse No. 72. A sharp fever reaction followed eleven days later. See Plate VII. This horse was apparently not susceptible to infection with the virus through the nasal membranes.

The results of the five preceding experiments dealing with the nasal secretion may be summarized as follows:

1. Infectious anemia may be transmitted by the subcutaneous inoculation of infective nasal secretion. This method was successful with horse No. 67 and probably also with No. 61.

2. The disease may be transmitted by douching the nasal cavities with nasal washings from an infected horse. This method succeeded in one case, No. 62, but failed in another, horse No. 69.

3. An attempt to transmit the disease by the subcutaneous inoculation of filtered nasal washings from an infective source, resulted negatively in the one case tried, horse No. 71.

II. AN ATTEMPT TO TRANSMIT SWAMP FEVER BY MEANS OF EYE SECRETIONS

Experiment 6. Horse No. 70, a small black mare, was brought, on June 8, 1920, from a ranch with no previous history of swamp fever. This horse was kept in an isolated lot until June 23rd; it was then placed in a screened cage, where it remained until it died on November 30, 1920.

The object of the experiment with No. 70 was to learn if swamp fever can be produced by the transfer of eye-secretion from an infected horse to the eye of a horse without the disease. The following method was employed: Some of the eye-secretion of horse No. 31 was collected from within the sac of the lower
lid; if the secretion was scanty the eye-ball was irritated gently with a camel's hair brush until the secretion increased. The secretion was then transferred by means of a pipette or a camel's hair brush to the conjunctiva of the upper eye-lid of horse No. 70.

The first transfer of eye-secretion was made on July 20th. Other transfers by the same method were made on July 24, 27, and 30, and on August 3, 6, 10, and 13. On August 24th eye-secretion was taken from horse No. 67, which had fever at this time, and transferred to horse No. 70. At no time did any evidence of swamp fever develop in No. 70. On November 26th she became noticeably ill, and was killed on November 30th when in an apparently dying condition. The station veterinarian diagnosed this last illness as due to "purulent hepatitis followed by general peritonitis". This horse had grown somewhat thin, due, no doubt, to the long confinement and lack of sufficient exercise.

If the virus is present in the eye-secretion, as claimed by Lührs, 1919, it is evident from this experiment that No. 70 either resisted the invasion of the virus or else tolerated its presence in the eye to such an extent that it failed to produce the disease. It is not safe to base a conclusion upon one test, for some horses undoubtedly have a high resistance to infection or else tolerate the presence of the organism to a very high degree, even after direct inoculation with the virus. Other horses with less resistance might become infected by the method described. Subcutaneous inoculation of eye-secretion from a virulent source would be more likely to give a positive reaction, provided the virus is present in this secretion.

III. EFFECTS OF INOCULATION WITH TABANID EXTRACT

Experiment 7. In Bulletin No. 133 it was mentioned that Tabanus septentrionalis appeared to be more effective in transmitting swamp fever than Stomoxys calcitrans. The tabanids used in those experiments were collected in the midst of a large alfalfa field at a distance of one-half mile or more from any pasture, which contained horses. By taking this precaution we were satisfied that the tabanids had had no chance of becoming in-
fected or contaminated with the virus of swamp fever before
they were used in our experiments. However, in order to avoid
any criticism, the following experiment was planned to test
whether such tabanids harbored the virus of swamp fever at the
time they were collected.

Horse No. 63, a roan gelding, was bought on July 9, 1919,
and was placed at once in an isolated lot. On August 4th six
tabanids were macerated in 25 cc. of normal saline. After stand-
ing one hour the extract was filtered through filter paper and 2
cc. of the filtrate was injected subcutaneously into horse No. 63
at 5:30 p.m. At 10 a.m. the next day, 5 cc. of the same extract
was injected into the same horse in a similar way. No after-
effects were observed. On August 13th an extract of four flies
was made in 20 cc. of normal saline. After filtering 12 cc. of
the extract was inoculated subcutaneously in the right breast of
horse No. 63 at 5:24 p.m. An anaphylactic shock developed
which was described briefly in my notes as follows:

"5:25 p.m. Horse became restless; noticed irritation at the
region or point of injection; started to lie down, tried to reach
point of injection with his mouth; bent body to that side, and
moved about. 5:26 p.m. Started to rear and continued restless
for about 3 minutes. 5:29 p.m. Stood still; yawned, that is,
opened his mouth wide, closing or partly closing his eyes until
about 5:32. Evidently is now recovering. Appeared about nor-
mal at 5:35 p.m., eleven minutes after the inoculation."

