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BLACKLEG IN KANSAS, and PROTECTIVE INOCULATION.

BLACKLEG, also known as quarter-ill, black-quarter, symptomatic anthrax, French *charbon symptomatic,* German *rausch-brand,* Dutch *spons ziekte* (sponge disease), was formerly regarded as a form of anthrax. The two diseases, however, are entirely distinct.

It has been long known that blackleg is not a contagious disease, and that the meat from affected animals could, without harm, be consumed as food by man or beast. Making *post mortem* examinations upon such animals or removing their hides are operations that are entirely without danger. It must, however, be borne in mind that anthrax, a very dangerous disease in man, also occurs in cattle, resembles blackleg in some respects, and must therefore be guarded against. Though the meat from blackleg carcasses must be regarded as absolutely harmless, it cannot be considered a wholesome article of diet. It decomposes readily, and, if preserved, will develop a disagreeably sweetish and rancid odor, reminding one of smoked herring (Kitt).

Clinical descriptions that were furnished many years ago show very plainly that blackleg and anthrax are two distinct diseases. But not until 1860, and again in 1875, when Feser discovered the presence of slender club- and spindle-shaped, rod-like organisms in the blood of blackleg victims, and proved them to be the cause of the disease, was it fully demonstrated that blackleg and anthrax were entirely distinct from each other. Feser produced the disease experimentally in rabbits, sheep, and cattle, by inoculating them with mud taken from blackleg regions in the Alps. In the same year similar experiments were made by Bollinger.

More recent investigations concerning the nature of blackleg and the biological characters of the blackleg germ were carried on in France by Arloing, Cornevin, and Thomas. The most important result of their work was the introduction of protective inoculation, or vaccination against blackleg.

Occurrence— Geographical Distribution. Blackleg occurs in both hemispheres of the world. The annual losses from this disease in the various countries of Europe and in the United States are something enormous. Although it seems that the Western states are especially unfortunate in sustaining heavy losses, perhaps no state in the union is entirely free from this disease. All but seven of the one hundred and five counties of Kansas have reported losses from blackleg, and there is no reason whatever for assuming that those seven counties are free from it. Blackleg is a stationary, infectious disease; *i. e.,* it is a disease that confines itself to certain restricted areas or localities—to certain sheds, stables, fields, etc. In these places it remains indefinitely, while certain adjoining places and buildings are entirely free from this disease. The disease occurs in uplands, in mountainous regions, and on lowlands. Low, black soils, in river bottoms, swamps, and other wet places, are most dangerous.

Climatic Conditions. The disease occurs in all seasons of the year, but causes greatest losses late in the spring, during summer, and early autumn. Stable feeding and pasturing no doubt influence infection, but in no case can the disease occur without the presence of the blackleg germ.

Influence of Class, Breed, Sex, Age, etc. Blackleg is a distinctly bovine disease. Sheep and goats are occasionally affected; rabbits, guinea-pigs and other animals can be infected experimentally. A few cases of natural infection in the horse have been observed. No one breed of cattle is more susceptible to blackleg than any other. However, from the manner in which infection occurs, thin-skinned animals, hence thoroughbreds and grades, are more susceptible than thick-skinned or common cattle. Native cattle are less liable to become infected than newly introduced stock, especially if the latter came from regions where blackleg did not exist. This may be explained by the fact that native cattle in blackleg regions have, either by exposure or through hereditary influences, acquired a certain degree of immunity. In mixed herds, it is easy to observe that the thoroughbreds and grades are the first to die of blackleg. Sex has little if any influence on the production of the disease. It is stated by some that male animals seem to be the more susceptible, but there may be other explanations for this.

No single factor exercises a greater influence than age. Young animals, especially those between the ages of six and eighteen months, are most liable to become infected. However, contrary to general opinion, the disease may occur at practically any age. From 502 reports received from practical stockmen,

2 reported undoubted blackleg in calves 7 to 10 days old.
4 reported undoubted blackleg in calves 2 to 3 weeks old.
24 reported undoubted blackleg in calves 4 weeks old.
46 reported undoubted blackleg in calves 6 to 8 weeks old.
75 reported undoubted blackleg in calves 10 to 14 weeks old.
75 reported undoubted blackleg in calves 3 months old.
67 reported undoubted blackleg in calves 4 months old.
51 reported undoubted blackleg in calves 5 months old.
71 reported undoubted blackleg in calves 6 months old.
88 reported that 7 to 18 months was the youngest age at which they ever observed this disease.

