AGRICULTURAL EXPERIMENT STATION

KANSAS STATE AGRICULTURAL COLLEGE
MANHATTAN, KANSAS

BLACKLEG VACCINES:
THEIR PRODUCTION AND USE

PRINTED BY KANSAS STATE PRINTING PLANT
B. P. WALKER, STATE PRINTER
TOPEKA 1923
9-5555

SUMMARY.

1. Blackleg is an acute disease of young cattle having such a short duration that curative measures should not be relied on.

2. Preventive treatment by early injection of blackleg filtrate or aggressin is highly important.

3. Preventive treatment should be given before losses occur, as the saving of one calf will pay for a large number of doses.

4. The treatment should be administered before changes are made to a more nourishing diet.

5. The immunity produced by the use of blackleg filtrate or aggressin does not develop for three to ten days after its injection, but after this immunity is produced it is of long duration.

6. Should losses occur before treatment is possible it is advisable to give all cattle antiblackleg serum, following this in 10 to 12 days with a dose of filtrate or aggressin.

7. The use of antiblackleg serum will stop all losses within 24 hours, but the protection produced will last only from ten days to three weeks.

8. The early treatment of all calves with blackleg filtrate or aggressin gives good assurance that there will be no losses from blackleg.

9. Vaccination by means of powder vaccines is unsatisfactory in certain respects.

10. The immunity produced by aggressin or filtrate is of a very high degree, both products having practically the same immunizing powers.

11. The absolute length of the immunity produced by aggressin or filtrate is as yet undetermined; however, field work covering over four years shows that an animal vaccinated when six months of age is usually protected until it is no longer susceptible to blackleg.

12. Losses following vaccination with blackleg filtrate or aggressin are less than one in 10,000.

13. Losses before any vaccination was practiced ranged from 3 to 25 per cent of all young cattle in affected districts in the West and Southwest.

14. Losses after powder vaccination was introduced were reduced to nearly 1 per cent.

15. All calves should be vaccinated before six months of age in districts where the presence of blackleg infection is suspected.

16. Calves vaccinated when less than six months old should be revaccinated six months after the first treatment.

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BLACKLEG VACCINES: THEIR PRODUCTION AND USE.[1]

JOSEPH P. SCOTT.

INTRODUCTION.

Prior to 1782 blackleg was considered as a particular form of anthrax, both diseases being treated in various ways, some of which bordered on pure witchcraft.

In 1782 Chabert (1790) differentiated clinically between the two diseases and described blackleg under the term "charbon symptomatique," or symptomatic anthrax. From 1782 till the isolation of the etiological factor by Bollinger in 1875, and Feser in 1876, very little progress was made. Superstitious treatments and ineffectual medical intervention were the rule. Since 1876 many workers have investigated the etiology and prophylaxis of blackleg, and numerous methods of vaccination have been developed.

Airlong, Cornevin, and Thomas (1887), 1876 to 1877, studied the causative agent, *Bacterium chauvaei,* and developed an attenuated muscle vaccine, which was used with success in France. This vaccine, with certain modifications, is used extensively at the present time, in the form of the double or Lyons vaccine or the modified single vaccine. Nocard and Roux (1887) used attenuated pure cultures of *B. chauvaei,* and Roux (1888) used filtrates of broth cultures. Kitt (1894) studied the organism under the term "Baccillus clostridium sarcophysematos bovis," or "Rauschbrandbacillus." He (1888) developed a single muscle-powder vaccine and also used semivirulent pure cultures. He also (Kitt, 1900) used an immune horse serum with some success, and was the first to develop a method of growing the organism aërobically. Kitasato (1889) immunized guinea pigs by the use of pure cultures. Duenschmann (1894) used filtered muscle juices for the immunization of guinea pigs. The cultural characteristics of the organism, *Rauschbrandbacillus,* were exhaustively studied by von Hibler (1908 and 1912), 1899 to 1908. Thomas of Verdun used threads impregnated in cultures of *B. chauvaei* in place of the Lyons vaccine. Grassberger and Schattenfroh (1908) used blackleg toxin in the immunization of rabbits,

1. Contribution No. 29 from the Department of Veterinary Medicine.

cattle, and sheep. Their toxin, however, proved unsatisfactory, as it caused intoxication and death in cattle. Later (1909) these workers used a toxin-antitoxin mixture and immune sera. Foth (1911) used powder vaccines and a filtrate prepared from pure cultures and precipitated with alcohol. Schöbl (1910 and 1912) studied muscle-juice filtrates or aggressins prepared from the affected tissues of guinea pigs and calves. Leclainchee and Vallée (1913) attenuated pure cultures by growing them at high temperatures, 42° to 43° *C.* In Japan experiments on the various methods of producing blackleg vaccines were conducted by Naoshi Nitta (1918), 1905 to 1918, work being done on spore vaccines, antisera and filtrates. A filtrate has been developed which is being extensively used. The basis for this filtrate is meat-piece culture media.