This horse showed no signs of becoming infected with swamp
fever and remained in good condition until near the end of Octo-
ber, when he was inoculated with blood from a virulent source.
(See Plate I.)

This experiment seemed to prove that tabanids (Tabanus
septentrionalis) do not naturally harbor the virus of swamp
fever. It also indicated that the tabanids used in our fly transmis-
sion experiments had not become contaminated with the virus of
swamp fever before they were collected.
IV. CASES OF APPARENT SPONTANEOUS ORIGIN

In our experience with more than eighty horses, two cases of infectious anemia, horses Nos. 55 and 58, have developed evidence of the disease soon after they were brought to our experimental lots. Our experience up to this time, in a number of cases, had repeatedly indicated that well horses can mingle with infected horses, eat from the same feed boxes, and drink from the same watering trough without contracting the disease. Hence, horses 55 and 58 were bought and turned in with infected horses. Both developed the disease. The results indicate that these horses were infected either before we bought them or very soon after they came into our possession. The evidence points to the former conclusion, but some further details are needed to make this clear.

Our experimental lots are situated a mile or more from any place suitable for the development of tabanids, and consequently one of these insects is rarely seen in our lots. Mechanical infection by this means is not probable under the conditions named. Late in the summer Stomoxys are common on certain days, but they are much more abundant in the cattle lots a short distance away. In general, then, our experimental lots were not favorable for the insect transmission of swamp fever, though it is possible that stable-flies transmitted the disease to No. 58. The exact circumstances, under which the disease was contracted in each case, so far as known, were as follows:

Horse No. 55 was bought on May 30, 1918, and immediately placed in a lot with some infected horses. Three days later one of these horses, No. 52, had a fever attack which lasted for several days, June 2d to 7th, inclusive. On June 18th the temperature of No. 55 suddenly rose to 102.5° F. and on the following day, at 1:30 p. m., 103.1° was registered; by the usual time of taking the temperature in the evening the fever had fallen to 101.7°. Other fever reactions occurred on July 22d and 29th, August 23d-24th, and September 20th, as well as at later periods. (See Plate VIII.) On September 1, 1919, so far as flesh and general appearance were concerned, No. 55 seemed to be in first-class condition. However, on September 30th, 22 cc. of his blood serum was injected subcutaneously into horse No. 63. After an
incubation period of thirteen days No. 63 developed the first attack of fever. See Plate I. The inoculation on October 1st of 30 cc. of blood serum from No. 55 also produced an attack of swamp fever in horse No. 53 after an incubation period of fourteen days. When No. 55 was killed on October 7th the station veterinarian reported “petechiae on the caecum, on the spleen and on the double colon, but no lesions of significant importance”. The history of the case, including the temperature record, indicated a characteristic case of infectious anemia which had apparently recovered. Careful inquiry developed the fact that three years previously this horse had been on a ranch on which one well-defined case of swamp fever had occurred. Considering all of the circumstances, I am inclined to believe that No. 55 was a comparatively mild, long-standing, chronic case of infectious anemia that had apparently recovered when he came into our possession.

The history of No. 58 was somewhat similar. He was bought from the same ranch on August 5, 1918, and on that date turned into the lot with our infected horses. On August 13th there began a mild fever reaction (101.5°), which rose to 102.0° on the 16th. See Plate IX. It will be noted that almost from the first the temperature record of this horse showed the irregular, “choppy,” character which is frequently found in chronic cases of infectious anemia. In the summer of 1919 the fever attacks grew more severe, and on September 1st No. 58 was thin, weak, and in very poor physical condition. His condition did not improve and he was killed on December 17, 1919. A post-mortem examination was made by the veterinarian, who reported “some serious exudate in the abdominal cavity, a parenchymatous nephritis of the kidneys, a spleen with a somewhat softened pulp and a few petechiae, evidence of a chronic hepatitis, and a number of petechial spots on the abdominal surface of the diaphragm”. Considering the character of the temperature curve and the previous history, this horse was probably infected some time before he came into our possession. However, it is possible that he became infected after he was put in with our experimental horses, either through the agency of biting flies or by other means.
Figure 2. Horse No. 58. A chronic case, probably already infected when bought on August 5, 1919. Picture shows condition on September 17, 1919.