Aside from class, breed, sex, and age, many other conditions no doubt influence the development of blackleg in cattle. Among the best known of these is a condition of general thriftiness and rapid growth. It is a matter of common observation among stockmen that the thrifty and well-nourished young cattle, especially in the spring and early summer, are always more liable than others to take blackleg. It must not be supposed, however, that animals in poor condition never take the disease. Greatly emaciated animals may take the disease and die.

Just why very young animals are more or less immune to the disease is not known. The fact that suckling calves receive animal nourishment was suggested by Arloing, Cornevin and Thomas to be a partial explanation. Another factor of importance is that suckling calves, by not being exposed to slight injuries, abrasions, etc., produced by eating rough food, escape one opportunity for infection. It is known, however, that artificial inoculations in young calves are never as successful in producing disease as they are with older animals. There is no doubt that the chemical and physiological condition of the blood and tissues themselves, at a certain age and under certain conditions, offers more favorable conditions for the growth of the disease germ than at other times. The exact nature of these conditions, however, has not yet been learned.

Animals Affected. Blackleg confines its ravages almost entirely to cattle, sheep, and goats, and in very rare cases horses also take the disease. Pigs, dogs, cats and rabbits are practically immune. The disease is not known to occur in human beings. When horses and mules are inoculated a local reaction (swelling) is the only result. The disease can be produced in cattle by inoculating them with the blood of an affected animal.

The Cause of Blackleg. The cause of every infectious disease is in the main twofold—(1) the direct or exciting cause, and (2) the indirect or predisposing cause or causes. In blackleg, the predisposing causes are due to racial as well as to individual peculiarities. As far as racial predisposition in cattle is concerned, it is limited to the condition of the skin. Thin-skinned animals are more easily wounded, and hence more liable to become infected. And, as before stated, breeds of cattle that have been exposed to the disease, by being raised in a blackleg district, possess a certain degree of immunity which is transmitted to the offspring. However, this would be individual rather than racial in character.

As to predisposition in individuals, there exists the greatest possible difference. Some animals are highly susceptible; others of the same breed are perfectly immune. Between the two extremes we find any number of intermediate conditions. Age always influences individual predisposition. Thus, while some animals are susceptible at almost any age, and others are immune at all times, the great majority of susceptible animals show the greatest predisposition at a certain age—six months to eighteen months or two years.

Finally, the individual animal's condition, by which we mean its general thriftiness, its rate of taking on new flesh, influenced by any change in diet, etc., is an important factor in determining susceptibility. It is known, for instance, that young, thrifty, rapidly growing animals, especially when this rapid growth is caused by a sudden favorable change in diet, as turning out on green pasture after dry winter feeding, are always in greater danger of an attack by this disease than other animals.

The Direct Cause of Blackleg. The direct or exciting cause of blackleg is a parasitic vegetable organism belonging to the bacteria, and known as the blackleg bacillus. Blackleg is caused by the entrance of the blackleg bacillus into the connective tissue of the animal's organism. This entrance is always effected by means of wounds. As a rule, the smaller and deeper the wound, the more favorable is the opportunity for successful infection; hence, wounds produced by the barbs of wire fences, thorns, awns of grains, etc., though usually invisible, are by far the most dangerous. As a rule, the production of the wound and the introduction of the disease germ occur simultaneously.

The Blackleg Bacilli. These are very minute, one-celled plant organisms. In shape they vary from slender rods to oval or spherical forms. The rods are usually enlarged at or near one end or the middle, thus assuming club- and spindle-shaped forms. They have the power of independent motion, which they produce with the aid of hair-like appendages called flagella. The enlarged portions bear the spores—organs that have been compared with the seeds of higher plants. These spores are the resting stage of the bacilli and are the most resisting organisms known. In size the bacilli range from five to fifteen micromillimeters* in length and about three micromillimeters in thickness.

