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INSECT TRANSMISSION OF SWAMP FEVER OR INFECTIOUS ANEMIA OF HORSES

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Insect Transmission of Swamp Fever
or
Infectious Anemia of Horses

JOHN W. SCOTT

INTRODUCTION

In Bulletin 121 of this Experiment Station, the writer ('19) mentioned that our work had conclusively demonstrated that swamp fever may be transmitted by certain biting flies, and that a detailed report of this work would be left to another paper. In this bulletin there will be taken up in order, (I) some features of the disease pertaining to the problem of transmission, (II) a somewhat detailed account of our experiments with insects, (III) a mention of some of the more important theories or suggestions made by other authors in regard to transmission, (IV) a discussion of the probable nature of natural transmission, particularly with reference to insects, and finally (V) some suggestions in regard to control.

I. GENERAL FEATURES OF THE DISEASE

The nature of swamp fever or infectious anemia of horses has been frequently described and is too well known to need more than a brief notice here. It is an obscure, insidious, septicæmic disease, apparently confined in nature to the equidæ. In some cases it does not admit of easy diagnosis even by veterinarians, and this is particularly true of horses that have apparently recovered from the disease. Whether such horses ever become entirely free from the virus is doubtful. We have one case in which the blood has remained virulent more than six years after the initial attack, and Schalk ('20) states that the blood of horse 636 was virulent on the twelfth anniversary of contracting the disease. Outside the work of the Japanese, which will be referred to later, it is generally agreed that the disease is due to a
filterable, probably ultravisible virus which so far has proved refractory to cultural and staining methods. The virus resists freezing, but is killed by heat for one hour at 60° C., and by exposure to direct sunlight; it is present in the blood and tissues apparently at all times during the progress of the disease, and is also found in the urine, nasal (Scott, '20) and eye (Lührs, '19) secretions, where it probably is not present in like amounts at all times. Whether it is commonly present in the feces seems to be doubtful.

Some characteristics of the disease include a more or less regular recurrence of fever which may be almost continuous in certain chronic cases, in many cases a marked decrease in the number of erythrocytes followed by either recovery to the normal number or by a progressive anemia, an increase of leucocytes at a fever period (Scott '22) due chiefly, as shown by Burnett, to an increase of the polymorphonuclears, and at times a progressive and frequently rapid emaciation. There are also certain other symptoms and pathological conditions frequently associated with the disease, which have been shown by many workers to be more or less inconstant and not to be depended upon for specific diagnosis.

Not less interesting for the study of transmission is the general relation of the disease to its environment. Various writers, in this country in particular, have noted that swamp fever is chiefly a disease of low swampy pastures and is more prevalent in wet seasons. For example, it has been especially noted in the wet coastal plain of Texas, in the low-lands of Mississippi, along the Platte River in Nebraska, Wyoming and Colorado, along the Red River in North Dakota, and in the valleys of the Big Horn, Laramie and Little Laramie Rivers in Wyoming. Other instances could be mentioned and the same general conditions appear to be true in Canada. Brimhall, Wesbrook and Bracken (’03) also noted the presence of swamp fever in a rolling country, which, in many places, was well wooded. Brickman in Europe (’07) mentions that infectious anemia is most frequent in wet regions. Ries (’08) states that it is found especially in wooded regions. The Seyderhelms recognize it as a pasture disease. The Report of the Japanese Commission states that no outbreak of the disease
occurred in districts where horses were kept in the stables and that the spread of the disease has been confined to pasturing districts. Other references could be mentioned.

The disease also has a marked seasonal distribution, a characteristic which has been mentioned by many observers. The great majority of field cases are first noticed in the summer or fall, and these horses usually have the more noticeable acute, or sub-acute form of the disease. The cases observed in the winter or spring appear to be all of a chronic nature and our experience indicates that these horses contracted the disease during the preceding summer or autumn. I have observed the origin of chronic cases in the summer and fall that ordinarily would never have been noticed until the following spring or later.

While infectious anemia appears to be less prevalent than formerly both in Wyoming and elsewhere, it still persists and its wide geographical distribution will make it important until more is known of its nature and suitable measures are taken to eliminate the disease entirely. It has been definitely reported from at least twenty states, ranging from North Dakota to Texas, and from Nevada and Washington to New York. It is reported from Mexico and from several Canadian provinces including the Yukon territories. Its presence in several countries of Europe, South Africa and Japan is also well known. Ordinarily the disease spreads slowly and tends to remain confined to individual farms or localities. Under certain conditions, however, it may assume great economic importance. For example, in Mississippi where swamp fever has long been prevalent in certain localities, it is a common practice to import mules from the north to cultivate the cotton fields. It is reported (Norton, '11) that these animals do not often take the disease the first year, but during the second year develop the disease and die in considerable numbers. This illustrates how the disease spreads more rapidly as the result of introducing healthy animals into infected territory. The experience of Frohner ('19) and Lühr's ('19) illustrates another way in which the disease may spread rapidly. Infectious anemia was present in 1917 in military horses and in certain herds on the eastern front. The movements of cavalry troops soon scattered the disease widely on the western front, and during 1918, large
losses were experienced. This shows the danger of transporting infected horses into new territory where the disease is not present. Any adequate measures of control must take into consideration these facts.

There seems to be a good deal of evidence to indicate that the horses of a given locality grow tolerant to the virus after the disease has been present for some years. Swamp fever has been known for over twenty-five years in the Little Laramie Valley. In recent years, the losses have not been nearly so great as formerly, though known cases have recurred almost annually. See front cover for cut of a chronic field case. On one ranch, with which the writer is personally acquainted, at least seven cases have developed within the past three years, but only one death has occurred and that one on the range during the rather severe winter of 1921-1922. From our experimental work it is known that various grades of the disease exist from those severe acute cases which quickly result fatally to the very light chronic cases in which the only evidence of the disease is a few mild attacks of fever. There seems to be no doubt that similar mild cases result from natural infection, and that there are some horses tolerant toward the virus or at least not readily susceptible to the disease. In general, from what one can learn of the history of the disease in this country, an outbreak of the disease is usually traceable to the introduction of infected horses into a new region where the conditions are favorable for transmission and for exposure to a large number of healthy horses. The disease wanes as the more susceptible horses die off and chronic cases become more common. In time these tend to disappear unless new opportunities, favorable for spreading the disease, arise.

Owing to its slow-going nature, it would be comparatively an easy matter to get rid of the disease if we knew a readily applied, adequate means of diagnosing the mild field case or "carrier." So far no satisfactory method of diagnosing such cases has been developed. The easiest and most accurate method of detecting the healthy "carrier" is by inoculation of blood, but this method is impracticable on account of the expense involved. The method of weakening a horse by drawing 5 to 8 liters of blood, as described by Lührs ('21) and Otto ('21) must await further
experiment to prove its value; according to these authors, if a horse after this treatment gives a fever reaction within 20 days that cannot be otherwise explained, it is to be suspected as having the disease. Under these conditions, it has seemed at the Experiment Station that the next best means of controlling the disease would result from a study of the means by which it can be transmitted. The fact that complete recovery rarely if ever occurs, that the lighter forms of the disease are frequently not so recognized, that the apparently recovered cases serve as virus carriers, and that the disease has a very wide known geographical distribution, all contribute to the serious character of the disease. If we add to this the fact that no efficacious remedy has been found, it becomes all the more important to find a means of preventing or controlling the disease.

II. EXPERIMENTS WITH INSECT TRANSMISSION

Before taking up a description of these experiments, it may be well to recall some of the references in the literature concerning the transmission of swamp fever previous to the year 1913. Carré and Vallée ('06, '07) were unable to establish any relationship between blood sucking animals and the distribution of the disease and their infective experiments ('08) with sucking and biting skin parasites were negative. Charlton ('07) suggested that the disease is probably carried by biting insects, and Ries ('08) held that the urine-contamination theory did not explain all cases. Mohler ('08) thought that the virus was probably spread by an intermediate host, such as flies, mosquitoes or internal parasites. Van Es ('10) recommends that horses with swamp fever be protected from biting insects on the suspicion that they may be agents in the transmission of the disease. The following year he repeated this warning suggestion, and Francis and Marsteller ('11) failed to transmit swamp fever by means of ticks. On the basis of these facts, the outlook for a successful transmission of swamp fever by means of biting insects did not appear particularly promising. Strong evidence, however, had accumulated to indicate that the urine contamination theory could not furnish a satisfactory explanation for all cases of swamp fever,
and it was decided in 1913 to study the possibilities of insect transmission. The remarkable work of the Japanese Commission, begun in 1909, was not published in English until December, 1914, and was not available to the writer until late in 1915; for this reason it will be discussed later.

Figure 1. A diagram to illustrate the arrangement of the screen wire cages, one and three. The stalls are separated by board partitions, and this portion of the cage is covered with a roof; the entry and the area in front of the mangers is covered by screen wire. Solid lines indicate board partitions or structures, and the broken line surrounding the whole represents screen wire. This screen wire has sixteen meshes to the inch.
In order to test the insect hypothesis, it was decided to construct a screened cage, 22 feet long by 20 feet wide. The general plan of this cage is shown in Figure 1. The entrance is ten feet long and is provided with a door at each end; this permits a horse or person to enter or leave the cage without allowing the escape or admission of insects. The screen wire used had 16 meshes to the inch; this provided a more nearly insect-proof cage than is constructed of ordinary house screening which usually has only 10 or 12 meshes per inch. In the course of the work, a second smaller cage was enclosed with ordinary wire screen, and a third large cage was built entirely similar to the first. In the description of the experiments these enclosures will be referred to as cages one, two and three respectively. Cages one and three have five stalls each, and cage two was large enough to confine three horses.

EARLY EXPERIMENTS

The experiments in the summer of 1914 were more or less general and preliminary in character. Though they have in part been previously reported, it is best to repeat here the main facts concerning them in order to show their connection with what follows.

Experiment 1. June 25 to July 30, 1914. Cage one used. The original purpose of this experiment was to test whether any of the various kinds of mosquitoes found in Wyoming could transmit the disease. Aquaria for raising mosquitoes were constructed in cage one and, at first, larvae and pupae were collected and put in the aquaria. Since the aquaria were not completed until June 23rd, and no pupae taken after July 2nd, it was necessary to collect adult mosquitoes for the rest of the experiment. It was found impossible to collect large numbers of the mosquitoes without including a few of the numerous wild flies found in the swampy places where the mosquitoes were abundant. Most of the wild flies died quickly in confinement. The house-flies and stable-flies, however, survived and bred in the cage, though they did not become abundant until near the middle of August. The mosquitoes were very abundant at first, and were more abundant than the flies until August 1st; after this date, they disappeared.
rapidly. Some larvæ and many pupæ were collected on June 25th and put in the aquaria. Many pupæ were collected June 29, 30, and July 1 and 2. Many pupæ had emerged by June 29th. On June 30th, horse No. 2, a long-standing chronic case, was put in the cage; within a short time many mosquitoes were noted filled with blood. On the following day, horse No. 12, another chronic case, was confined in the cage. Mosquitoes were collected and turned lose in the cage on July 3, 6, 7, 8, and 10. On July 4th horses 2 and 12 were taken out, and three horses without the disease, Nos. 21, 22 and 23 were put in. On July 7, these three horses were taken out and No. 2 put in again. On July 9th, No. 2 was taken out, and a new horse No. 24 put in, where he remained until the thirtieth of the month. So far as one could tell by observation, mosquitoes alone were involved in the experiment. Since horses 21, 22, and 23 gave no indication of taking the disease after 27 days, they were used in the next experiment on August 3rd. No. 24 had showed no signs of taking the disease by August 26th when he was used in experiment three.

