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KANSAS STATE AGRICULTURAL COLLEGE.

DEPARTMENT OF VETERINARY MEDICINE.

METHODS OF CONTROLLING BLACKLEG.

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The veterinary department of the Kansas State Agricultural College began a series of experiments in 1912 to increase the efficiency of the blackleg vaccine which the College had been making. The department was led to undertake these investigations because of the losses which were occurring from blackleg following the use of the old spore vaccines, administered in powder or pill form. The work was under the direction of Dr. F. S. Schoenleber, with Dr. O. M. Franklin and Dr. T. P. Haslam in charge.

The first phase of the investigations involved an extensive and critical test of the strength and composition of the spore-containing vaccines produced by the leading laboratories of the United States and Europe. This work extended over a period of two years and a summary of the results was published in *The Journal of Infectious Diseases*.¹ It was found that spore vaccines were at best more or less unreliable because of the narrowness of the range of virulence between a good and a poor vaccine; that is, between a vaccine that will immunize and one that will kill. It was found, also, that the degree of susceptibility of calves varied greatly, which decreased the efficiency of the spore vaccines. As a result of this work the department succeeded in increasing the efficiency of its vaccine to a small degree. This investigation shows the weakness of spore vaccines, while the work done by Kitt, Leclainche and Vallee, Grassberger and Schattenfroh, Foth,

1. Vol. 19, No. 3, September, 1916, pp. 408-415.

and others gave encouragement for the production of an immune serum, which had been tried in an experimental way. As a result of further investigations, covering an additional period of three years, a serum has been produced from the horse which will stop immediately the losses in a herd in which calves are dying from blackleg. Also, a germ-free fluid vaccine or aggressin has been produced from calves, which when used upon healthy calves will produce a more durable immunity against blackleg.

BLACKLEG SERUM.

In the preparation of blackleg serum, pure cultures of *B. chauvæi* are grown upon a liver bouillon brain infusion. The culture medium is inoculated from a test tube of stock culture, then grown for twenty-four hours, and passed through gauze. This material is injected intravenously into the jugular vein of a horse in increasing doses, beginning with 50 cc., followed at intervals of seven to ten days with 150, 250, 400 and 400 cc. doses. Nine days after the fifth injection about 20 cc. of blood is drawn from the jugular vein. This is allowed to clot. Fifty-five hundredths cc. of the clear serum is injected subcutaneously into each of three guinea pigs. About fifteen hours after this the guinea pigs are injected subcutaneously with 125 mg. of dried, finely ground muscle from a blackleg lesion of a calf. If none of the three guinea pigs dies within the three following days, the horse is bled upon the third day. The blood is drawn from the jugular vein into sterile 2½-inch glass tubes, 20 inches high. Weights are placed upon the clot within from two to three hours after bleeding. The following day the clear serum is poured off, and after the addition of one-half percent of phenol it is filtered through an infusional earth filter. The filtrate is then bottled and tested for sterility. Upon the second day serum is again poured from the tube.

Each horse is bled 1,200 cc. to each 100 pounds of weight. Ten days after bleeding the horse is again injected with 400 cc. of culture and again tested in nine days. Should the blood fail to be potent, as is true in about 20 percent of the cases, the horse is again injected with 400 cc. of culture on the third day following the test. Some horses will produce a potent serum following one injection after bleeding, while others require two or three injections. The injections are sometimes

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followed by local and general disturbances, resulting in some cases in the death of the horse.

The following table shows the time and amount of injection of cultures in one case:

TABLE I.

March 8	50 cc. cultures of <i>B. chauvæi</i> .
March 16	150 cc. cultures of <i>B. chauvæi</i> .
March 24	250 cc. cultures of <i>B. chauvæi</i> .
March 31	400 cc. cultures of <i>B. chauvæi</i> .
April 7	400 cc. cultures of <i>B. chauvæi</i> .

April 16, 20 cc. of blood was drawn. Fifty-five hundredths cc. of the serum was injected into each of three 12-ounce guinea pigs. Fifteen hours later they were injected with 125 mg. of dry muscle virus, 2 mg. of which would kill a guinea pig not protected with serum. All three of the guinea pigs survived the test. April 19, 13,400 cc. of blood was drawn from this horse.

In the production of serum from three to eight horses are bled at the same time. The serum from all horses bled at one time is mixed prior to filtration.

The following table shows the protective properties of blackleg serum upon calves:

TABLE II.

Number of calf.	Date of injection of serum.	Dose (serum). cc.	Date of injection of virus.	Dose (virus). mg.	Results.
712	2-16-15	20	2-20-15	450	No symptoms.
19	2-16-15	22	2-20-15	250	No symptoms.
25	2-16-15	20	2-20-15	450	No symptoms.
43	2-16-15	15	2-20-15	250	No symptoms.
331	2-16-15	15	2-20-15	1000	No symptoms.