At the time they were bought both horses, 55 and 58, appeared in good health and free from disease. However, as mentioned in a previous bulletin, certain chronic carriers show, even after the most careful examination, little or no evidence whatever of infectious anemia. We have always inquired into the early history of the horses used, and, except as a source of the virus, in no case have we used in our transmission experiments any horses that seemed to have had direct or indirect contact with the disease, or that came from ranches where the disease had been previously reported.

V. EXPERIMENTS WITH OFFSPRING OF AFFECTED MARES

The Japanese Commission ('14) have stated that the virus of infectious anemia is present in the blood of the foetus and the milk of affected mares. Experiments with feeding the milk
to stallions and colts resulted negatively, but the injection of milk (30 cc.-100 cc.) into each of three horses gave three positive cases after 24, 42, and 46 days' incubation, respectively. Blood from four foals, born of affected mothers, was inoculated with one positive, two doubtful, and one negative result. Blood from one young foal, taken before it began sucking, was inoculated intravenously and produced symptoms of the disease in two out of four cases. Lührs ('19) produced the disease by inoculating intravenously with 10 cc. of milk from an infected mare; the incubation period in this case was 57 days.

In our experience with this disease, we have had two mares abort at the close of rather severe fever reactions, the foetus in each case being small and immature. In a third case, after a fever reaction, a foal was born dead apparently near full term. In two other cases healthy colts were born of affected mares, and since their history turned out to be somewhat different from what was expected in the light of the above-mentioned results, they will be described in some detail.

On July 19, 1916, an old mare was bought and was designated as horse No. 39. This horse was exposed to infective tabanid bites on August 7, 8 and 14, and on August 15th the first fever reaction occurred. A full account of the comparatively mild chronic case of infectious anemia that developed is given in Bulletin 133. On May 30, 1917, she foaled a rather small but vigorous and apparently healthy male colt. No. 39 grew thin and emaciated during the following summer and fall while suckling her colt, and died, as a result of the disease, December 7, 1917. The colt thrived and daily temperature records were kept from August 29, 1917, to October 12, 1919. Its temperature range was variable and rather high throughout the period. During the first two summers the range was ordinarily from 100.5° to 102.5°; during the third summer the range was usually between 100.1° and 102.2°. In mid-winter the average was about a degree lower than in summer. Rather high temperatures were recorded in 1917, on October 24th, 104.2°, and October 31, 103.9°; in 1918, on May 31, 103.0°; August 9 and 10, 103.0°; and in 1919, on June 26, 103.6°, and on July 11, 102.7°. While this colt appeared
Figure 3. Colt of No. 39, foaled May 30, 1917. This picture taken in November, 1917. No. 39, an old gray mare, became infected by means of tabanid bites during the summer of 1916.

Rather stunted during the first year, it later made a better growth, and was in good flesh on September 1, 1919. During the whole period while it was under observation it ran in the lot with infected horses, but at no time did it afford any clear evidence that it had infectious anemia. An attempt was, therefore, made to test whether its blood was virulent. On September 30, 1919, 25 cc. of its blood serum was inoculated subcutaneously into horse No. 54. No reaction having occurred within the next four weeks, on October 28th No. 54 was again inoculated, this time with 40 cc. of blood from the same source. On the twenty-fifth day
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No. 54 developed a one-day fever period, the temperature rising to 102.8°; his average for some time had been about 99°. No other fever reaction occurred in No. 54 until January 31, 1920, fourteen days after he had been inoculated with blood from No. 65. See Plate IV. Also tending to confuse the result was the fact that the previous year, November 9, 1918, No. 54 had been inoculated with 15 cc. of blood from horse No. 50, a known virulent source; no definite fever reaction resulted until November 22, 1919, after being twice inoculated with blood from No. 39's colt, as mentioned above. In October, 1919, a veterinarian who knew something of the history of the case, gave the opinion that this colt did not have infectious anemia. The following facts, however, suggest that he may have been a carrier of the virus: His mother was highly tolerant of or resistant to the disease; the rather irregular and variable temperature curve of this colt is similar to that of some known chronic cases; the fact that a fever reaction was apparently produced in No. 54, a horse known to be highly resistant to the disease, indicates that the colt was a carrier of the virus. It is unfortunate that limited funds made it necessary to dispose of this colt before accurate tests could be completed, or that we did not have more horses into which his blood could be inoculated. Plate X shows his temperature record.