In discussing the results of this experiment, it should be stated that none of these horses were immune to the disease as shown by subsequent results. On the face of the experiment, it appeared that mosquitoes either could not transmit the disease, or else the conditions of the experiment were not suitable for transmission. If swamp fever can be transmitted by mosquitoes, it must be done either mechanically or after an incubation period. The conditions for mechanical transmission were not favorable except from July 27th to 30th when No. 2 and No. 24 were both in the cage. Later work with biting flies shows that this may explain the negative result. With horses 21, 22 and 23, the possible incubation period was from 1 to 7 days; while in the case of horse 24, it was possible for an incubation period to vary from 1 to 30 days. While the experiment was not conclusive it would seem to indicate that mosquitoes are not ordinarily capable of transmitting swamp fever, or else only in a mechanical way. Another experiment with mosquitoes was tried the following year.

Experiment 2. July 30 to October 9. Cage one used. Since the first experiment had resulted negatively, a still more ex-
tensive test was planned involving both mosquitoes and flies. Cage one was again used. The conditions in this cage on July 30 were as follows: There were present some mosquitoes and a comparatively small number, perhaps a few dozen, of house-flies and stable-flies, combined. It will be remembered that horse 24 was taken out of the cage on this date thus closing up the first experiment. Mosquitoes and flies were captured and introduced into the cage on July 30, 31, August 1, 6 and 13. On August 15, my notes record that there were many, estimated at several thousand, house-flies and stable-flies present; that the number of these had increased rapidly since August 4, but that there were very few mosquitoes left, and practically no other flies of any kind. The house-flies and stable-flies continued to increase for about two weeks longer; they began to die, however, early in September as the nights grew cool, and disappeared very rapidly after September 12th, when considerable ice was found outside the cage. The experiment closed October 9th.

Infected horses were exposed in the cage as follows: Horse No. 2 had been put in the cage July 27 and remained there until he died of swamp fever on August 3; several times during this period his temperature was above normal. On July 30, horse No. 26 showed fever after an incubation period of 13 days and was put in the cage; he was taken out August 3rd, but was put in again August 20th where he remained until September 11th when he died, possibly by accident since he was found down in the manger with neck in a cramped position. See Plate I for his temperature during this period. This horse had been inoculated subcutaneously on July 17th with 15 c.c. of blood serum furnished through the courtesy of Dr. Van Es from his case No. 1276. On August 2nd, horse No. 20 was injected subcutaneously with 21 cc. of blood serum drawn the day previous from the jugular of No. 2. After an incubation period of only four days, he took the fever and was placed in the cage August 6 where he remained until August 11; he was again placed in the cage August 13 and remained there until September 12th. Plate II shows the temperature record of this horse from June 9 to December 14, 1914. In this experiment, therefore, infected horses
were in the cage from July 27 to September 12, except on August 4, 5, and 12.

Horses 21, 22 and 23 were again used since they had given no indication of taking the disease for 27 days after they had been last exposed to the bites of mosquitoes in the previous experiment. These horses were put in the cage August 3rd and taken out August 6th; they were put in again August 7th; No. 22 was taken out September 11 after a second attack of fever and the other two October 9th. Horse 23 was a big, vigorous, healthy animal and at no time up to May 8th, 1915, showed any signs of taking the disease; he was then used in another experiment. Both the other horses gave indications of being infected. On August 22nd, No. 21 without any other apparent cause showed a temperature of 103° F. On August 28th, No. 22 had a fever with a temperature of 102.8°; eleven days later there began another fever reaction in this horse that lasted for three days, with a temperature rising to 103.3°. On October 3rd, a third reaction began in horse 22, which resulted in his death two days later; the day before his death his temperature registered 103.4°. Horse 23 did not at any time show loss of flesh; on September 1st, it was noticed that No. 21 had lost some flesh, while in No. 22 emaciation was very marked, and rapid loss of flesh continued until his death. Apparently two out of three of the well horses used in experiment 2 had developed swamp fever. These two cases will be considered in detail.

It was believed that horse No. 22 had died of swamp fever. See Bulletin 121, Plate I for his temperature record. In order to confirm or disprove this opinion, the station veterinarian was asked to make a post-mortem examination; he stated in his report that none of the conditions found could account for the death of this horse on any other theory than that of swamp fever. In order to further verify this conclusion, some blood was drawn on October 5th from the jugular vein of horse 22 after death. On October 10th No. 29, a very hardy pony, was injected subcutaneously with 35 c. c. of blood serum that had been drawn on October 5th. The serum had an offensive odor, and was not filtered before injection; that it contained septic bacteria was shown by an immediate rise in temperature, which is not true of swamp
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fever virus. However, No. 29 soon regained a normal condition, and outside of a few slightly abnormal irregularities in the temperature curve, notably in December, January and February, this horse did not appear to take the disease. Since the result was doubtful, it was decided to use another horse in this test. An old horse, No. 30, used several years as a light dray horse, was procured, and on November 21 was injected subcutaneously with 35 cc. of blood serum that had been drawn from the jugular of No. 22 on the date mentioned. Here again was an immediate initial rise in temperature which reached 103° on the afternoon of the same day. Returning to normal for two days, on the third day after the injection, a fever began which lasted two days; eleven days later fever was noted again, though the temperature did not register above 102°. Further attacks of fever were recorded in 1915 on January 2-4, 14, 23, February 8-11, 15, 17-18, 21, 28, March 4-5, 8-10, 30, and April 8th and 25th. By this time, No. 30 had become extremely emaciated and weak. After May 2nd, his temperature remained sub-normal until death. He got down on the morning of May 5th and was unable to rise. His temperature on the afternoon of May 7th registered 96°; he was then killed and blood drawn from his jugular. See Plate III for temperature record. His spleen was found enlarged and flecked with numerous petechial spots; the heart was large and contained stringy ante-mortem clots; the liver had a soft, mushy consistency; the lymph glands were somewhat enlarged and had an abnormal appearance. Two or three dozen sclerostomes were found in the coelome or in its lining tissues, and large numbers were found in the intestine, while 10 or 12 bot-fly larvae were present in the stomach. Under all the conditions mentioned, it is quite probable that parasitic complications were involved. While No. 30 was not obtained until a few days before he was used in the experiment, the previous history given by the owner indicated that he was free from swamp fever when he was brought to the station. However, it is certain that he had the disease as shown by the following experiment. On May 8th, No. 29 was again inoculated subcutaneously with 30 cc. of the blood drawn the day before from No. 30. At the same time, No. 23, which we have stated was a vigorous healthy animal, was inoculated in a similar
manner with 24 cc. of the same blood. Neither of these horses showed any rise in temperature due to bacterial sepsis and No. 29 gave no evidence of taking the disease within a period of 51 days. On the ninth day after inoculation, No. 23 showed fever which lasted for eight days. This proved to be an acute case; he died of swamp fever on the 47th day after inoculation, and some of his blood was saved. Four days later, June 29th, 20 cc. of this defibrinated blood was injected subcutaneously into No. 29. From this third inoculation, he developed a typical case, and after being used in other experiments was finally killed in an advanced stage of the disease on April 30th, 1917. We had thus established by proof that horse 22 had died of swamp fever.

It was stated above that on August 22nd (1914) No. 21, while in cage one developed a fever that rose to 103°. By September 1, he had lost some flesh but except for slight temperature irregularities in October, there was no further significant indication of fever until August 2nd, 1915; however, his temperature was not recorded from November 14, 1914, to March 1, 1915. Between August 2 and October 25, 1915, he had seven attacks of the disease during each of which his temperature rose above 103°. In this period he became much emaciated, and though his temperature then remained normal until March 25, 1916, he regained only part of his former weight during the winter. During the remainder of 1916, his temperature curve showed the “choppy” condition characteristic of the later stages of chronic cases, and only twice, April 3rd and August 14th, did it rise above 103°. He again lost some flesh during the summer and fall and during the following winter failed to get back all that he had lost. In 1917, he had no fever and his condition to June remained about stationary or slightly improved. He was killed at this time.

Summary and Conclusions from Experiment 2.

(1) The flies and mosquitoes in the cage had three sources of infection: horse No. 2, July 27 to August 3; horse No. 26, July 30 to August 3, and again August 20 to September 11; and horse No. 20 August 6 to August 11 and again August 13 to September 12.

(2) During this time three well horses were exposed in the cage, Nos. 21, 22 and 23 from August 3 to August 6; No. 22
from August 7 to September 11; and Nos. 21 and 23 from August 7 to October 9.

(3) On August 28, No. 22 developed an acute case of swamp fever which resulted in his death October 5. No. 21 showed fever August 21 and after a long latent period developed a long standing chronic case.

(4) Under the conditions of the experiment, it appeared that certain insects, stable-flies or mosquitoes, were capable of transmitting swamp fever. Transmission by contact or by contamination was not entirely excluded.

(5) Certain facts tended to implicate the stable-fly, *Stomoxys calcitrans*, rather than the mosquitoes, as the carrier of the disease. Since the mosquitoes were abundant and had fed freely during the first experiment, which had resulted negatively; and since they were getting scarce and were comparatively inactive during the second experiment, it was thought they had nothing to do with transmission. On the other hand, soon after the first of August, the stable-flies became abundant and were very active in biting both sick and well horses. The house-flies must be eliminated, since they do not bite, and the disease is rarely, if ever, transmitted by contact. It was concluded that the stable-fly was probably responsible for the transmission and later results have confirmed this conclusion.

**FURTHER EXPERIMENTS WITH STOMOXYS CALCITRANS**

*Experiment 3.* August 26th to October 9th, 1914. Cage two used. While experiment 2 was still in progress, it was decided to try a third experiment in which stable-flies alone were used. For this purpose, a second small screened cage was finished August 26th. Immediately, some flies were transferred from cage one. As horse No. 24 had given not the slightest indication of taking the disease since the close of the first experiment July 30, he was again used. On the same day, August 26th, a new healthy horse, No. 27, was also put in cage two. Two days later a less vigorous, but apparently healthy old mare, No. 28, was put in with the other two. Stable-flies were transferred from cage one to cage two on August 29th, and on September 4, 5, 11 and 12. It was estimated that 5000-7000 flies were so transferred. All three
horses were taken out of the cage October 9th, after all flies were dead.

The results of this experiment were doubtful and at first were thought to be entirely negative. Horse 24 showed no trace of taking the disease and on November 21, he was inoculated subcutaneously with 24 cc. of blood serum drawn on August 2nd from horse No. 2. However, he proved very resistant to the virus and showed no indication of taking swamp fever even after this treatment. Possibly the virus had become inert. Horse 27, with an average normal temperature of 99.5° F., showed light fever October 11-14, 16-17, 24-26, 28, 30-31 and on November 11; the temperature reached 101.8° on the 17th, 102.0° on the 24th and 25th but only 101.0° on November 11. A study of his temperature record suggests the possibility of infection. See Plate IV, page 1. No particular significance was attached to these irregularities and as he gave no further indications of the disease in the course of several months, it was believed he had not been infected. No. 28, with a normal temperature of 99.7°, also showed light fever October 13-19, 25-29, 31, November 2-4 and 11, but the temperature registered never exceeded 101.6° and this too, at the time, was considered a negative case. From the results given we see that none of the horses in cage two gave any distinct evidence of taking swamp fever and this experiment closed up the first season's work. The subsequent history of these cases will be given later.