The foregoing table shows that 15 to 20 cc. protected the five calves against infection from the 250 to 1,000 mg. doses of virus. This amount of virus will kill most calves which have not been protected previously. The test shows that a serum which will protect guinea pigs in doses of .55 cc. against 125 mg. of muscle virus will protect calves in doses of 15 to 20 cc. of serum against 250 to 1,000 mg. of virus.

The material is an anti-blackleg serum, and therefore will produce only a passive immunity. In order to produce active immunity the serum must be followed in three days with a virus. From experiments and field observation it is deemed unwise to administer virus in doses exceeding 4 to 8 mg., as the susceptibility of calves varies greatly.

The virus is made up in pellet form. The pellets weigh from 4 to 5 mg. each. They are made by selecting the darkest meat from a blackleg lesion of a calf. This is ground and passed through an 80-mesh sieve. It is then made into pellets with a pill machine, after which the pellets are attenuated at 60° C. for one hour to kill all nonspore-forming organisms which might be present.

As shown by Table II, blackleg serum produces within five days an immunity in calves which will protect them against large doses of virus. Observations in the field show that in nearly every case serum will stop death from blackleg within twenty-four hours after it is administered. On that account serum treatment is valuable for use upon infected herds. Blackleg serum has curative properties when administered in large doses. An injection of 150 cc. of serum will cure blackleg if the calf is treated in the early stages of the disease. Recoveries have taken place in about 50 percent of the cases treated.

The serum and pellet vaccine produced by the College has been used upon about 60,000 animals in the past eighteen months. During this time information has been received of the death of twelve animals upon six farms. These deaths occurred in the earlier tests. These instances of loss might suggest that pellets used after serum are not of sufficient strength to give immunity that will protect all animals. However, the percentage of loss is small, and the immunity is remarkably good when compared with the immunity produced by use of other biologics.

GERM-FREE FLUID VACCINE.

Germ-free fluid vaccine, a tissue aggressin, is made by the inoculation of calves with muscle virus in doses of one gram, or by the use of 20 to 30 cc. of pure cultures of *B. chauvæi*. After the death of the calf the affected tissue is removed, ground, frozen, thawed, and drained; then filtered through infusorial earth filters. The filtrate is bottled after the addition of one-half percent of phenol or one percent of chloroform, and is then tested for potency and sterility. Three guinea pigs are each given 10 cc. of the vaccine, subcutaneously. They are kept under observation for four days to determine whether the material contains pathogenic organisms or toxin. Should any one of the guinea pigs die the material

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is condemned, although it may have passed the sterility test required by the Bureau of Animal Industry of the Department of Agriculture.

One of the most difficult problems in the production of these vaccines is the obtaining of filters which will remove the various microorganisms with which the material becomes contaminated prior to filtration. The material is also difficult to filter because of the high content of albumins.

Table III shows the protective properties of germ-free fluid vaccine given in 8 cc. doses.

TABLE III.

Number of calf.	Weight.	Date of vaccination.	Dose aggressin.	Date test dose.	Size test dose.	Results.
325	<i>lbs.</i> 300	7-3-'15	<i>cc.</i> 8	7-27-'15	<i>mg.</i> 1	No symptoms.
307	300	7-3-'15	8	7-27-'15	1	No symptoms.
311	325	Not vaccinated	7-27-'15	1	Died blackleg (7-29-'15).

It is seen that the vaccine protected calves against a dose of virus which killed a nonvaccinated calf.

The station has sold over 50,000 doses of germ-free fluid vaccine. Field reports indicate that it produces within three days an immunity of high degree and long duration. A standard potency test has not been adopted. The field use of 50,000 doses indicates that it has been highly potent. At present tests are being made which may show, when completed, the average degree of potency to be expected.

There are several problems in connection with the production of these vaccines which require additional study. Further investigations are under way at the present time.

CONCLUSIONS.

Blackleg Serum.

1. Blackleg serum is curative in the early stages of the disease.
2. It will check outbreaks within twelve to twenty-four hours.
3. The use of virus pellets on the third day following the injection of serum produces an active immunity.

Germ-free Fluid Vaccine.

1. It is toxic.
2. It will not cure blackleg.
3. It produces immunity within four to five days after injection.
4. The immunity is of much longer duration than that produced by the powder or pill form of vaccine. The length of immunity conferred by the germ-free fluid vaccine has not yet been determined.

Use of Serum, Serum and Pellets, and Germ-free Fluid Vaccine.

1. Serum should be used upon animals with symptoms of blackleg.
2. Serum and pellets should be used upon herds in which losses are occurring at the time of vaccination.
3. Germ-free fluid vaccine should be used as an annual vaccination upon calves at weaning time or earlier, should conditions indicate necessity.