In the diseased animal these bacteria can be found in enormous masses in the affected subcutaneous connective tissue and muscles, but they are not found in the blood of the living animal. They occur also in large numbers in the bile and in the contents of the intestines. Some time after death they are also found in the blood. The blackleg bacillus is a so-called anaerobe; that is, it develops only in the more or less complete absence of oxygen.† In artificial cultures and in the animal body large quantities of gases are developed during its growth. These gases give rise to the characteristic blackleg tumors referred to later on. The first pure cultures of this germ were made in chicken broth by Arloing.

According to Kitasato, the most favorable temperature for its development is 36 to 38 degrees C. (96.8 to 100.4 degrees F.), but it will grow at as low a temperature as 16 to 18 degrees C. (60.8 to 64.4 F.) Spores develop most rapidly at high temperatures. In the living body no spores seem to be formed, but they appear a very short time after death—at most, an hour or less.‡

Arloing, Cornevin and Thomas have shown that blackleg virus (blackleg bacilli and spores) has a remarkable power of resisting the injurious influences of external agencies. When dried this power of resistance is even increased, and, in this condition, it may retain its virulence or disease-producing power for a long time without being mitigated in the least.

Buried cadavers of blackleg victims harbor the active germ indefinitely. Low temperatures have no effect whatever in decreasing its virulence. According to Kitt, fresh virus (fresh meat from blackleg carcasses) loses its disease-producing power when exposed to 100 degrees C. for twenty minutes. Dried virus will resist the action of

*A micromillimeter is the one-thousandth part of a millimeter; a millimeter is about one-twenty-fifth of an inch.

†Kitt has succeeded in cultivating it, artificially, under aerobic conditions.

‡Personal observations.

110 degrees C. for nearly six hours. Fresh virus is destroyed by boiling for two minutes; dried virus requires boiling for two hours to destroy it.

Dried virus is destroyed by the action of solutions of one part of corrosive sublimate in 5000 parts of water, salicylic acid (1: 1000), thymol (1:800), carbolic acid (1:50), boracic acid (1:5), hydrochloric acid (1:2), etc. On the other hand, alcoholic solutions of carbolic acid, unslaked lime, copperas, chloride of zinc, sulphuric acid and other disinfectants in common use have little value as destroyers of the blackleg bacillus.

Finally, it has been shown that the continued action of high temperatures and of certain chemicals has the power of reducing the virulence or disease-producing power of these bacteria. Bacilli thus reduced in virulence can be made to regain their original power by the addition of lactic acid, calcium lactate, acetic acid, dilute alcohol, etc. Blackleg bacilli are always found in the infected parts of animals that have blackleg. There they grow and multiply. In the soil they find a resting-place from which they enter the bodies of other animals and produce the disease. Whether or not the blackleg bacilli multiply in the soil is still an open question. That they remain alive and virulent in the soil for an indefinite period has been demonstrated repeatedly.

Manner of Infection. Blackleg is a wound-infection disease; that is, it is caused by the entrance of the blackleg bacilli into the tissues through wounds in the skin or other parts of the body (mucous membranes). These wounds must be deep enough to reach the subcutaneous or submucous connective tissue. Virus introduced merely under the skin but not into the subcutaneous connective tissue will, as a rule, not produce the disease. The blackleg bacilli being anaerobic, deep wounds are best suited to serve as ports of infection. Such wounds can easily be produced by barbs of wire fences, thorns, the beards of grains and other grasses, or any other sharp-pointed objects or instruments. They can occur in the stable as well as in the field. In both places infected food may be the medium of infection. In the pasture the soil itself may play this role. As soon as the germs have entered the tissue of the animal and found a favorable place for growth they begin to develop and multiply rapidly. They increase in numbers at an enormous rate, and at the same time produce a number of gases which accumulate and give rise to the peculiar gassy, crackling swellings characteristic of blackleg.

Period of Incubation. The time between the entrance of the germs into the body and the appearance of the first recognizable symptoms of disease is called the period of incubation. On the average this is about two days, but varies according to the amount of virus introduced, the individual animal, and other conditions. Sometimes it requires five days, and experiments at this Station show that distinct elevation of temperature and the characteristic lameness may appear less than seven hours after inoculation and death result in less than twenty-one hours.