The general results described so far were summed up in a preliminary paper in the following words: "In view of the facts presented, we reach the conclusion that swamp fever can be, and under natural conditions probably is, transmitted by biting insects. If this proves to be true, it is very probable that the agent of transmission is the widely-distributed and well-known stable fly, Stomoxys calcitrans."

Experiment 5. July 19 to September 30, 1915. Cages one and three used. A third screen cage, similar to cage one, was built early in the spring of 1915. It was first used for a second mosquito experiment, which resulted negatively; this will be described later.
Cage one had not been used for several months, since the close of experiment two. At the beginning, cage three had a few mosquitoes, which soon died, and as a matter of fact had disappeared before this experiment had really begun. The object of experiment 5 was to make a more careful test of whether or not *Stomoxys calcitrans* was capable of acting as an agent in transmitting swamp fever. The plan involved keeping cage one stocked with stable-flies and keeping cage three free from flies. Both cages contained well horses and diseased horses. By a system of rotation, both cages were exposed alike to the same diseased horses, so that the results obtained cannot be accounted for on the theory of unequal contamination. Two out of three horses became infected in cage one where the stable-flies were present in large numbers and there was no definite evidence to indicate that either of the two well horses in cage three became infected. A more detailed description will be interesting. *Stomoxys* were caught and put in cage one on July 14, 17, 19, 24, 26, 29; August 5, 6, 7, 9, 11, 12, 13, 14, 18, 19, 21, 23, 25, 26, 27, 30; and September 1, 2, 3. They were scarce until August 7; they were then abundant until September 1, after which they rapidly disappeared. Our records show that the total number introduced from August 9 to September 3 inclusive, was estimated at more than 4000 flies. Some also bred in the cage. By mishap due to carelessness of the man employed in feeding the horses, a few wild flies got into cage three during the summer, but they were always killed daily as soon as discovered. However, some laid eggs, for late in the summer, flies hatched out rapidly. From August 27 to the end of the experiment, nearly 3000 house flies were destroyed. On August 27, among 200 house flies killed, there was discovered for the first time eight stable-flies, three of which had taken blood. During the following two weeks, a total of seventy-five *Stomoxys* were captured, a majority of which were killed before they had fed upon blood. No infection was observed in this cage.

Diseased horses were put in as follows: On July 19, horse No. 29 was placed in cage one and horse No. 20 in cage three. On July 26, horse No. 21, which had recently developed high fever, was also put in cage three. On August 2, there was begun the
series of daily changes, and rotation through the cages, that resulted in the equal exposure of the diseased horses in the two enclosures. These changes were continued until the close of the experiment September 30th. The purpose of this procedure was to treat the two cages alike except in reference to the presence of Stomoxys in cage one.

Healthy, well horses were put in these cages as follows: Into cage one, horse 32 was placed on July 26, horse 33 on July 28, and horse 35 on August 16. Into cage three, horse 28 was placed July 26. We have seen that this horse was used in experiment 3, and that we concluded there had been no infection. On August 5, a new horse, No. 34, was also put in cage three. A third healthy horse for this cage could not be obtained. All these horses remained in their respective cages until September 30, except No. 28, which was turned out for one day, August 19, when it had the colic.

That the diseased horses had active cases of swamp fever is shown by their temperature records. No. 20 had attacks of fever August 3-6 and 27-29, inclusive. No. 21 had fever August 2-6, 9-13, 16, 23-28, 30; September 9-12 and 27-30. No. 29 had fever July 26, 29-30; August 1-5, 9, 14, 16-18, 27, 28, 31; September 9-11, 15, 18-20, 24, and 30.

Horse No. 32, on August 31st, suddenly showed an attack of fever lasting one day in which the temperature rose to 103.8°. His average normal temperature had been 99.9°. He showed mild attacks September 13, 20, and October 2, 9, 13 and 26-27; he gave further evidence of the disease in loss of flesh. His temperature then continued "choppy" but about normal for several months; he never regained all the flesh he had lost, and the following spring and summer he became extremely emaciated. See Figure 2. A further series of fever reactions began on July 28. Early in August, the number of red corpuscles began to diminish rapidly and he died September 9th, 1916. Dr. Lehnert, the Station Veterinarian, pronounced his death was directly due to gangrenous pneumonia, and indirectly to swamp fever. This horse became extremely emaciated, and his blood count fell to a little over four million before he died. See Plate V.
Horse 33, a fine, big gelding in good flesh, was put in cage one July 28, 1915. His average normal temperature was 100.2° F. On the 9th and 10th of August he gave the first evidence of taking swamp fever when his temperature rose as high as 102.6°. His temperature was also slightly above normal on the 14, 16 and 26, and on the 27 it rose to 102.4°. On August 30 began an attack of fever which lasted through September 1st, the temperature rising to 104.7°. There was also slight fever September 4, 6, 9, 15, 17-21, 24 and 30. Mild fever also occurred October 1, 6, 7, 14, 19, 20, 23, 25, November 5, and December 22nd. His temperature then remained about normal until June 19, 1916, when he had a slight attack. Three days later the fever recurred and lasted for three days. On August 14th, there began a six-day period in which the temperature rose to 104.0°. On September 18th, his temperature was slightly above normal. On October 3rd, there began a five-day period of fever, the temperature rising at one time to 106.0°. On the 22nd of the same month, occurred slight fever, and on the 28th began another severe fever reaction which showed a temperature of 105.6° on the 31st. He died...
during the following night. On examination, the veterinarian
pronounced a hemorrhage from the spleen as probably being the
immediate cause of death. This organ was two or three times
normal size, and was gorged with blood. The peritoneum showed
what were apparently ante-mortem fibrous clots, and the greater
omentum and stomach in the region of the spleen showed bloody
discolorations due to the hemorrhage. His blood count had fallen
to less than five million on October 14th. See Plate VI.

Horse No. 35, during the experiment, and for more than six
months afterward, gave no sign of taking the disease. Subsequent
inoculations showed him to be very resistant, though not entirely
immune, to the virus.

In cage three, horse 34 had given no indication of taking the
disease up to May 13, 1916. While he was in poor flesh when we
bought him, this was due to a sort of eczema. His temperature
was always fairly steady, and seemingly normal in every way
except slight fluctuations due to poor flesh. At the same time, he
was not immune to the disease. Several months later, May 13,
1916, he was inoculated subcutaneously with 12 cc. of blood
serum that was obtained from a chronic field case in the last
stages of the disease. Eleven days later, from a normal average
temperature of 99.4° F. he showed fever with a record of 102.0°.
Several attacks of fever followed in which the temperature varied
from 102.0° to 104.2° and he died on July 12, 1916. During this
time, his pulse rose from 48 per minute until it averaged between
60 and 70 per minute, and respirations from 14 to 24-30 per min-
ute. His blood count had dropped from nine million to about
four million on the day he died.

There was no indication that horse 28 took the disease in this
experiment. She was in better flesh than the previous season and
her normal temperature averaged higher (102.2°). She had mild
fever, May 8 (101.6°), June 3 (101.3°), August 20 (101.8°),
September 9 (101.2°), October 13, (101.3°), October 21
(101.4°), April 8, 1916, (101.6°) but she was subject to attacks
of indigestion and it was thought this accounted for the fever
noted. It is fairly certain that she did not become infected with
the disease in experiment 5. Inoculations of blood from this
horse should have been made after experiment 3. It seems pos-
sible from a study of the temperature chart that she may have developed a mild case of swamp fever at that time. On June 30, 1916, she was inoculated subcutaneously with about 15 cc. of blood serum from horse 32, and six days later showed a temperature of 103.7°. Except for slight attacks on July 10, 11, and 13, she showed no more fever until August 3rd and 4th, and she was found dead on the 5th. Death was apparently due to impaction of the small intestine a little below the stomach. Whether this was the sole cause of death is not known. She was not apparently anemic.

Considering all the data in this experiment, there seemed to be good reason for the conclusion that horses 32 and 33 had become infected with swamp fever by means of the bites of Stomoxys calcitrans. No. 35 did not become infected after such exposure and later inoculations showed that it was hard to infect him even with a mild form of the disease. It seems clear that 34 did not become infected in spite of the fact that a few flies were found in cage three. Since these flies were killed as soon as found, there was no chance for the virus to undergo an incubation period and of the total number found (40) which had taken some blood, it was probable on the law of chance that there had been little, if any, interrupted feeding from sick to well horses.

Experiment 6. September 11, to September 18, 1915. The manner of transmission was not determined in experiment 5. Accordingly, it was decided to see if infection could be brought about by means of interrupted biting. It was late in the season and the only two horses available were No. 27 and No. 31. Both of these horses had been used in experiment 4, from June 27 to July 26, 1915, but since they were in good flesh, their temperatures very regular and uniform, and they had since that experiment given no evidence of being infected with swamp fever, it was decided to use them. It will be remembered that No. 27 was used in experiment 3, Sept. 1914, and that it was considered doubtful whether he had become infected or not at that time. His blood should have been tested by inoculations.

In experiment 6, stable-flies were confined in "hazel" jars covered with mosquito netting. The flies in each jar were exposed to the back of a diseased horse for a time, until they had
begun feeding usually one-half to three minutes and then were transferred to the back of No. 27 and to No. 31. This procedure was repeated with the same jar as long as the flies would bite. To facilitate biting, the hair was cut short on a spot on the back of each horse. In all 15 jars were used.

On September 12, flies bit best in jars 5, 4, and 7 in the order named. On the 13th, all experiments worked well, and it was found that a few flies in a bottle gave better results than if many were present. On the 14th, flies in jars 8 and 9 fed well, and spots of blood formed where flies bit No. 31. After this date, there was not much success in getting the flies to bite. An objection to the method of conducting the work in this experiment lies in the fact that individual flies were not observed to bite both diseased and well horses. However, the number of bites secured frequently exceeded the number of flies in the jar, and so some of them must have bitten more than once. It was found later that flies ready to bite will usually bite several times in succession, and considering all the facts, there is no doubt we had instances of interrupted feeding between sick and well horses.

Horse 27 gave no clear indications of taking swamp fever, though his temperature curve shows an increasingly "choppy" character from the 8th of October up to the time when decided fever reaction occurred in July and August, 1916. Whether he became infected in this experiment or in experiment 3 is not certain. In either case, stable-flies were concerned. See his temperature record, Plate IV. At the time it was believed he had not become infected.

Horse 31 gave little evidence immediately that he was contracting the disease. His temperature had been running very uniform since the 19th of May and averaged 100.3°, but on October 6th, it rose to 101.5°. It soon settled back to normal (See Bulletin 121, Plate IV and Fig. 1), but in January 1916, the temperature curve began to assume the "choppy" appearance usually characteristic of a chronic case, and on July 12, the first violent fever reaction set in, resulting in a temperature on the 14th of 106.2°. His chart shows that not less than six fever reactions occurred between this date and the last of October, 1916. Between this date and June, 1918, there were only two days in which the tem-
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Temperature was above 102.0°, April 28 and August 2, 1917; at times it was somewhat variable but usually approximately normal, though not so steady as before he took the disease. During the fall of 1916, his blood count fell at times below five million, and he lost considerable flesh. Later he regained the flesh lost, and has repeatedly given a blood count of eight or nine million. However, his blood was highly virulent as late as August, 1921. He is now in fair flesh (Fig. 3) but is lame and occasionally shows oedema. See Plate VII.