On July 19, 1916, horse No. 40, a good-sized, thrifty-looking mare, was bought and placed in a screened cage. How she became infected with infectious anemia through the agency of stable flies (*Stomoxys*) has already been described in Bulletins 121 and 133. The disease developed rather gradually and she did not die until September 13, 1918; in spite of suckling a colt during the summer of 1917, she remained in fairly good flesh up until November of that year. See cover of Bulletin No. 121. On May 23, 1917, No. 40 foaled a good-sized, strong, apparently normal mare colt. The colt thrived and at three years old it weighed about 1200 pounds. Daily temperature records were kept from August 29, 1917, to June 29, 1920, when it was turned over as a work horse to be used on the experimental farm. During the period under observation, nearly three years, the temperature seldom varied from the normal, averaging about 101.5°
at first and about 100.5° at three years of age; the temperature was slightly more variable during the second summer but even then it rarely varied as much as a degree above or below the average. Fever temperatures were recorded as follows: In 1917, on October 26th, 104.3°, on October 31, 104.1°; in 1918, on June 7th, 103.4°, on June 27th, 104.0°, on October 11th, 103.5°; in 1919, April 29th, 102.9°. In May and June, 1919, the colt, grown now to be a strong young mare, was put to work. On working days the temperature usually rose to 102° to 103° F., but it returned to normal after two or three hours' rest. On non-working days the temperature usually ranged between 100° and 101° F.

Some attempts were made to prove whether or not this colt was a carrier of the virus of infectious anemia. Its mother became infected while it was carried in uteri, it suckled from the infected mother, it showed fever occasionally, as mentioned above, and it lived in the same lot with infected horses for three years, eating from the same mangers and drinking from the same water trough. At eight years of age it is still working on the farm, has shown no evidence of the disease, and in the intervening five years no cases of infectious anemia have occurred among the work horses. The first attempt to prove that its blood contained the virus of swamp fever was made on June 26, 1918. On this date 30 cc. of its blood was inoculated subcutaneously into horse No. 56. No definite evidence of fever developed in No. 56. On February 16, 1920, 33 cc. of its blood was inoculated subcutaneously into horse No. 66. No reaction having occurred within the next three weeks, No. 66 was again inoculated on March 8th, this time with 40 cc. of the colt's blood. No indication of infectious anemia had developed by April 2d, when horse No. 66 was inoculated with 25 cc. of blood from No. 31, a known virulent source. After an incubation period of fifteen days a clear case of the disease developed. See Plate XI. This result seemed to show that the blood of No. 40's colt at about three years of age was not a carrier of the virus of infectious anemia. The subsequent history of this animal in the past five years, though not under close observation, seems to have added evidence toward this conclusion.
In the light of the above results, our evidence with reference to the relation of infectious anemia to pregnant mares and their offspring may be summed up as follows: Severe onsets of fever during pregnancy produced abortion in three cases. Both No. 39 and No. 40 had only mild attacks of fever during pregnancy. The colt of No. 39 was probably a carrier of the virus, though adequate proof of this was not established. The colt of No. 40 was probably not a carrier of infectious anemia, considering the tests made on its blood in 1920 and the subsequent history of this animal.