In the United States the Lyons vaccine was used with some success from the time of its introduction. In 1896 Norgaard (1898), head of the Department of Pathology, Bureau of Animal Industry, United States Department of Agriculture, studied the field results obtained by the use of the Lyons vaccine and developed a more satisfactory method of attenuating the powder vaccine. He also introduced a single vaccine. This vaccine is still extensively used and until recently was distributed by the Bureau of Animal Industry. Doctor Norgaard's report says: "It appears that blackleg causes greater losses in the Southern and Western states than all other diseases combined. The losses are placed at from 10 to 20 per cent of the young stock." The same report further states that after the use of 100,000 doses of the improved single vaccine, losses were reduced to 1 per cent or less.

The Kansas Agricultural Experiment Station started its blackleg experiments in 1897, at which time N. S. Mayo (1897) experimented on both the double and single forms of vaccination. Later Mayo and Barnes (1904) developed an improved method of producing and testing both vaccines. From 1911 to 1916 extensive field and experimental work was done by O. M. Franklin and T. P. Haslam (1916) on the use of powder vaccines. In 1914 a highly efficient anti-blackleg serum was produced. The Twentieth Biennial Report of the Kansas State Board of Agriculture, 1915-1916, reports that 25,000 head had been treated with perfect results. In 1916 blackleg aggressin was developed and placed on the market. Since the introduction of the aggressin 150,000 doses have been distributed, and reports of losses have been very small, 10 or 12 having been received. In 1917, L. W. Goss (1917 and 1919) and the writer used

filtrates from brain-liver cultures of *B. chauvaei*. Since testing this product, in the field and on experimental animals 100,000 doses have been sold, with the report of the loss of eight calves, or less than one in 10,000.

In 1917, Eichorn (1917) introduced into the United States an improved Japanese method of producing blackleg filtrate.

BLACKLEG.

DEFINITION.

Blackleg—also known as black quarter, quarter ill and symptomatic anthrax—is an acute, infectious, but noncontagious disease of cattle, and exceptionally of other ruminants. It has been reported to occur in swine. This disease is characterized by a sudden appearance of lameness, followed by a rapid development of muscular and subcutaneous swellings containing gas. These symptoms are followed by prostration and death in from 12 to 48 hours.

OCCURRENCE.

Blackleg is found throughout the western half of the United States, especially in the Southwestern states and the eastern slopes of the Rocky Mountains. In Canada blackleg is found in the western provinces; in Europe, in the mountainous districts of Germany and Austria, and to a less extent in France, Switzerland, and England.

MORTALITY.

The mortality from blackleg is high. Before vaccination for blackleg was introduced, and at the present time in unvaccinated herds, losses from blackleg are from 3 to 25 per cent of the young cattle in affected districts. Thrifty calves from six months to two years of age are most susceptible. Calves less than six months of age are rarely affected, although cases of very young calves, a few weeks or even a few days old, have been reported. Cattle over two years of age may become affected; a few cases of old cattle dying from blackleg have been reported. Good, fat, beef calves are very susceptible, while poor, thin calves and calves of the dairy breeds are not as commonly the victims of the disease. Losses from blackleg are greatest during the seasons of the year when calves are making their best gains—in the spring after the calves have been put on pasture and in the fall when they are taken off the dried pastures and put in the feedlot.

ETIOLOGY.

Description of Organism. Blackleg is caused by an anaërobic bacillus, *Bacillus chauvaei (Bacillus gangrenæ emphysenzatosœ, Bacillus clostridium sarcophysematos bovis,* or *Rauschbrandbacillus),* or the blackleg bacillus. This organism is found in rich, moist soil, from which it enters the animal body either through wounds, bruises, or the intestinal tract.

B. chauvaei is a large, slender bacillus, 2 to 6 microns in length and 0.5 micron in thickness. The ends are rounded. The bacillus is motile by means of peritrichic flagella. The spores develop usually at the end of the rod. They are oval in shape and cause a buldging of the organism. A small amount of the cell surrounds the spore. The organism is easily stained by aqueous solutions of aniline dyes. As a rule it is gram positive, though some cultures show a gram negative reaction.