Figure 3. Horse No. 31, June 28, 1922. Infected by Stomoxys. Is decidedly stiff and lame in hind quarters. Contracted the disease in 1915. See Plate VII. Also compare Bulletin 121 page 95 and Plate IV. Occasionally shows oedema of sheath, belly and limbs. His blood is still virulent.

It will be seen that this experiment added further proof to the theory that the stable-fly is capable of transmitting swamp fever. It also appeared to indicate that a purely mechanical transmission of a very small amount of infected blood is sufficient to produce the disease, and that an incubation period within the body of the intermediate host is not necessary. However, an incuba-
tion period was not entirely precluded, for some of the same flies were used three days in succession. Again, it seemed that the incubation period in the horse, following infection with minute quantities of the virus, may be several months before the active appearance of the disease. The following year, 1916, there was found further confirmation of the hypothesis with reference to transmission by biting flies.

Experiment 8. July 15, 1916, to September 30, 1916. Cages one and three used. If our conclusion was correct in experiment 6, with reference to a mechanical transmission by means of Stomoxys, the question arose, what is the smallest amount of blood needed to produce infection? Would a hypodermic needle, contaminated by puncturing the skin of a horse with swamp fever, carry enough of the virus to produce the disease when the needle was immediately used to puncture the skin of a well horse? That is, if one used a hypodermic needle to imitate the interrupted biting method of experiment 6, would it be possible to produce swamp fever. To answer this question, the following plan was adopted.

On July 15, a thrifty, vigorous horse, No. 38, was placed in cage three with other horses without the disease. Several horses, Nos. 21, 27, 29, 31, and 32, with swamp fever, were confined in cage one. Beginning August 1, a medium fine hypodermic needle was used to puncture the skin of a horse in cage one and then carried into cage three where it was immediately stabbed into the skin of horse 38. This was repeated 3 or 4 times each week, usually once a day, using in turn different infected horses as a possible source of the virus. On August 9, No. 38 was stabbed twice, once with needle from horse 31 and once from horse 32. On August 30, he was stabbed twice, once from horse 29 and once from horse 31; on August 31, from horse 21, on September 2, he was again stabbed twice, once from horse 27 and once from horse 32. In all the skin of horse 38 was punctured twenty or more times.

The fever record of this horse is shown in Plate VIII. From June 23rd to September 11, both before and after he was placed in cage three to prevent possibility of infection by biting flies, or by contact, the temperatures show the steadiness of a healthy.
horse. On September 12, the first fever reaction began; a more severe fever reaction followed September 18 to 21, inclusive. Observe that the temperature is more erratic or variable after taking the disease. As he gave evidence of developing into a long-standing chronic case, he was killed November 1, 1917, while still in a vigorous condition. The fact that he had swamp fever was further substantiated by inoculation of some of his blood on April 13, 1917, into a healthy, vigorous horse No. 42, which developed swamp fever thirteen days later. No. 42 had several other comparatively mild attacks of fever, but there were practically no other symptoms of infectious anemia. See Fig. 5, Bulletin 121.

From this experiment, it was demonstrated that mechanical transmission can be brought about by means of extremely small amounts of infective material. The experiment also furnished substantial evidence to indicate that the mouth parts of biting flies could carry mechanically a sufficient amount of the virus to produce the disease.

However, not all horses are readily infected by this method. A similar experiment in 1919 resulted negatively, or at best, was inconclusive. The only horse available No. 59 was an old mare in poor flesh, and not much vitality; she had been worked hard and not well fed, but otherwise pronounced sound by the Station Veterinarian. She was bought on June 16 and immediately put in cage three. On June 30, her blood count showed 8,300,000 erythrocytes and 7,850 leucocytes. Between July 14 and July 24 the skin of 59 was pricked nine times with a fine hypodermic needle after it had been used to puncture the skin of No. 31. No distinctive fever reaction having occurred by August 31, for the following fourteen days her skin was punctured three times daily with a needle that was contaminated each day by pricking in turn the skin of horses 31, 55 and 58, all horses with swamp fever. This was repeated September 17, so that in all this series there were forty-five chances for infection, fifteen from each horse mentioned. There was a mild fever reaction (101.9°F.) on September 9 and another (101.7°F.) on September 17, but the temperature showed great variability from day to day, a characteristic of horses in poor flesh and with little vitality. During the progress of the experiment No. 59 became more and more emaciated,
and finally got down October 1, when she was killed. If this horse became infected with swamp fever there was too little vitality to produce more than a mild reaction. Unfortunately her blood was not tested to prove whether it was virulent or not.

*Experiment 9.* July 15, 1916, to September 27, 1916. Cages one and three used. The same general plan was used in this experiment as in experiment 6. However, to facilitate handling the *Stomoxys* small screen-wire boxes with removable bottoms were used. During this fly season, as mentioned in experiment 8, the horses with swamp fever were kept in cage one. Two well horses were placed in cage three, No. 35 on July 15 and No. 40 on July 19; it will be remembered that No. 35 did not contract the disease in 1915, experiment 6.

Though several trials were made, no successful results were obtained with *Stomoxys* until August 15. On that date one stable-fly after biting No. 21 was transferred to the back of horse No. 40 where it was observed to bite again. Other successful bites on horse 40, following interrupted feeding on diseased horses, were obtained as follows: August 18, one from horse 31; August 19, one from horse 21 and two from horse 31; August 22, one from horse 27 and three from horse 31; August 23, one from horse 21; August 24, two from horse 29. As no reaction had occurred by September 4, the experiment was continued as follows: September 4, ten bites from horse 31 and three bites from horse 32; September 5, four bites from horse 31 and three from horse 32; September 6, six bites from horse 29. September 9, four bites from horse 27 and three from horse 31; September 12, four bites from horse 29 and three from horse 31; September 13, six bites from horse 27; September 15, seven bites from horse 27 and nine from horse 31; September 20, five bites from horse 31; September 21, one bite from horse 31. This made a total of 80 successfully interrupted bites each one of which was thought to be potentially capable of transmitting the virus of swamp fever.

On August 29, horse 35 was bitten by two flies that had previously bitten horse No. 27, also by two flies that had previously bitten horse No. 29. Other successfully interrupted bites were as follows: August 31, one bite from horse No. 27; September
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1, one bite from horse No. 21 and one from horse No. 27; September 2, one bite from horse No. 21; September 4, three bites from horse 29, two from horse 31 and three from horse 32; September 5, seven bites from horse 31 and three from horse 32; September 6, three bites from horse 29; September 9, two bites from horse 21, and two from horse 29; September 11, four bites from horse 21; September 12, three bites from horse 27; September 13, three bites from horse 31; September 14, one bite from horse 29; September 15, seven bites from horse 27; September 20, four bites from horse 27; September 21, four bites from horse 31; September 23, two bites from horse 31. This made a total of 61 bites, which were completed on No. 35 after they were begun on horses with the disease.

As the result of this treatment, horse No. 40 became infected but horse 35 gave no evidence of taking swamp fever. In experiment 6, horse No. 35 had shown itself highly resistant to infection and the result in experiment 9 was therefore not surprising. See Wyoming Experiment Station Bulletin 121, Fig 2; also Plate III for the temperature record of horse No. 40. While there was gradual emaciation and an increasing variability in the temperature from day to day, the mild fever reactions were not sufficient to demonstrate the presence of the disease. Accordingly, on May 29, 1917, twelve cubic centimeters of blood from No. 40 were inoculated subcutaneously in horse No. 44, in which a severe case of swamp fever was developed after an incubation period of 22 days. Since contamination by food and drink, or infection by other means, was considered highly improbable, it was concluded that we had in horse No. 40 a case illustrating the mechanical transmission of the virus of swamp fever by means of the interrupted bites of the stable-fly, Stomoxys calcitrans.

It is barely possible that No. 35 became infected as the result of this treatment. On October 17, his temperature rose to 101.6°, the highest it had been since he was bought on August 4, 1915; after this date the temperature was also slightly more irregular than before. If he did have the disease, it was a very mild case; at the time it was believed that he did not have swamp fever and so no inoculations of his blood were made. If he did not have the disease, he was at least very highly resistant to it, or tolerant
of the virus, as shown by the following tests. On April 7, 1917, he was inoculated subcutaneously with 50 cc. of blood from No. 39, a mild but ultimately fatal case that will be described later. From an average of about 99.7° his temperature rose to 101.8° on April 20 and again to the same height on May 4. This was not then considered important, and on June 19, he was inoculated with 48 cc. of blood from horse No. 21, another comparatively mild case. Fever did not follow after the usual incubation period but on August 3, his temperature rose to 101.8°. Later fever was registered as follows: August 23, 102.2°; September 18, 102.2°; October 12, 102.5° F. It was then decided from this record and certain other symptoms that he had contracted swamp fever.

Experiment II. August 1, 1917, to October 8, 1917. Cages one and three used. Horse No. 44, which had a severe chronic case as the result of inoculation from No. 40, was put in cage one. Three well horses, No. 48 and No. 49, bought August 1, and No. 50, bought September 6, were confined in cage three. Stomoxys,
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confined in screen wire boxes, were allowed to bite No. 44, then their feeding was interrupted and they were allowed to continue feeding on the back of one of the other horses mentioned. From August 25 to 31, No. 48 received fifty-one such bites, but this horse did not stand confinement well and he developed digestive trouble from which he died September 2nd.

Horse No. 49 received the following bites from *Stomoxys* after interrupted biting on horse 44: August 30, fifteen; August 31, three; September 1, two; September 3, ten; September 4, two; September 5, thirty-four; September 6, six; September 7, ten; September 8, two; September 12, five; September 14, twelve; September 17, ten. This made a total of 104 bites. No important symptom of the disease developed and on May 23, 1918, 30 cc. of her blood were inoculated subcutaneously into a new horse, No. 54. Since no reaction was produced horse 54 was again inoculated with 25 cc. of her blood on June 19. Slight rises in temperature occurred on June 30, August 27, September 20, and October 10, but the symptoms were not sufficiently pronounced to make an unqualified diagnosis of swamp fever.

Horse No. 50, from September 8 to 19, inclusive, had a total of 39 *Stomoxys*’ bites following interrupted feeding on horse 44. No evidence of the disease developed in the course of several months and he was used the following summer in another experiment.

In considering case 49, it is well to know that horse 54 was a vigorous, hardy animal, very tolerant of or highly resistant to the virus of swamp fever as shown by the later record of his case. See Experiment 13. Considering all the facts connected with this case, and other experience as well, I am inclined to the opinion that horse 49 had a mild case of swamp fever; the evidence was not sufficient, however, to prove that this was true.

The results obtained with this series of experiments with the stable-fly has led to the conclusion that infectious anemia of horses may be mechanically transmitted from sick to well horses by bites of *Stomoxys calcitrans*. The conditions of transmission require horses with virulent blood in close proximity with susceptible well horses, an interruption of the feeding operations of the flies and the presence of probably large numbers of *Stomoxys*. 
In 1914, the Japanese Commission had attributed the chief role in transmitting infectious anemia to Tabanid flies. In 1916, we began a series of experiments to test whether this held true for the chief Tabanid species of this region, *Tabanus septentrionalis*.