VI. PROBABLE ROLE OF THE EXCRETIONS IN THE NATURAL TRANSMISSION OF SWAMP FEVER

From the previous experiments and from the literature cited in the introduction it is clear that the virus of swamp fever is commonly present in not less than four excretions from infected horses. That it is present in urine has been repeatedly demonstrated; the preceding experiments have shown that it is present in the nasal secretions; the Japanese Commission produced infectious anemia by the inoculation of milk from infected mares; and Lührs has shown that the eye secretion contains the virus. Transmission experiments with saliva, sweat and feces have so far given negative results. A pathological condition in the lining of the alimentary canal may occasionally allow the escape of blood or the exudation of bloody serum which contains the virus, but such presence in the feces would be of a purely accidental character.

The probable role of urine in the transmission of swamp fever has been discussed at considerable length in a preceding bulletin, No. 133, and need not be repeated here. In contrasting the urine-contamination theory with the theory of transmission by means of certain biting insects, it was concluded that the evidence very greatly favored the latter theory; that while it would be possible in nature to transmit swamp fever by means of food or drink contaminated with infective urine, this method of transmission was not probable and could not consistently account for all cases of the disease.
In view of the negative results obtained, saliva and sweat probably do not play any role in nature in the transmission of swamp fever. The same is true of feces; even if the virus should later be found in feces, we would expect transmission of the disease in and about stables more frequently than in pasture, which is not the case. Infective milk also affords no satisfactory explanation of transmission; for the Japanese did not succeed in transmitting the disease by feeding milk, the only natural method by which this secretion is transferred from one animal to another.

There remains, then, a discussion of the possible or probable roles that the eye and nasal secretions play in the natural transmission of the disease under consideration. There appears to be no doubt that the eye and nasal secretions both contain the virus. Indeed, it is possible that our own experiments simply supplement those of Lührs. For if the virus is present in the eye secretions, it is probably conducted into the nasal passages through the nasolachrymal ducts. Or, the virus may be excreted in both nasal and eye secretions. In either case the excretion may reach the outside by two paths. If the eye secretion is abundant, it tends to collect and overflow at the inner angle of the eye. Here it may be readily fed upon by houseflies which may transfer it to the eyes of well horses. If the nasal secretion is abundant, it tends to run down and out of the nostril. In certain cases of infectious anemia, with high fever present, a bloody nasal secretion has sometimes been so abundant that it collected in drops on the edge of the nostril, and flies have been observed feeding on this secretion. Under these circumstances it would be easy for the virus to contaminate the food or drink of other horses. However, it has been shown in a previous bulletin that infection by way of the alimentary canal is always a difficult matter; considered in the light of experiments, described in Bulletin No. 133, this may be possible, but hardly a probable method of natural transmission. Under the circumstances mentioned, it would also be an easy matter for the housefly to transfer the virus from an infected horse to the corner of the eye or to the edge of the nostril of a well horse. Here, too, we have a possible method of transmission, but whether it is effective or not must be left to further ex-
perimentation. As a means of natural transmission, it probably plays at best a minor role, since the disease can be effectively transmitted by means of biting flies. Whether a horse may become infected by inhaling the organism that produces swamp fever is also a matter of speculation.

Note:—For references and general acknowledgments, see Bulletin No. 133. There should be stated here an additional word acknowledging the very faithful service of Mr. E. C. O'Roke, formerly Assistant in Parasitology, and of Mr. Louis Revell, student assistant, in helping to carry out some of the details of this work.

EXPLANATION OF PLATES

Degrees in temperature, Fahrenheit, are shown at the left margin of each plate. Dates are given below each section of the temperature record; the day of the month is given on every tenth line, except that the name of the month is substituted for the second place in each month; the year is given at convenient intervals. The temperature record is read from left to right, and, when necessary, is continued from page to page. Unless otherwise stated, all temperatures were taken between four and six p. m.

Cover Plate. Horse No. 72, a chronic field case. Contracted the disease during the summer of 1919. Apparently recovered during the third year of the disease and was in good flesh. Used as a source of virus in several experiments. This photo shows condition in July, 1923, a few days before death. Note the extreme emaciation and the edematous condition of lower lip, belly, sheath and breast.