Cultural Characteristics. *B. chauvaei* is an obligate anaërobe. It may be grown in fresh Martin's broth or dextrose broth (Goss, 1921), in either medium rapidly developing a cloudiness, and later forming a flaky, white precipitate. In milk *B. chauvaei* causes a fine clot, not always readily detected. Gelatin may be liquefied by the organism, while insipisated blood serum or other solid albuminous media are not digested. (Deep colonies in agar media are quite characteristic of *B. chauvaei.* These colonies are small, solid colonies having a smooth outline. In moderately hard agar the colonies appear as small pinpoints. They are spherical or slightly lenticular in shape. Atypical forms may show a slight woolly outline.) In the presence of the organism Hibler's brain medium remains white and becomes acid; most sugars are fermented and large amounts of gas are produced. The organism is not particularly sensitive to the reaction of media. It grows equally well in media that are slightly acid or slightly alkaline. Cultures of *B. chauvaei* have a characteristic sour or butyric-acid odor, which is not particularly unpleasant. This organism may be grown aerobically in media containing brain or meat pieces. A very luxuriant growth is produced in a brain-liver medium.[2]

2. This medium is a modification by T. P. Haslam, Kansas State Agricultural College, 1913, of von Hibler's (1908) brain media and of Pfuhl's (1907) liver broth. It is prepared from equal parts of beef or calves' brains and liver. The brain is ground, a small amount of water is added, and the mixture steamed for one to two hours. Broth is made from the liver by steaming the ground liver for one hour and straining out the solid material. Peptone and salt are added to the liver broth to make a 1 per cent peptone solution and a one-half of 1 per cent salt solution. Test tubes and culture flasks are filled with from one-half to one inch brain stew and an equal amount of liver broth added. The medium is then sterilized at 15 pounds for one to three hours, depending on the size of the culture flasks used.

Pathogenicity. Cattle from six months to two years of age are very susceptible to natural infection. Very young calves a few days old occasionally contract the disease, and old cattle may die from blackleg. Susceptible animals are killed by inoculation of from one-half to one gram of powdered muscle virus, or 5 to 10 mil.[3] of culture virus. Guinea pigs are very susceptible to inoculation and are killed by injections of 1 mg. of powder virus or 0.05 to 0.2 mil. of culture virus. *B. chauvaei* is nonpathogenic for rats; it may be pathogenic for rabbits in large doses. It is very resistant to heat and disinfectants, and pastures once infected remain so almost indefinitely.

SYMPTOMS.

The symptoms develop rapidly. There is loss of appetite and rumination, rise in temperature, increased heart beat and respiration, swelling of muscular parts of the body, usually in one of the hind quarters, and marked lameness. The swelling is at first painful, crepitating and tympanitic to the touch, later becoming insensitive and firmer, the skin being stretched as tight as a drum. In some cases the swelling may involve a considerable portion of the subcutaneous tissue, especially over the abdomen; occasionally only a very slight swelling is noticed, the lesions developing in the depths of the muscle. The lesion develops rapidly, the temperature reaching 103° to 105° F. The animal is unable to rise; respirations become labored; and death occurs 12 to 36 hours after the symptoms develop.

POST MORTEM.

After the skin has been removed the carcass will be seen to have a characteristic diffused pink color. In the vicinity of the lesion the connective tissue is found to be full of dark-red fluid or semigelatinous material. The characteristic blackleg odor is very noticeable, especially after the muscles are cut; the affected muscle shows black spots or streaks intermixed with lighter areas, giving the characteristic mottled appearance. Some muscles may be very light and contain numerous small gas bubbles.

The internal organs are fairly normal in a fresh carcass. The peritoneum is injected and the peritoneal cavity contains from a pint to a quart of dark-red exudate. The liver is usually normal. It may contain numerous gas bubbles, especially in an old carcass, in which case it is soft and yellowish. The spleen, usually normal, in an old carcass may be enlarged. The pleura is injected and the

8. "Mil." is used for "milliliter," the one-thousandth part of a liter, formerly referred to as cubic centimeter (cc.).

pleural cavity contains some dark-red exudate. The heart contains a large black clot. The pericardial sac usually contains some exudate. The blood is rather dark, and possibly coagulates a little slower than normally.

BLACKLEG VACCINES.

The prevention of blackleg is of very great importance, as very large numbers of cattle are affected by this disease. Sometimes as high as 5 to 25 per cent of the young stock are affected. Curative measures have not stopped the ravages of the disease. Successful control can be accomplished only by the use of blackleg vaccines.

POWDER VACCINE.

Prevention of blackleg by means of vaccination has been practiced for forty years. Airlong, Cornevin, and Thomas (1887) discovered that cattle could be immunized against blackleg by the use of virulent material in various forms. In 1883 they developed the attenuated muscle or Lyons vaccine. This vaccine has been used extensively in Europe (Leclainche and Vallèe, 1900 and 1909) and in this country.