**Experiment 10.** July 18, 1916, to September 30, 1916. Cages one and three used. Horse No. 39 was bought on July 18, and immediately placed in cage 3 with other horses without the disease. Tabanid flies, after feeding for a time on Horse 31, which was confined in cage one, were disturbed and then allowed to complete feeding on horse 39. Two such interrupted bites were completed on August 7, one on August 8, and one on August 14. Eight days after the first exposure to the flies there was a sharp rise in temperature to 102.0°. See Plate IX. After this reaction the temperature became more variable from day to day, but the next definite fever reaction did not occur until October 22, (102.4°). A few mild fever reactions occurred in the following May and June. This horse lost some flesh, but the symptoms were not sufficiently clear to diagnose swamp fever. Consequently the experiment was continued in the summer of 1917. Flies were first allowed to bite horse 44, which was confined in cage one, then transferred to the back of horse 39 to complete their feeding. Tabanids were much more abundant this season, and successful bites were completed as follows: On July 26, seven; July 27, seventeen; July 28, nine; August 1, sixteen; August 2, nine; this made a total of 58 bites. The next mild reaction (101.9°) took place September 9 and 12. In spite of the mild fever reactions No. 39 continued to lose flesh and, though blood counts showed little evidence of anemia, died on December 8, 1917. Perhaps it might be mentioned that No. 39 suckled a colt during the summer of 1917, and this probably had something to do with her loss of flesh. See Fig. 5. There is no doubt, however, that she had swamp fever. On June 11, 1917, a vigorous young horse, No. 45, was inoculated with 35 cc. of her blood. This produced mild fever reactions on June 25, 28 and 30; on July 4, 5, 24, and 31, on August 2, 7, and 16. On August 18, No. 45 was inoculated with 24 cc. of blood from the same source. A definite rise in temperature (102.5°)
occurred August 28. This virus was not highly virulent for horse 45, for on September 24 he was inoculated with 25 cc. of blood from No. 46, a horse with a very severe type of the disease. On October 8 there began a severe fever lasting six days, which reached its highest point (106.5°) on October 12. Milder fever reactions followed later. That the blood of No. 39 was highly virulent for some horses was shown by inoculating subcutaneously on December 8 20 cc. of her blood serum into horse No. 52. A three day fever, rising to 102.8°, began December 31; other fever reactions were as follows: January 5-8, reaching 105.0°; January 12-14, reaching 104.4°; January 30-February 4, reaching 105.4°; after this date the fever reactions were not so high and usually at greater intervals. From these experimental results it was clear that No. 39 had swamp fever though the symptoms were so indefinite and obscure as to make that conclusion uncertain until after her death and the inoculation tests had been completed.
Experiment 12. June 5 to October 6, 1917. Cages one and three used. Horse No. 46 was bought June 5 and placed in cage three; horse No. 47 was brought in June 16 and put in the same cage. Horses with swamp fever were kept in cage one. The Tabanids were handled in small screen wire boxes and the same method of procedure was used as that described in experiment 10. After allowing the flies to begin feeding on No. 44, which had a severe case of swamp fever, they were transferred to the back of No. 46 or to the back of No. 47, and were observed to continue the feeding process. Flies completed feeding on No. 46 as follows: July 26, eleven; July 27, sixteen; July 28, eleven; July 31, two; August 1, fifteen; August 2, twenty-five. No reaction had occurred by August 24, when ten more Tabanid bites were successfully completed. The temperature of No. 46, which had been remarkably uniform since June 5 suddenly rose to 104.7° on September 8, reached 105.7° on the next day, and fell to normal September 12. Another severe reaction occurred September 18-22 in which the temperature rose to 106.0° on September 19. A third attack of fever which reached 105.5° on October 2, resulted in death on October 5. See Plate X. As previously mentioned No. 46 had highly virulent blood, as shown by the inoculation of some of her blood into horses No. 35, No. 38, and No. 45. See Fig. 6.

No. 47 received Tabanid bites as follows: July 27, seventeen; July 31, five; August 1, eight; August 2, sixteen; August 25, one. This made a total of forty-seven bites successfully completed on horse 47 after partial feeding on horse 44. After August 14 the temperature from day to day showed a rather wide range; for example, on August 14 the temperature was 101.4°; on the 17th, 100.1°; August 18, 101.0°; August 19, 99.4°; August 20, 101.4°; August 23, 101.9°; August 25, 98.9°. This tendency toward variability continued until the following April, but no definite symptoms indicating swamp fever were developed. The experiment was continued in 1918 as follows: On July 19, six bites were received after exposure of the flies to horse 31, four after exposure to horse 40, and eleven after exposure to horse 52. On July 22 there were forty-three transfer bites, eleven from horse 31, three from horse 40, and twenty-nine from
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Figure 6. Horse No. 46, October 5, 1917. Infected by means of Tabanids. Was nearly ready to drop down when this picture was taken a few hours before death. Died after third attack of fever. See Plate X. Also compare Figure 3 in Bulletin 121.

horse 52. On July 23, there were eleven bites from horse 31, two from horse 40, and six from horse 52; on July 24, one from horse 40, and six from horse 52; on July 24, one from horse 40, and two from horse 52; July 27, three from horse 31; July 30, five from horse 40. This was a total of 94 bites. Beginning August 26 there was again greater than normal variability in the temperature range which lasted until in December; for example on the date given the temperature reached 102.0° and was also rather high on September 21 (102.2°). However, there were no other definite symptoms of swamp fever.

It may be added that 25 cc. of blood from No. 47 was on May 24, 1918, inoculated subcutaneously into No. 53, a horse which had been under observation since December 5, 1917. Again on June 19, 1918, No. 53 was inoculated with 25 cc. of blood from the same source. No reaction followed this treatment in the course of several months, and during 1919 No. 53 was allowed to mingle with horses with the disease. Finally on October 1, 1919,
he was inoculated with 30 cc. of blood from horse No. 55; thirteen days later he reacted strongly with a four-day fever. From all of these results it was concluded that we had probably been unable to transmit the virus of swamp fever to horse 47 by the bites of T. septentrionalis. This is not certain, for occasionally the inoculation of blood, from a known virulent source produces no infection. We should have inoculated his blood into at least two other horses; horses, however, were not available at that time. During 1919 No. 47 was allowed to mingle in the same lot with horses infected with swamp fever, but there was no evidence of the disease other than occasional irregularities such as were previously noted. Since the temperature irregularities in this horse were nearly as great as those in No. 39, which was known to have had swamp fever, it is quite possible that No. 47 was a “carrier” of the virus.

**Experiment 13.** May 30, 1918, to October 1, 1918. Cages one and three used. In experiment II it was mentioned that late in the fly season horse No. 50 was used in an attempt to transmit swamp fever by means of Stomoxys. This horse had shown no fever reactions when it was again placed in cage three on May 30, 1918. Infected horses 31, 40, and 52 were kept in cage one. Tabanids were used and the following transfer feedings were made. On July 19, fourteen flies bit after previously feeding on horse 31, fourteen after feeding on No. 40, and two after feeding on No. 52; on July 22, three flies bit after feeding on No. 31, thirteen after feeding on No. 40, and nineteen after feeding on No. 52; on July 23, seventeen flies bit after feeding on No. 40; on July 24, eight flies bit after feeding on horse No. 40. This made a total of 90 bites within six days. A sharp reaction followed on July 31, just twelve days after the first fly feeding, in which the temperature rose to 104.2°. In addition to fever No. 50 developed other symptoms of swamp fever and died October 28, 1918. A few days later, November 9, horse No. 54 was inoculated subcutaneously with 15 cc. of blood from No. 50, but no definite reaction followed. See Plate XI.

Horse No. 54 was very tolerant of, or highly resistant to the virus of swamp fever. Following the inoculations of blood from horse 49 (Experiment II) there was only one slight variation
(100.5°) from the normal temperature before August 27; during the next two months his temperature was more variable, rising to 101.6° on three occasions, and falling to 97.6° once and to 98.0° at another time. His temperature settled down to a normal condition before he was inoculated with the blood of No. 50, and nothing unusual was noted before May, 1919; then for five months it was variable the extremes being 98.0° and 102.0° F. On September 30, 1919, No. 54 was inoculated with 25 cc. of blood from the colt to which No. 39 had given birth on May 30, 1917. This colt while suckling had been kept with swamp fever horses and had upon several occasions shown unexplained fever reactions. On October 28 No. 54 was inoculated with 40 cc. from the same colt. There was one fever reaction (102.8°) on November 22, but otherwise the temperature continued variable as during the preceding five months. On January 17, 1920, No. 54 was inoculated with 36 cc. of blood from horse No. 65, which recently had been having some severe attacks. A three-day fever reaction began January 30, in which the temperature rose to 103.0°. A temperature of 103.2° was registered on February 13 but succeeding fever attacks grew less severe, and while seemingly on the road to recovery No. 54 was killed May 4, 1920.

In closing this account of experiments with *Tabanus septentrionalis* I should state that we were apparently more successful in transmitting swamp fever by means of *Tabanus* than by means of *Stomoxys*. This is rather to be expected for the mouth parts of *Tabanus* are larger, the wound made in the skin is more extensive, and following the bite blood trickles out more frequently and in larger amount. The bite is also so severe that it tends to initiate interrupted feeding. Consequently there is more chance of transferring the virus by means of contaminated mouth parts. We may sum up this series of experiments as follows: Swamp fever was transmitted to two horses, 39 and 46, by means of *Tabanus septentrionalis*, and this was probably true of a third horse, No. 50; we had failed to transmit the disease by this means to horse 47, or at least the result was doubtful.

In this connection it may be mentioned that we collected two species of *Chrysops* but we did not succeed in getting them to feed and they died in a short time in captivity.
In experiment one purely negative results were obtained in attempting to transmit swamp fever by means of mosquitoes. A second experiment was arranged the following year to make a further test of this particular question.

Experiment 4. June 22 to July 26, 1915. Cage three used. Cage three, which was entirely similar to cage one, was finished on June 20. On this date several aquaria were placed in the area in front of the mangers; these aquaria were sufficiently ample to take care of several thousand mosquito larvae and pupae with which they were stocked on June 22 and 23. Two horses with swamp fever No. 20 and No. 24, were placed in this cage on June 22. About 1000 mosquitoes pupated on June 24; the following day many more pupated and some were observed to bite the two horses. More larvae and pupae were collected and placed in the aquaria on the twenty-sixth. On June 27 horse No. 27, which had been used in experiment three, was put in cage three; since he had not shown any fever in several months it was believed that he did not have swamp fever. (See also experiment six). On the following day there was also put in cage three a new horse, No. 31, which had been under observation since May 19. After July 6, larvae and pupae being no longer available, large numbers of mosquitoes were collected and turned loose inside the cage. Mosquitoes were not numerous outside of the cage by July 17. From this date the number of mosquitoes in the cage rapidly dwindled until July 26 when the experiment was brought to a close.