Plate I. Horse No. 61. This horse was inoculated subcutaneously, on the dates indicated by arrows, with normal saline that had been previously used to wash out the upper nasal passages of a long-standing chronic case, horse No. 31. No. 61 was a locoed animal, and the failure to produce any very sharp fever reactions was probably due to his low vitality.
Horse No. 63. Arrows on August 4, 5 and 13 indicate dates when he was inoculated with tabanid extract. These experiments were made to show that tabanids, such as we used in our insect transmission experiments, were not previously infected with the virus of swamp fever. On September 30th (I) No. 63 was inoculated with 22 cc. of blood from horse No. 55, a mild chronic case.

Plate II. Horse No. 62. This horse had the upper portions of his nasal cavities douched with normal saline that had previously been used to wash out the nasal cavities of horse No. 31. The arrows indicate dates on which the treatment was applied. Symptoms of swamp fever developed. See text.

Plate III. Horse No. 65. This horse was used to verify the diagnosis of swamp fever in horse No. 62. The arrows indicate dates on which he was inoculated subcutaneously with blood from No. 62.

Plate IV. Part of the later temperature record of horse No. 54. Part of the earlier record indicates that he was already infected. (See text for description of experiment with the colt of No. 39.) No. 54 was a hardy animal, very resistant to or tolerant of the virus. However, inoculation with blood from No. 65 on January 17, 1920, produced an important series of reactions.

Plate V. Horse No. 67. On the dates indicated by arrows this horse was inoculated subcutaneously with normal saline that had previously been used to wash out the nasal cavities of horse No. 31 and then filtered through filter paper. This proved to be a decisive case. See text for further explanation.

Plate VI. Horse No. 71, pages 1 and 2. On page 1, arrows indicate the dates on which this horse was inoculated subcutaneously with normal saline that had previously been used to wash out nasal cavities of infected horses and then passed through a Berkefeld earthen filter. On page 2 the arrow indicates the date on which he was inoculated with blood from No. 31, a known virulent source.

Plate VII. Horse No. 69, pages 1 and 2. On page 1 the arrows indicate dates on which this horse was given nasal douches
with normal saline which had been previously used to wash out the nasal passages of certain infected horses, Nos. 31, 67 and 72. On page 2 the arrow indicates the date on which he was inoculated subcutaneously with 18 cc. of blood from horse No. 72, a severe chronic field case shown on the cover of this bulletin.

*Plate VIII.* Horse No. 55, pages 1 and 2. This represents the temperature record of one of two cases of apparent spontaneous origin which developed in our experimental lots. Inquiry developed the fact that this horse, as well as No. 58, came originally from a ranch on which a case of swamp fever was present three years before, in 1915. See text.

*Plate IX.* Horse No. 58, pages 1, 2 and 3. The temperature record of the second case of apparent spontaneous origin. See text and explanation of Plate VIII.

*Plate X.* A temperature record of a colt foaled by a mare, horse No. 39, that became infected during pregnancy. This colt was probably infected *in uteri*, and was therefore probably a carrier of the virus. Limited funds did not permit the experiments which were necessary to give definite proof of the latter conclusion.

*Plate XI.* Horse No. 66. On February 16th and March 8th, 1920, this horse was inoculated with blood from the colt of No. 40, without inducing any fever reaction. On April 2, 1920, he was inoculated with blood of No. 31. A fever reaction occurred after fifteen days. It was concluded that the colt of No. 40 was not a virus carrier.

The temperature records between January 10th and March 7th, 1919, cannot be depended upon as correct, for the assistant employed at that time proved to be unreliable. This applies to Plates IV, VIII, IX and X.
Plate I. Horses Nos. 61 and 63.
Plate II. Horse No. 62.
Plate III, page 1. Horse No. 65.
Plate III, page 2. Horse No. 65.
Plate IV. Horse No. 54.
Plate V. Horse No. 67.
Plate VI, page 1. Horse No. 71.
Plate VI, page 2. Horse No. 71.
Plate VII, page 2. Horse No. 69.
Plate VIII, page 1. Horse No. 55.
Plate VIII, page 2. Horse No. 55.
Plate IX, page 1. Horse No. 58.
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Plate IX, page 2. Horse No. 58.
Plate X, page 1. Horse No. 39's Colt.
Plate X, page 2. Horse No. 39's Colt.
Plate X, page 3. Horse No. 39's Colt.
Plate XI. Horse No. 66.