Production of Powder Vaccine. To produce powder vaccine the blackest muscle fibers in a lesion of blackleg are obtained. This muscle is dried, ground up in a fine mill, and the powder mixed with a certain amount of water and heated in an oven for six hours at about 96° C. This attenuated material or vaccine is put up in the form of pills or powders. These vaccines are sold in two forms–the double-treatment vaccine and the single-treatment vaccine. The double-treatment consists of two vaccines, a weak and a strong. The weak vaccine is given first and the strong vaccine ten clays later. The single treatment consists of a vaccine intermediate in strength between the weak and the strong vaccines of the double treatment.

The results from the use of attenuated vaccines have not been entirely satisfactory. Numerous cases of loss either immediately following vaccination or several weeks after have been observed. In affected district's the use of the attenuated vaccines has reduced loss to about 1 per cent.

Use and Results from Use of Powder Vaccine. In 1912 the Veterinary Department of the Kansas Agricultural Experiment Station conducted numerous experiments on various makes of powder and pill forms of attenuated muscle vaccine. These experiments showed that spore vaccines were at best somewhat unreliable because of the narrowness of the range of virulence between a good and 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 showed the weakness of spore vaccines, while the work done by Kitt in 1900, Leclainche and Vallèe in 1900, Grassberger and Schattenfroh (1908 and 1909) in 1907, and Foth (1911) gave encouragement for the production of an immune serum, which had been tried in an experimental way.

ANTIBLACKLEG SERUM.

As a result. of further investigations by Drs. 0 .M. Franklin and T. P. Haslam, covering an additional period of three years, 1913 to 1915, a serum was produced from horses which will immediately stop the losses in a herd in which calves are dying from blackleg. A germ-free fluid vaccine or aggressin was also produced from calves, 1912 to 1916. This material, when used upon healthy calves, will produce a durable immunity against blackleg.

Production of Antiblackleg Serum. In the preparation of blackleg serum, pure cultures of *B. chauvaei* are grown upon brain and liver medium. 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 mil., followed at intervals of seven to ten days with 150,250,400, and 450-mil. doses. (Table I.) Nine days after the fifth injection about 20 mil. of blood is drawn from the jugular vein. This is allowed to clot. Into each of three guinea pigs 0.55 mil. of the clear serum is injected subcutaneously. About 15 hours after this the guinea pigs are injected subcutaneously with 125 mg. (60 M.L. D.[4]) of dried, finely ground muscle from the 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 2.5-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 of 1 per cent of phenol it is filtered through an infusorial earth filter.

Each horse is bled 1,200 mil. to each 100 pounds of weight. Ten days after bleeding the horse is again injected with 400 mil. of cul-

4. "M. L. D.," minimum lethal dose, determined as in Table VII.

ture and tested nine days later. Should the blood fail to be potent, as is true in about 20 per cent of the cases, the horse is again injected with 400 mil. 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 to three injections. The injections are sometimes followed by local and general disturbances, resulting in some cases in the death of the horse.

Table 1 shows the dates and amount of injection of cultures in one case—horse No. 23.

TABLE I.—Injection of cultures of *B. chauvaei* in the hyperimunization of horse No. 23.

Date	Dose, mil.	Strain No.
March 8, 1917	50	5
March 16, 1917	150	6
March 24, 1917	250	3
March 31, 1917	400	1
April 7, 1917	400	2

TABLE II.—Potency of serum obtained from horse No. 23.

Guinea pig		Serum		Virus		Results, first, second, and third days		
No.	Weight, grams	Dose, mil.	Date	Dose M. L. D.	Date	First	Second	Third
627	450	0.55	4-16-'17	62	4-17-'17	(a) 3 X	3 X	2 X
628	250	.55	4-16-'17	62	4-17'-17	3 X	2 X	1 X
629	375	.55	4-16-'17	62	4-17-'17	1 X	1 X	1 X

(a) "X" shows the degree of swelling produced by the virus. 1X, slight swelling; 2X, moderate swelling; 3X, large swelling.

This experiment shows that 0.55 mil. serum of horse No. 23 has a protective strength of 62 immunizing units; 1 mil., therefore, has a strength of 111 immunizing units.

On April 16, 1917, 20 mil. of blood was drawn from the jugular vein. Into each of three 12-ounce guinea pigs 0.55 mil. of the serum was injected. Fifteen hours later they were injected with 125 mg. of dry muscle virus. (Two mg. of this virus would kill a guinea pig not protected with serum.) All three of the guinea pigs survived the test. (Table II.) On April 19, horse No. 23 was bled, 11,350 mil. being drawn from the jugular vein. On April 20 the serum from four

horses, Nos. 23, 24, 25, and 26, was mixed and filtered after the addition of perservative. This filtered serum was put up as antiblackleg serum, serial No. "Y."