During their sojourn in the cage horse No. 20 had one attack of fever lasting from June 29 to July 4, and horse 24 had five attacks as follows: June 27 to July 2, July 4-6, July 10-15, July 17-18, and July 20-21. Mosquitoes were observed to bite freely of both diseased and well horses. Not the slightest indication of infection with swamp fever had developed in horses 27 or 31, by September 11 when they were both used in experiment six previously described. The negative result in this experiment is probably not hard to explain. Compared with Tabanus or Stomoxys little pain is produced by the mosquito in piercing the skin, so feeding is not often interrupted and conditions are therefore not
favorable for mechanical transmission. The more slender arrangement and structure of the mouth parts of the mosquito may also be less likely to become contaminated with the virus. Lührs ('19) claims that a species of *Anopheles* in Germany can transmit infectious anemia. Most of our common species of mosquitoes belong to the genus *Aedes*.

**EXPERIMENT CONCERNING THE SUPPOSED RELATION BETWEEN GASTROPHILUS LARVAE AND INFECTIOUS ANEMIA.**

In 1914 the Seyderhelms brought forward the theory that botfly larvae were the cause of infectious anemia. They produced an anemia in horses by means of extract of bot larvae and were unable to distinguish this malady from infectious anemia. After a careful reading of their report the writer was convinced that they were not working with swamp fever, and soon the opportunity came in our own work to test the Seyderhelm hypothesis.

**Experiment 7.** March 21 to 28, 1916. During the principal part of the bot-fly season horse No. 21 had been confined for two years in screened cages, August 3 to October 1, 1914, in cage one, and July 26 to September 30, 1915, in cages one and three. We have seen that he contracted swamp fever while in cage one, August, 1914, and that he had severe recurrent attacks while confined in cages one and three in 1915. If these recurrent attacks were due to bot-fly larvae the larvae should still be in the alimentary canal in March, 1916, for in this climate the larvae do not leave the stomach or intestine until May, June and July. Horse 32 was in cage one from July, 26, to September 30, 1915. On August 31, thirty-six days after being put in the cage, this horse gave the first evidence of contracting swamp fever. At no time were bot-flies present in the cage and all flies were dead before the horses were turned out. If the initial attacks of swamp fever in horse 32 were due to virus from bot-fly larvae, these larvae should still be present in the stomach the following March, and if expelled should be large enough to be easily found in the feces. Horses 21 and 32 mere given the following treatment for bots:

"First day (March 22). Water only in the morning; no food or water the rest of the day."
"Second day. During the first hour of treatment gave each horse three capsules, each capsule containing 10 cc. of carbon bisulphide. Four hours later gave each horse three quarts of wheat bran mixed with three quarts of water." They ate only part of this feed.

"Third day. In the morning gave each horse a powder consisting of powdered aloes 6 dr., calomel 1 dr., ginger 2 dr. At noon gave each horse four quarts of bran mixed with two quarts of warm water, and afterwards drinking water. At evening the same food was given as at noon." Not all the bran was eaten at either time.

"Fourth, fifth and sixth days. Fed three times each day, each time with four quarts of bran moistened with water. The horses were also given hay and drinking water."

"Seventh day. Turned out of closed stall."

During the experiment each horse was confined in a closed box-stall with wooden floor, and all feces passed were carefully examined on the evening of March 23, and on the morning and evening of each of the following days during the progress of the experiment. Not a single bot-fly larva was found. Since the treatment given is a standard treatment for ridding horses of bots, we may assume that at least some of the larvae, if present, would have been expelled. Consequently swamp fever as developed in horses 21 and 32 did not appear to have any relation to bot-fly larvae.

The *Gastrophilus* theory, of course, has been abundantly disproved by other investigators. Favero ('16) claimed that his researches had disproved the claims of the Seyderhelms. Van Es and Schalk ('17) after numerous experiments concluded that there was no reason to believe that *Gastrophilus* species play a specific part in the causation of infectious anemia. Du Toit ('19) and Marxer ('20) believe their experiments show that there is no causal relation between *Gastrophilus* larvae and infectious anemia, and other workers have been equally emphatic.
III. IN REFERENCE TO THEORIES OF TRANSMISSION

Considering the obscure and insidious nature of the disease, it is not surprising that the early theories to account for the transmission of infectious anemia were matters of pure conjecture. As knowledge of the disease increased and as observations and experimental evidence accumulated, two principal theories of natural transmission became prominent. By the theory of internal transmission the infection is believed to be introduced through the alimentary canal by means of contaminated food and drink; by the theory of external transmission, biting insects are believed to play an important part in carrying the virus from one horse to another. A brief reference is given here to the more important suggestions that have been made in regard to natural transmission.

Zschokke ('83) as a result of his experience with the disease in Switzerland, advanced the view that the disease was caused by an agent similar to malaria. Köpke ('01) believed the disease due to an infection.

Brimhall, Wesbrook and Bracken ('03) made some careful observations of the ecological relations surrounding two foci of the disease, and suggested that insects and arachnoids possibly play a part. Jarmatz ('04) suggests that inbreeding and insufficient nutrition share in producing the disease. Carré and Vallée ('04, '05, '06, '07, '16), who established the principal facts with reference to the nature of the disease, showed that both blood and urine are virulent, and put forward the urine-contamination theory which was so generally accepted as the natural means of transmission until recent years. Beebe ('05) states that one horse contracted the disease after being driven with a probable case. Ries ('06) believed intestinal helminths bore an important relation to the disease, by carrying the disease, by opening a port of entrance, or by weakening the resistive power. Later ('08) he noted the prevalence of the disease in wooded regions and observed the abundance of flies; he held that the urine-contamination theory did not explain all cases, and his own observations pointed to Gastrophilus larvae as the other mode of transmission. Charlton ('07) suggested, from analogy, that the disease is very
probably carried from one animal to another by biting insects. Ostertag ('08) failed to transmit the disease by saliva. Mohler ('08) states that probably the virus is spread by an intermediate host, such as flies, mosquitoes, or internal parasites. Hempel ('09) did not get transmission by standing two well horses with two latently sick horses from June 22nd to December 8th; these horses ate together and breathed the same air. Kinsley ('09) observed that the disease occurred equally on upland and lowland; that it is more prevalent in wet seasons, that the initial attack occurs in July, August or September; that the disease occurs regardless of the source of water, or of food, and tends to remain localized on individual farms. However, since adjacent horses were in certain cases not infected, he states that flies and mosquitoes appeared to have no relation to the disease. It is interesting to note that practically all of these observations agree with the theory of insect transmission. Acres ('09) states that he never has seen an outbreak caused by putting a diseased horse in a stable with healthy horses, but has seen horses feeding on high dry pastures with good water contract the disease; healthy horses became infected in this pasture after a diseased animal was turned out with them. Melvin ('10) mentions that a healthy horse kept in a stall adjacent to one containing a sick horse for seven months did not become infected. Halverson ('10) makes the statement, "It is considered that flies, mosquitoes and internal parasites act as intermediate hosts for the parasites that cause pernicious anemia." Schlathölter ('10, '11) conveyed the disease to healthy horses by contaminating hay or straw with infective blood or urine, and favored the urine contamination theory. Van Es, Harris and Schalk ('11) concluded that the disease is not due to poisonous plants or to helminth intoxication, and while they do not deny transmission by insects they believe that horses contract the disease naturally through food and water contaminated with urine. Norton ('11) mentions that the disease is hardly ever found in mules the first year after they are imported from the North. At first he thought it was carried by flies and mosquitoes, but later doubted this and believed that unsanitary conditions and long continued hard work had much to do in developing the disease. Wolbach ('12) expresses the opinion that swamp
fever is transmitted by some intermediate host, as yet undiscovered. Swingle ('13) found great difficulty in transmitting the disease by way of the alimentary canal, though he repeatedly fed large quantities of both blood and urine. The Seyderhelms ('14) advanced the theory that the disease was due to certain poison found in the bodies of *Gastrophilus* larvae. This theory was soon disproved by others and the symptoms developed were shown to have an anaphylactic nature. Scott ('14) was the first to show that the stable-fly, *Stomoxys calcitrans*, could probably transmit the disease. Later this relationship was definitely established and *Tabanus septentrionalis* was also found capable of transmitting swamp fever. The Japanese Commission ('14) on the basis of extensive field studies and penning experiments, came to the conclusion that infectious anemia was transmitted by means of insects; they attributed the chief role to certain species of *Tabanus* and *Chrysops*, since these flies appeared abundant during the infective season. Schmidt ('15) obtained a negative result from an experiment in a poorly drained pasture where conditions favored transmission on the urine-contamination theory. Theiler and Kehoe ('15) discovered equine infectious anemia in South Africa and stated methods of natural transmission, mentioning contamination by urine and by insects. Howard ('17) probably succeeded in transmitting the disease by means of biting flies, the stable-fly being the species suspected. Miyagawa, Taniguchi, Nagano and Takemoto ('17) found an organism which they considered was a spirochæte, in various organs of a sick horse; they reproduced the disease they were studying by inoculating cultures of this organism into healthy horses. This work remains to be substantiated. Wirth ('18) took a horse that showed a normal temperature for ten and one-half months and on October 5, 1916, put it in a roomy stall with four other horses, two of which had the fever. The first fever reaction appeared in this horse after 16 days. Wirth states that these horses ate from a common box and drank from a common tank, but says nothing about the presence or absence of flies. Fröhner ('19) failed to get transmission by contact experiments, and favors the insect theory as the natural method of transmission. Lührs ('19) made extensive observations and experiments with the disease. Endo- or Ecto-parasites
which take up body fluids of sick horses may be bearers of the virus; he demonstrated that *Gastrophilus* larvae, filaria, biting flies and mosquitoes may contain the virus. He transmitted the disease by feeding, by introducing infectious material under the eye-lids, and proved that the eye-secretion contained the virus. He also believes that *Anopheles* must be considered as transmitters of the disease. Hadwen ('20), working in conjunction with Ransom, found no evidence of a relation between parasitic worms and swamp fever, though frequently complicating the disease. Habersang ('21) states that, since spirochaetosis of the horse produces symptoms similar to those of infectious anemia, as discovered by Theiler, Dodd and Stoidy in South Africa, he suspects that the Japanese ('17) were working either with a spirochaetosis or else with a mixed infection containing both spirochaetes and the virus of infectious anemia. Scott ('22) and Jones were unable to find spirochaetes in any of the sick horses studied and failed to cultivate such organisms on media known to be favorable for the growth of spirochaetes. This brief summary of the theories of transmission of infectious anemia is sufficient to open the discussion in regard to the probable nature of natural transmission in the next section.

IV. ON THE NATURE OF NATURAL TRANSMISSION

We owe to Carré and Vallée (1904-07) the first demonstration of most of the fundamental facts with reference to the nature of the virus and the characteristics of infectious anemia of horses. The theory of natural transmission by ingestion of food and drink contaminated with urine from infected horses has been based in part upon the findings of these investigators. Since they succeeded in producing the disease as the result of feeding 20 cc. of blood to one horse and noted another doubtful case that probably resulted from the feeding of urine, they put forward the theory that the spread of the disease is due to food and drink contaminated with urine from diseased horses. While they succeeded in transmitting the disease by means of intravenous injection of filtered blood serum, they failed to find any relationship between blood-sucking insects and the distribution of the disease. Sub-
sequent writers have confirmed most of these results and, as a rule, have accepted the contamination theory. Since the writer of this paper began the study of the problem in 1913, he has found it necessary to examine critically the evidence upon which the theory is based. Hempel (1909), Schlathölter (1910-11), Van Es, Harris and Schalk (1911), Francis and Marsteller (1911), the Japanese Commission (1914) and Theiler and Kehoe (1915), have all succeeded in transmitting the disease by feeding blood. Schlathölter, Van Es, Harris and Schalk, the Japanese Commission, and Theiler and Kehoe, have also succeeded in transmitting the disease by feeding urine. It should be added too, that Van Es, Harris and Schalk, the Japanese Commission, and Theiler and Kehoe, have produced the disease by the subcutaneous injection of urine.