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. Each filtration forms a serial. The potency of antiblackleg serum, serial Nos. 45 to 54, has been found to have a protective strength of 70 to 100 units at the present time (September, 1921) as compared to strength of 111 units when produced in 1917 and 1918. (Scott, 1922.)

Use of Antiblackleg Serum. Table III shows the protective properties of antiblackleg serum on calves.

TABLE III.—Protective properties of antiblackleg serum.

Calf No.	Injection of serum		Injection of virus		Results
	Date	Dose, mil.	Date	Dose, mg.	
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	1,000	No symptoms

Table III shows that 15 to 20 mil. of serum protected each of the five calves against infection from the 250 to 1,000 mg. doses of virus. This amount of virus would have killed most calves which had not been previously protected. The test shows that a serum which protected guinea pigs in doses of 0.55 mil. against 125 mg. (62 M. L. D.) of muscle virus also protected calves against 250 to 1,000 mg. blackleg virus, when used in doses of 15 to 20 mil.

This material is an antiserum, and therefore will produce only a passive or short immunity. To produce lasting immunity a dose of blackleg filterate or aggressin is given 10 to 12 days after the serum. A strong muscle virus is sometimes used three days after the serum, but its use has the same objection as those for the attenuated powder vaccines.

As shown by Table III, antiblackleg serum produces immunity in calves within five days, which will protect them against large doses of virus. Observations in the field show that in nearly every case serum will prevent death from blackleg. On that account serum

treatment is valuable for use upon infected herds, as losses are stopped within 24 hours. Blackleg serum has curative properties when administered in large doses. Field reports show that an injection of 150 to 300 mil. of serum will cure blackleg if the calf is treated in the early stages of the disease. Recoveries have taken place in about 50 per cent of the cases treated.

BLACKLEG AGGRESSIN.

Roux (1888) in 1887 experimented with filtrates of cultures of *B. chauvaei,* and Schöbl (1910) worked with filtrates from the muscle juices of calves and guinea pigs artificially inoculated with blackleg. The immunity produced by Schöbl's aggressin was very high. At the Kansas Agricultural Experiment Station, O. M. Franklin and T. P. Haslam while working with antiblackleg serum also experimented with aggressins, 1913 to 1916, at which time they perfected the product now known as blackleg aggressin.

Production of Blackleg Aggressin. Blackleg aggressin is prepared from a typical blackleg lesion artificially produced in a healthy calf by the injection of from 15 to 25 mil. of pure culture of *B. chauvaei.* All the juices which can be pressed out from the affected muscles and connective tissue are phenolized and filtered through Mandler filters. The product is then tested for sterility on culture media, and for toxicity in guinea pigs. Two pigs are used for this purpose, one receiving 5 and the other 7 mil. of aggressin. Both pigs must live and should not show any symptoms of any kind. (Table IV).

TABLE IV.—Toxicity test of blackleg aggressin and blackleg filtrate on guinea pigs.

Guinea pig		Product		Results—first, second, third, and fourth days			
No.	Weight, grams	Dose, mil.	Date	First	Second	Third	Fourth
BLACKLEG AGGRESSIN							
857	500	5	10-20-'19	O. K.	O. K.	O. K.	O. K.
858	400	7	10-20-'19	O. K.	O. K.	O. K.	O. K.
307	400	15	1-14-'20	O. K.	O. K.	O. K.	O. K.
308	450	23	1-14-'20	2 X	1 X	O. K.	O. K.
BLACKLEG FILTRATE							
285	300	5	1-13-'20	O. K.	O. K.	O. K.	O. K.
286	400	7	1-13-'20	O. K.	O. K.	O. K.	O. K.
305	500	15	1-14-'20	O. K.	O. K.	O. K.	O. K.
306	500	25	1-14-'20	O. K.	O. K.	O. K.	O. K.

Use and Properties of Blackleg Aggressin. Blackleg aggressin is used in doses of 5 mil., the same dose being used for cattle of all ages. Field reports on several hundred thousand head have shown almost perfect results; less than one in 10,000 cattle vaccinated with aggressin have been reported as having become affected with blackleg.

The high degree of immunity produced by blackleg aggressin is shown in Table V.

TABLE V.—Immunity produced by the use of blackleg aggressin.

Calf No.	Injection of aggressin		Injection of virus		Results—first, second, third, and fourth days			
	Dose, mil.	Date	Dose, mg.	Date	First	Second	Third	Fourth
1256	5	11-21-'18	5	2-3-'19	O. K.	O. K.	O. K.	O. K.
1266	5	11-21-'18	5	2-3-'19	O. K.	O. K.	O. K.	O. K.
1267	4	11-21-'18	5	2-3-'19	O. K.	O. K.	O. K.	O. K.
1268	3	11-21-'18	5	2-3-'19	O. K.	O. K.	O. K.	O. K.
1269	Not vaccinated		5	2-3-'19	Death			

The fact that this substance is an aggressin and not a toxin is shown in Tables IV and VI. Table IV shows that very large doses, 25 mil., are harmless to guinea pigs, thus showing that the product

is not a toxin. Table VI shows that small amounts of washed culture when added to blackleg filtrate or aggressin produce typical blackleg. Washed cultures are nonpathogenic to guinea pigs even in large doses. (Table VI.)

TABLE VI.—Pathogenicity of washed cultures of *B. chauvœi*.

Guinea pig		Products injected				Results—first, second, third, and fourth days			
		Washed culture		Dose, mil.	Date				
No.	Weight, grams	Dose, mil.	Date			First	Second	Third	Fourth
223	325	3.0	12-20-'19	.....	.....	O. K.	O. K.	O. K.	O. K.
224	425	.5	12-20-'19	.....	.....	O. K.	O. K.	O. K.	O. K.
255	300	6.0	1- 5-'20	.....	.....	O. K.	O. K.	O. K.	O. K.
256	300	5.0	1- 5-'20	.....	.....	O. K.	O. K.	O. K.	O. K.
257	300	2.5	1- 5-'20	.....	.....	O. K.	O. K.	O. K.	O. K.
330	500	10.0	1-19-'20	.....	.....	O. K.	O. K.	O. K.	O. K.
				Blackleg Filtrate.					
365	200	1.0	1-21-'20	5	1-21-'20	Death	.....	.....	.....
366	200	1.0	1-21-'20	4	1-21-'20	Death	.....	.....	.....
367	225	1.0	1-21-'20	3	1-21-'20	1 X	O. K.	O. K.	O. K.
368	250	1.0	1-21-'20	2	1-21-'20	O. K.	O. K.	O. K.	O. K.
				Blackleg Aggressin.					
360	300	1.0	1-21-'20	5	1-21-'20	3 X	Death	.....	.....
361	200	1.0	1-21-'20	4	1-21-'20	Death	.....	.....	.....
362	225	1.0	1-21-'20	3	1-21-'20	O. K.	O. K.	O. K.	O. K.
363	300	1.0	1-21-'20	2	1-21-'20	O. K.	Death	.....	.....
364	250	1.0	1-21-'20	1	1-21-'20	O. K.	O. K.	O. K.	O. K.

Test for Potency. The standardization and potency of blackleg aggressin is still somewhat under discussion (Ward, 1919). An immunization test in which guinea pigs are immunized by various doses of aggressin and later injected with a certain dose of virus has been suggested. On account of the considerable individual variation in the resistance of guinea pigs to blackleg and the fact that the only satisfactory test of potency—a calf test—is prohibitive in cost, another method of testing the potency of aggressins and filtrates was worked out. This test has been called the neutralization test (Goss and Scott, 1918). In this test it is attempted to determine how much aggressin will neutralize a known amount of antiserum and allow a small dose of virus to kill a guinea pig. This test is shown in Table VII.

TABLE VII.—Neutralization test to determine the aggressive strength of blackleg aggressin and filtrate.

Guinea pig		Normal serum		Virus		Product injected		Results—first, second, third, and fourth days			
No.	Weight, gm.	Dose, mil.	Date	Dose, mil.	Date	Dose, mil.	Date	First	Second	Third	Fourth
18	400	.....	.....	0.1	11-13-'19	.....	.....	1 X	1 X	1 X	O. K.
19	300	.....	.....	.2	11-13-'19	.....	.....	O. K.	3 X	(*a*) Death	.....
20	300	.....	.....	.3	11-13-'19	.....	.....	2 X	Death	.....	.....
				M. L. D.							
36	400	0.5	11-17-'19	4.0	11-18-'19	.....	.....	1 X	2 X	O. K.	O. K.
37	300	.5	11-17-'19	5.0	11-18-'19	.....	.....	1 X	1 X	O. K.	O. K.
38	500	.5	11-17-'19	6.0	11-18-'19	.....	.....	2 X	1 X	O. K.	(*b*) O. K.
39	300	.5	11-17-'19	7.5	11-18-'19	.....	.....	2 X	3 X	Death	.....
						Blackleg Aggressin					
195	200	1.0	12-17-'19	1.0	12-18-'19	5	12-18-'19	3 X	Death	.....	.....
196	275	1.0	12-17-'19	1.0	12-18-'19	4	12-18-'19	3 X	(*c*) Death	.....	.....
197	300	1.0	12-17-'19	1.0	12-18-'19	3	12-18-'19	1 X	1 X	1 X	1 X
						Blackleg Filtrate					
205	300	1.0	12-19-'19	1.0	12-20-'19	5	12-20-'19	3 X	(*d*) Death	.....	.....
206	225	1.0	12-19-'19	1.0	12-20-'19	4	12-20-'19	2 X	2 X	2 X	O. K.
207	200	1.0	12-19-'19	1.0	12-20-'19	3	12-20-'19	1 X	O. K.	O. K.	O. K.
208	400	1.0	12-19-'19	1.0	12-20-'19	2	12-20-'19	1 X	1 X	1 X	O. K.