The easiest way, however, to produce the disease experimentally has been by the subcutaneous or intravenous inoculation of virulent blood. Carré and Vallée, and Francis and Marsteller, succeeded in producing the disease by the intravenous and hypodermic injection respectively of as low as 1 cc. of blood. Lührl's ('19) states that a small part of a cubic centimeter of blood or serum is sufficient to produce the disease. Writers generally agree that it is more difficult to induce the disease by way of the alimentary canal, and this is true whether blood or urine is used. Ries ('06) denied that urine can infect by the mouth. Hempel found that four times the quantity of virulent blood needed to infect subcutaneously failed to produce a reaction when given by the mouth; in another experiment an intravenous dose of 120 cc. produced a slight form of the disease, while 300 cc. of the same blood given by the mouth produced only one day of fever after 21 days incubation. Van Es ('11) states that when same blood is used the incubation period is somewhat shorter after intravenous than after subcutaneous injection; if the virus is given by the mouth the period is still more prolonged, and in general the period of incubation is longer when urine is used for infecting purposes than when blood is employed. He produced the disease by feeding 50 cc. of blood daily for 10 days following doses of 25 cc. per day for a like period, and another horse became infected after being fed a dose of 2000 cc. of urine. Francis and Marsteller fed
4 oz. of blood to each of three cases; two horses took the disease after 12 and 26 days respectively. Swingle ('13) failed after repeated attempts to get infection by feeding virulent blood and urine; in his experiments each of three horses were fed blood, from two or four doses each, in amounts ranging from 150-450 cc. To two other horses he fed urine at various intervals, in amounts ranging from 15-200 cc., to one horse seven doses, to the other eight; one of these cases showed a doubtful reaction. The Seyderhelm's injected intravenously 40 cc. of urine from a sick horse with negative result. The Japanese Commission were more successful. To each of two horses they fed 100-200 cc. of urine daily until they became infected after 30 days. To another horse they fed 100 cc. of urine twice daily for 120 days with no infection. Feeding each time 100 cc. of blood mixed with feed, daily or on alternate days, they produced the disease in two out of four horses after about 50 days. They also fed daily extract of feces mixed with feed over a long period, but none of the five horses used became infected. They conclude that the virus may enter through the alimentary canal of a healthy horse, by such unnatural methods mentioned above, but that contact infection in nature, if any, must be a very slow and limited process. Theiler and Kehoe fed defibrinated blood to two horses. One reacted on the sixteenth day after being fed 500 cc. The other failed to react within 27 days after receiving a similar amount; again it was fed 500 cc., which failed to produce a reaction within 21 days; an additional 900 cc. was then given and a reaction occurred on the fourteenth day. They also fed urine of affected horses to one healthy animal; 300 cc. failed to produce a reaction within 24 days; the same animal was then fed 500 cc. without positive result inside of 13 days; it was then fed 600 cc. on each of two successive days, and not having reacted within 6 days it was given an additional 500 cc.; as a result of this treatment the horse reacted after an additional 25 days. It is probable that a horse would not ordinarily obtain one hundredth part of this amount of urine on his food or in his drink within a whole year under normal pasture conditions where the disease most frequently occurs. These data show the difficulty that investigators have invariably experienced in transmitting the disease by way of the alimentary canal.
There are various reasons for rejecting the contamination theory as the only method of transmission. (1) It involves infection by way of the alimentary canal, and we have just seen the great difficulty with which this is accomplished. This is true no matter what sort of infective material (blood or urine) is used. (2) The theory does not account for the seasonal occurrence of the disease. In fact, since the virus is not seriously affected by freezing, one would expect the disease to be more prevalent in the winter when horses, as a rule, are confined and fed in closer quarters. But in America and Japan and as reported by some in Europe, the disease is most prevalent in the latter part of summer and early autumn. (3) On the contamination theory, we should expect swamp fever to be a stable as well as a pasture disease. Certainly the chance for contamination of food and drink in and about stables and feed lots is greater than in the open pasture. (4) In the experiments mentioned above, it was noted that it is apparently easier to infect by feeding blood than by feeding urine. But there is very little chance of the blood of a sick horse reaching the food or drink of another. It is true that horses sometimes cut the mouth on sharp-edged grass, and a small amount of bloody serum sometimes escapes from the nostrils of a diseased horse, but the quantity is small and it is hard to see how this could account for the disease in pastures more than in stables, in summer more than in winter, or for epidemics of swamp fever. (5) The virus in the blood is apparently pathogenic at all times. On the contamination theory, this fact argues for infection at any time rather than for the well-known pasture, or seasonal distribution of the disease. (6) Direct sunlight soon kills the virus. This fact is unfavorable to the contamination theory, for virus in accessible urine or blood would soon be destroyed. (7) Infection rarely has occurred under conditions favorable for contamination. During seven years we have never had a case to develop that could not be explained on another theory, and we have had a number of cases that cannot be explained on that theory. Altogether these reasons make it appear very improbable that the usual natural method of transmission of the disease is by way of the alimentary canal. Certainly it does not seem possible on this theory to account for some known epidemics.
of swamp fever. We have noted the great difficulty experienced in transmitting the disease by way of the alimentary canal. At the same time, there is nothing to indicate air transmission and autopsies do not show any pathological change in the air passages. Other methods of internal transmission have been suggested as follows:

Ries ('06) first suggested the theory that *Gastrophilus* larvae are in some way connected with infectious or pernicious anemia. More recently, the Seyderhelms, confirmed by Van Es and Schalk ('17), have shown that the extract of botfly larvae is capable of producing anemia. We have already stated excellent reasons for believing that the anemia produced by this method is not the disease known as swamp fever, or infectious anemia. Various investigators have recently shown that the Seyderhelms were dealing with phenomena of anaphylaxis. Besides, there are other valid objections to the theory that *Gastrophilus* larvae are either the agents or the cause of swamp fever.

Glage ('06) called attention to the importance of intestinal worms in producing anemia. Several other writers have emphasized the importance of sclerostomes as a possible etiological factor in producing swamp fever. While certain helminths can undoubtedly produce anemias in horses, and in some respects the diagnosis is quite similar to infectious anemia, the fever produced is of a different type and there is no evidence that these parasites bear any etiological relation to swamp fever. Hadwen ('20) working in cooperation with Ransom, found no evidence of a relation between parasitic worms and swamp fever; they believe it necessary, however, to give careful attention to parasitic conditions in order to avoid misleading deductions. I wish here to confirm these conclusions.

If we find the theories mentioned unsatisfactory, or unable to give an adequate explanation for natural transmission of swamp fever, we are left with the quest for a substitute. Considering the data favoring internal transmission insufficient, the writer in taking up the problem in 1913, decided to study the relation of the disease to the agents that might possibly be concerned in external transmission. The idea that abrasions of the skin could afford a means of transmission was rejected because of
the accidental character of such injuries. By this method of elimination there was left, if our reasoning was correct, only one means for transmitting the disease, that through the agency of some biting parasite. But if an intermediate host is necessary it must satisfy certain conditions commensurate with the disease. It must have a wide geographical distribution; it must have an altitudinal distribution from sea level to near 9000 feet. It must satisfy the seasonal occurrence, and the low-lying, wet, or swampy pasture conditions of the disease. It must be capable of making a ready transfer of virus from one horse to another. It must satisfy the conditions of transfer with a habit of feeding more than once during its lifetime, or if only one feeding occurs this must be completed on a different host, following one or more interruptions. It is well to remember, too, that two or more intermediate hosts are possible, one satisfying the conditions in one locality, the other in another.

Of the external parasites of the horse in Wyoming comparatively few fill all the conditions mentioned. For one or more reasons lice, mites and ticks must all be excluded as possible carriers of the disease. So far as can be learned, the only biting parasites of the horse that satisfy the wide geographical distribution characteristic of swamp fever include certain species of mosquitoes and certain biting flies. After arriving at this conclusion it was decided to put the insect hypothesis to a test.

In view of the experiments described in this paper, taken in connection with the work of other investigators, it is believed that certain biting flies provide the usual means for the natural transmission of infectious anemia of horses. It has been shown that Tabanus and Stomoxys are mechanically able to transmit the disease. The needle experiments have shown that the transfer of a very small amount of blood is required to produce swamp fever, an amount that can readily be carried on the contaminated mouth parts of the flies mentioned. The seasonal occurrence of the disease is another fact that agrees with the insect theory of transmission. With two or three exceptions where the full history of the cases was not clear or could not be obtained, all field cases with which the writer is acquainted have developed between July 15 and December 1, and most all of these between August 15 and
October 15. In some cases, where the disease apparently developed at other seasons, inquiry as a rule has brought out the fact that these horses had not done well or had shown symptoms of the disease in previous years. This agrees with the history of certain experimental cases. In Wyoming Stomoxys is commonly present from July 15 to October 1, and in greatest abundance from August 1 to September 15. They bite most freely between August 15 and September 15. The season for Tabanus septentrionalis extends from about July 1 to September 1, with the flies most abundant from July 10 to August 25 in the region of the Laramie Plains; in some other parts of the state the season is somewhat longer. Not only does the seasonal character of the disease agree with the theory of insect transmission, but it is difficult to explain on any other theory. When outbreaks occur they may take on almost the character of an epidemic; in the writer’s experience such outbreaks have invariably occurred in seasons and localities especially favorable for the development of biting flies. Still another fact of importance is the observation that swamp fever is essentially a pasture disease. The following account of an outbreak illustrates these points.

Ranch M is situated on bench land about two miles from the Big Horn River; the year of the outbreak, 1915, M. had leased a low-lying pasture on the A. place, consisting mostly of bottom land, along the river. The disease was first observed in April; two three-year-olds, No. 1, a mare and No. 2, a gelding, were noticed as not doing well; the mare died about July 19 and the gelding about two months later. No. 1 and No. 2 had never done as well as expected; they were bought as yearlings from W., who two years previously had lost eleven head of horses. Case No. 3, a yearling, developed swamp fever and died the latter part of July. In August No. 4 and No. 5 were noticed as not doing well; No. 6 was also sick. About September 15, the horses from the lower pasture were brought to the ranch. On September 24, they were put to work putting up alfalfa hay. While at work the horses began getting weak and thin; the disease developed the worst among these horses between the first and the fifteenth of October and most of the sick horses died in November. Case No. 16 did not die until January 5, 1916. Cases No. 6 and No. 17 had
not apparently been seriously sick and both survived; they were seen by the writer on January 29 and neither showed any definite symptoms of swamp fever; they were in fairly good flesh and there was no indication of anemia. However, a temperature record was kept of horses on this place and three showed a return of the fever, No. 6 in April and No. 17 and one other not previously suspected, in May. These three horses were killed, and for a known period of four years swamp fever did not again appear on the M. ranch. Perhaps the most interesting fact about this outbreak is the fact that the horses that were kept on the M. ranch all summer, that were worked, and fed in the stables, did not take the disease, while the horses that were out in pasture between hayings were the ones that died. Cases 1 and 2 were undoubtedly chronic and probably brought swamp fever to the M. ranch; since they were not put on the low-lying pasture until 1915, there was probably no good opportunity of transmitting the disease until that time. There was one other possible opportunity of introducing the disease on the M. ranch. During the haying season of 1914, M. leased horses from F., who lived at that time on the A. place and who is said to have lost some horses that year; since F. had moved away definite information along this line was not obtainable.