(*a*) Two-tenths mil. of this virus is found to be the minimum amount necessary to kill or the minimum lethal dose—*M. L. D.*
(*b*) One mil. normal serum protects against twelve *M. L. D.* virus.
(*c*) Pig No. 196 received 12 immunizing units of serum (*b*) and 1 *M. L. D.* of virus plus 4 mil. of aggressin. Four mil. of aggressin, therefore, neutralized eleven immunizing units of serum. One 5-mil. dose of blackleg aggressin has a strength of 13 aggressive units.
(*d*) Five mil. of blackleg filtrate neutralizes eleven immunizing units of serum; therefore, this filtrate has an aggressive strength of eleven units per 5-mil. dose.

It is seen in Table VII that 0.5 mil. of normal serum protects a guinea pig against 6 M.L.D. of balackleg virus, and that 7.5 M.L.D. of virus just kills a guinea pig which received 0.5 mil. of normal serum 15 hours previously. One mil., therefore, protects a guinea pig against 12 M.L.D. of blackleg virus. The amount of antiserum or of normal serum required to protect a guinea pig against 1 M.L.D. of blackleg virus is one antiblackleg unit. Therefore, 1 mil. of serum contains 12 antiblackleg units.

The administration of 1 mil. normal serum, and 15 hours later of 1 M.L.D. virus plus 4 mil. of blackleg aggressin, caused death of the guinea, pig from blackleg. The 4 mil. of aggressin increased the virulency of the single M.L.D. of blackleg virus sufficiently to neutralize the action of the 12 antiblackleg units of serum. Counting one aggressive unit as that amount of blackleg aggressin that will neutralize one antiblackleg unit, it is seen that 4 mil. of aggressin have an aggressive action equal to 11 aggressive units, and that one 5-mil. dose of this aggressin has an aggressive strength of 13 units.

BLACKLEG FILTRATE.

Artificial culture filtrates have been used by various workers, notably by Roux (1888), who immunized guinea pigs by means of old broth cultures heated to 115° C. and filtrates of such cultures. Duenshmann and also Grassberger and Schattenfroh produced a filtrate from cultures and had variable success with its use. Nitta in 1905 produced a filtrate that has been used extensively in Japan. Eichhorn (1917), using Nitta's method, produced a filtrate in this country. During 1917, L. W. Goss and the writer, of the Veterinary Department of the Kansas Agricultural Experiment Station, produced a filtrate which showed very high immunizing properties.

Production of Blackleg Filtrate. This filtrate is produced by growing several virulent strains of *B. chauvœi* in brain-liver media for a period of nine days, at which time there is very great spore formation and the medium is apparently saturated with the products of growth. The fluid part of the culture is then removed, phenolized, and filtered in the same way as the blackleg aggressin. The finished filtrate is tested for sterility on brain-liver media and glucose broth in the same way as the aggressin, and the same guinea-pig tests are made. Table IV shows that blackleg filtrate is absolutely nontoxic, not even an absorption lesion developing in the pig that received 25 mil.

Use and Properties of Blackleg Filtrate. Blackleg filtrate produces a high degree of immunity in calves, as shown in Table VIII.

TABLE VIII.—Immunity produced by the use of blackleg filtrate.

Calf No.	Product injected				Results—first, second, third, and fourth days			
	Filtrate		Virus					
	Dose, mil.	Date	Dose, mil.	Date	First	Second	Third	Fourth
1260.....	(a) 5	1-22-'18	5	2-3-'18	Death	.....	.....	.....
1261.....	5	1-22-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.
1262.....	4	1-22-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.
1263.....	3	1-22-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.
1264.....	Not vaccinated	.....	5	2-3-'18	Death	.....	.....	.....
1099.....	5	1-12-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.
1100.....	5	1-12-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.
1101.....	5	1-12-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.
1102.....	5	1-12-'18	5	2-3-'18	O. K.	O. K.	O. K.	O. K.

(a) The loss of calf No. 1260, following vaccination with the normal dose of filtrate, may have been due to unusual susceptibility of the individual calf, together with the large dose of virus used in this case, as in other cases included in the table.

Tests for Potency. The relation of the potency tests (Neutralization and guinea-pig immunization tests), as run on blackleg filtrate, to the immunity produced in calves, is shown in Table IX, in which the same serial is subjected to all three tests.

TABLE IX.—Comparison of calf immunity, guinea pig immunity, and neutralization produced by blackleg filtrate.

Animal injected		Normal serum		Blackleg filtrate		Virus		Results—first, second, third, and fourth days			
No.	Weight, gm.	Dose, mil.	Date	Dose, mil.	Date	Dose, mil.	Date	First	Second	Third	Fourth
CALF IMMUNITY TEST											
Calf 1				5	10-23-'17	10	11- 7-'17	O. K.	O. K.	O. K.	O. K.
2				5	10-23-'17	10	11- 7-'17	O. K.	Lameness	O. K.	O. K.
3				5	10-23-'17	10	11- 7-'17	O. K.	O. K.	O. K.	O. K.
1020						10	11-12-'17	O. K.	Lameness	Death	
1021						10	11-12-'17	Lameness	Lameness	Death	
GUINEA PIG IMMUNITY TEST											
Guinea pig 988	275			2	1-30-'18	*M. L. D.* 2	2-11-'18	Death			
989	250			3	1-30-'18	2	2-11-'18	Death			
990	275			3	1-30-'18	2	2-11-'18	3 X	Death		
991	300			4	1-30-'18	2	2-11-'18	Death			
992	375			4	1-30-'18	2	2-11-'18	Death			
993	250			5	1-30-'18	2	2-11-'18	O. K.	O. K.	O. K.	
994	300			5	1-30-'18	2	2-11-'18	O. K.	O. K.	O. K.	
NEUTRALIZATION TEST											
514	330	(*a*) 1	5-27-'18	1	5-28-'18	1	5-28-'18	O. K.	O. K.	O. K.	O. K.
515	240	1	5-27-'18	2	5-28-'18	1	5-28-'18	O. K.	O. K.	O. K.	O. K.
516	360	1	5-27-'18	3	5-28-'18	1	5-28-'18	O. K.	O. K.	O. K.	O. K.
517	270	1	5-27-'18	4	5-28-'18	1	5-28-'18	1 X	(*b*) Death		
518	270	1	5-27-'18	5	5-28-'18	1	5-28-'18	O. K.	O. K.	O. K.	O. K.

(*a*) One mil. of the normal serum was found to have a strength of 15 antiblackleg units.
(*b*) This guinea pig, receiving 4 mil. of filtrate plus 1 *M. L. D.* of virus, is killed; therefore, 4 mil. of filtrate has an aggressive strength of 14 units and a 5-mil. dose an aggressive strength of 18 aggressive units.

Table VIII shows that a filtrate having an aggressive strength of 18 will give calves sufficient immunity to withstand large doses of virus.

Blackleg filtrate acts as an aggressin in the same may as blackleg aggressin, in that a few milliliters will activate small doses of inert washed blackleg culture. (Table VI.) The purity of blackleg filtrates may be determined by means of the complement fixation test (Gouchenour, 1920).

Blackleg filtrate is given in doses of 5 mil. to all cattle, and has been shown to protect such animals till they are no longer susceptible to blackleg, Field reports show that losses are no greater than following the use of blackleg aggressin; that is, a loss of less than one in 10,000.

DOSAGE OF BLACKLEG VACCINES.

The point of inoculation for blackleg vaccines is preferably the skin in front of the shoulder. Powder vaccines are usually put up in 10 or 25-dose packages. This powder is mixed with boiled water, and the straw-colored fluid obtained by filtration through filter papers is divided into the requisite number of parts. One dose of powder vaccine is 10 mg. Blackleg filtrate and aggressin are given in doses of 5 mil. to all animals. Antiblackleg serum is given in doses of from 15 to 35 mil., as follows:

Minimum dose.	Weight of calf.
15 mil.	300 pounds or under.
20 mil.	300 to 500 pounds.
25 mil.	500 to 600 pounds.
30 mil.	600 to 700 pounds.
35 mil.	700 to 900 pounds.

Ten days after the administration of serum a dose of 5 mil. of blackleg aggressin or filtrate is given.

TIME AND AGE FOR TREATMENT.

It is advisable to treat calves which are on pasture with their dams, in the early fall before the fall rains make a fresh start of grass, as losses usually occur with or following an improvement in pasture conditions. If calves are in badly infected pastures or lots it is advisable to treat them as young as three or four weeks of age. Animals treated at this age should be treated again when five to eight months old; otherwise one treatment has proved to be sufficient. All calves should be vaccinated before six months of age in districts where the presence of blackleg infection is suspected.

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