Inoculations have shown that the virus of infectious anemia is in the blood at all seasons of the year. The virus is therefore probably present in the urine or other excretions at all seasons and this relation would appear to favor the urine contamination theory. In spite of this fact we have a seasonal incidence of swamp fever. While it is certain that the virus is present at all seasons of the year, it may not be present at all times. For negative results sometimes follow the inoculation of blood from a known virulent source. This is a problem that remains to be solved. A number of secondary factors undoubtedly influence the seasonal incidence of the disease. Many ranchmen have told me that swamp fever developed in their horses when they were turned out in pasture after the haying season was over; the principal haying season in this region is usually over by the 25th of August. In an over-worked, run-down condition these horses are more susceptible to the effects of the virus. The dim-
Finishing food supply in the fall is frequently an unfavorable condition and the subnormal temperature that follows a fever is further aggravated by the chill, cool nights. While these secondary factors are important in inducing active onsets of swamp fever, they have nothing whatever to do with the transmission of the disease.

It is well known that the virus of infectious anemia is readily killed by exposure to direct sunlight. In Wyoming there are not many days on which the sun does not shine for a good portion of the day. This is a condition unfavorable for the urine-contamination theory, and infection in most cases certainly does not occur in stables or other places where the virus would be best protected from the rays of the sun.

Finally, in view of the facts mentioned above and the experiments described in this paper, taken in connection with the work of other investigators, the writer is convinced that certain biting flies furnish the most important means of natural transmission. The fact that the virus has been found in the eye and nasal secretions may possibly be of significance when more is known of this matter. According to the insect theory of transmission, the most favorable conditions for infection are the following: First, the intimate mingling of virus carriers with well horses under circumstances which permit the fighting off of biting flies and so the production of interrupted feeding; second, the presence of large numbers of biting flies, particularly *Tabanus* and *Stomoxys calcitrans*. Even under these conditions, infection may not occur; some horses are highly resistant to or tolerant of the virus, and the inoculation of rather large quantities of blood sometimes does not produce the disease.
V. SUGGESTIONS IN REGARD TO CONTROL.

The experiments described in this paper emphasize the importance of certain parasitic insects in any measures looking toward the control of infectious anemia. Whatever other natural means there may be, it is believed that the preceding experiments afford an explanation of how infectious anemia of horses can be transmitted and that this hypothesis agrees best with all the known facts concerning transmission. In any system of control, the detection and identification of the disease, particularly in the mild chronic or apparently healthy carrier, is of prime importance. Until a satisfactory method of diagnosing such cases is discovered, we may expect swamp fever to persist in certain regions of Wyoming, with occasional outbreaks when the conditions are favorable. However, our knowledge of infectious anemia has advanced to such a degree that if the measures suggested below were strictly enforced, losses from the disease would soon be greatly diminished and the disease possibly exterminated.

In Bulletin 121 of this Experiment Station the subject of the control of swamp fever of horses in Wyoming is discussed at some length. With one or two exceptions the following suggestions differ only in minor details from the instructions given in that bulletin. Since writing that paper the writer has had the opportunity to observe a number of field cases and more emphasis needs to be laid upon the methods used in detecting the healthy carrier. On the other hand, aside from desirable sanitary measures, I would lay perhaps less emphasis on precautions taken to prevent infection as the result of the contamination of food and drink with urine. Since no cure for the disease has been found, and treatment involves only palliative measures, there is no opportunity of gaining a control of the disease in this way. These instructions apply principally to Wyoming conditions.

1. All horses with the disease should be condemned and killed. As an added incentive to aid in carrying out this measure the owner should be in part compensated for his loss by the State. All infected horses, if left alive, serve as carriers; consequently they should be killed to avoid the danger of further spreading the disease and the expense of care and feed. Isolation serves to pre-
vent the spread of the disease, but such horses are usually of not much value unless worked and chronic cases do not stand up well under work. Owing to the persistent nature of the virus the carcases of all horses that are killed or that die of swamp fever should be carefully burned or deeply buried.

2. All suspected carriers, including all horses belonging to herds in which the disease has existed within a year, should be kept under observation and not allowed to mingle with well horses. While under observation the temperature of each horse should be taken at least once each day. If the period of observation lies between July 15 and December 1, taking the temperature of a chronic carrier for three months will usually disclose some evidence of the disease; if the period of observation lies between December 1 and July 15, the temperatures should be taken for at least five months. However, putting a horse to work, or giving him occasionally severe endurance tests, will usually induce onsets of the fever in much shorter periods. Some mild cases, if vigorous and healthy, may stand considerable work and may be worth keeping on that account. However, such horses are virus carriers, and should be isolated or protected from biting flies during the infective season. There seems to be very little danger of transmitting the disease unless biting flies are present; the larger the number of flies present, the more favorable are the chances of transmission.

3. Ordinarily, swamp fever is a slow-spreading disease. The chronic case or healthy carrier mingling with well horses is probably the only means by which the disease is spread from one district to another or from one ranch to another. Unless the precautions mentioned in the two preceding paragraphs are taken, a ranch on which swamp fever has existed should be quarantined for at least two years; no horses should be sold from it within this period and none should be allowed to run on the public range, or mingle with horses from adjacent farms between July 1 and October 1; if there is no recurrence of the disease within this period and all horses continue in a thrifty condition, it is probably safe to lift the quarantine though carriers might still be present provided there had been no conditions favorable for transmitting the disease or for inducing onsets of fever.
4. Sanitary conditions should, of course, always be provided. A pure water supply is important; water from springs, from open running streams, or from watering troughs filled from fenced ponds, all are satisfactory arrangements. Wet, swamp pastures should be drained or should be avoided for pasturing horses; such places usually furnish excellent conditions for the propagation of horse parasites, both external and internal. A horse infested with numerous sclerostomes has its resistance lowered, and well drained pastures are unfavorable for the development of biting flies. In the barn yard, the stable and watering place should be kept in a sanitary condition, and stables in which diseased horses have been kept should be disinfected.

5. There remains the possibility of infection by means of food and drink contaminated with infective urine. For reasons previously given in this paper the writer believes that this possibility is comparatively remote, and apart from the sanitary measures suggested in the last paragraph it is believed that there is comparatively little value in measures that attempt to control transmission of swamp fever when based on this hypothesis.

REFERENCES
Wyoming Agricultural Experiment Station Bull. 133


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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 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 where necessary is continued from page to page.

Plate I. Horse No. 2. The latter part of the temperature record of a fatal chronic case in which there was great emacia-
This horse was used as a source of infective material in experiments one and two. Died August 3, 1914.

Horse No. 26. Inoculated with 15 cc. of virulent blood serum on July 17, 1914. Used as a source of infective material in experiment two, from July 30 to September 11, 1914, when death was caused probably by an accident.

Plate II. Part of the temperature record of horse No. 20. Inoculated on August 2, 1914, with 21 cc. of blood from horse No. 2. Was used as a source of infective material in experiments two, three, five and six. The latter part of his record is omitted. Note the unusually short incubation period, four days.

Plate III. Record of horse No. 30. Inoculated subcutaneously on November 21, 1914, with 35 cc. of blood serum that was drawn October 5 from horse No. 22. The immediate fever reaction, which began on the same day, was due to bacterial sepsis and recovery occurred in a few days; the disease, swamp fever, developed later. The attendant failed to keep some of the temperature records, and this shows on the plate. This case undoubtedly was complicated with sclerostomiasis. See Figure 6 in Bulletin 121.

Plate IV. Horse No. 27, pages 1 to 4. Arrows indicate dates on which this horse was exposed to infective bites of *Stomoxys calcitrans*. Shows how the disease may have a very gradual development with a long latent period. Probably became infected in 1914. See experiments three and six. Also compare with Plate VI following.

Plate V. Horse No. 32, pages 1 and 2. Placed in cage one (1) July 26 and taken out (0) September 30. *Stomoxys* were most abundant in the cage between August 7 and September 1. See experiment five and Figure 2.

Plate VI. Horse No. 33, pages 1 and 2. Showed the first fever reaction thirteen days after first being exposed to infective *Stomoxys* bites. Note that after three reactions the disease followed a mild course for several months. See experiment five.

Plate VII. Part of temperature record of Horse No. 31, which was infected by means of *Stomoxys* in September, 1915. See Bulletin 121, Figure 1 and Plate IV, for his appearance in 1918, and the earlier part of his record. See Figure 3 for his
appearance in June, 1922. He shows a good deal of stiffness and lameness in the hind quarters, and occasionally shows oedema of sheath, breast, belly and limbs. Used as a source of infective material in several experiments.

Plate VIII. Horse No. 38, pages 1 to 3. Infected by means of a hypodermic needle, which had been contaminated with the virus of infectious anemia by pricking the skin of diseased horses. Arrows indicate dates on which the skin of No. 38 was punctured, once each day except where otherwise numbered. See experiment eight.

Plate IX. Horse No. 39, pages 1 and 2. Infected by means of Tabanus septentrionalis. Arrows indicate dates of Tabanid bites. See experiment ten for further explanation and Figure 5 for appearance of this horse in November, 1917. The fact that this mare suckled a colt during the summer and fall of 1917, probably reduced her vitality, and helped to produce emaciation. Note the absence of severe fever reactions. Her blood, however was highly virulent.

Plate X. Horse No. 46. Inoculated with infectious anemia by means of Tabanid bites. Arrows show the dates on which this horse was exposed to the biting flies. A severe case developed in which death resulted after the third fever reaction. See Figure 6 and experiment twelve. Also see Figure 3 in Bulletin 121. Her blood was highly virulent.

Horse No. 37. This brief chart illustrates the temperature record of a field case that was brought in for observation a few weeks before its death. How this horse became infected is unknown.

Plate XI. Horse No. 50, pages 1 and 2. The arrows on page 1 indicates an unsuccessful attempt to transmit swamp fever by means of the stable-fly, Stomoxys calcitrans. See experiment eleven. The arrows, page 2, indicate the dates on which Tabanids bit this horse after interrupted bites on diseased horses. Note the sharp, definite fever reaction which followed. See experiment thirteen.
Plate I. Horses No. 2 and No. 26.
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Plate II. Horse No. 20.
Plate III. Horse No. 30.
Plate IV, page 1. Horse No. 27.
Plate IV., page 2. Horse No. 27.
Plate IV, page 3. Horse No. 27.
Plate IV, page 4. Horse No. 27.
Plate V, page 1. Horse No. 32.
Plate V, page 2. Horse No. 32.
Plate VI, page 1. Horse No. 33.
Plate VI, page 2. Horse No. 33.
Plate VII, page 3. Horse No. 31.
Plate VII, page 5. Horse No. 31.
Plate VIII, page 1. Horse No. 38.
Plate VIII, page 2. Horse No. 38.
Plate VIII, page 3. Horse No. 38.
Plate IX, page 1. Horse No. 39.
Plate IX, page 2. Horse No. 39.
Plate X. Horses No. 46 and No. 37.
Plate XI, page 1. Horse No. 50.
Plate XI, page 2. Horse No. 50.