Symptoms. Blackleg has a very rapid course, death usually occurring within one and one-half to three days after the appearance of the first symptoms, or sooner. The principal symptoms are the appearance of rapidly developing crackling tumors, fever, enlargement of the neighboring lymphatic glands, and lameness.

1. The blackleg tumors may appear in various parts of the body, but chiefly at the thighs, shoulders, neck, chest, flank, and rump. They do not occur below the knee or hock. Sometimes they are found in the mouth, at the gums, tongue, or in the throat. At first the tumors are small and very painful, but they increase in size rapidly and within a few hours can attain enormous proportions, and may even extend over the entire body. These tumors are characterized by the fact that when they are touched, or when the hand is rubbed over them, they produce a peculiar crackling or crepitating sound; percussion produces a clear tympanitic sound. The middle portion of the tumor becomes cold and painless, sometimes dark colored, and resembles parchment when touched. When cut into with a knife the animal exhibits little or no sign of pain, and from the wound there exudes a dark red, nearly black, foamy fluid, with a disagreeable, somewhat sweetish odor. This fluid contains millions of bacteria. One or several of these swellings may be observed. The lymphatic glands near these tumors become enlarged, and can often be distinctly felt as hard swellings under the skin.

2. The principal general symptoms are as follows, viz.: Sudden loss of appetite, suspended rumination, general depression. To these are added high temperature (105 to 107 degrees F.), increased pulse and respiration. The visible mucous membranes, particularly those of the eye, become dark red, even purplish, or of a dirty leaden color. As a result of the pain produced by the tumors, the animals have a stiff and awkward gait. Finally they get down, are unable to rise, respiration becomes more labored, they groan, sometimes show symptoms of colic, the extremities get cold, they get weaker and weaker, and death occurs with a fall of the body temperature. The duration of the disease, when it terminates fatally, is six to forty-eight hours. Sometimes the blackleg tumors, sometimes the general symptoms just described, set in first. When the general symptoms are less severe,

animals have been observed to recover in a day or two, or after four or five days from the appearance of the first symptoms.

Post-mortem Appearance. Beneath the skin, where during life the tumors were observed, we find puffed-up connective and muscle tissue of a dark red or nearly black color. From the cut surface there exudes a blackish, frothy mass, consisting of the blood and tissue juices charged with gases. The affected muscles have a porous, spongy texture, crackle when handled, and when exposed to the air for some time take on a peculiar iridescence. The escaping gases will burn with a bluish flame.

The lymphatic glands near the tumors are enlarged, gelatinous in texture, and contain numerous small hemorrhages. The lymphatic vessels are often distended with gas.

In the abdominal cavity we often find large quantities of bloody exudate or only slight quantities of colorless serum. The walls of the stomach and intestines may be swollen and covered with hemorrhages to a greater or less extent. The kidneys are similarly affected, large hemorrhagic spots being frequent in the surrounding tissues and in the walls of the abdomen. When the stomach and intestines are affected their contents are often bloody. The liver is congested. The spleen shows no abnormal changes.

In the thoracic cavity we find similar changes, viz.: the accumulation of bloody exudates, and a few large or innumerable small hemorrhagic spots in the lungs, heart, pericardium, and pleura. If the muscular substance of the heart is affected it is soft and friable. The air passages of the lungs also show hemorrhages. The number, size and distribution of these hemorrhages and the quantity of the bloody and gelatinous exudates vary greatly.

The blood itself (except in the tumors) has its normal color and is distinctly clotted. The other muscular parts (those not containing tumors) show few changes from the normal.

The whole cadaver is, as a rule, considerably distended, from the accumulated gases. These gases result in part from the development of the blackleg germs, in part from fermentative processes in the stomach and intestines. As a result of the accumulation of these gases, a blackleg carcass that has been dead for several hours has a peculiar and characteristic appearance. The germs of blackleg, during the life of the animal, are found in the muscle and connective tissue only. After death they also occur in the blood.

Differential Diagnosis. A number of other diseases, but malignant œdema and anthrax in particular, may be mistaken for blackleg.

Anthrax. In this disease the tumors do not crackle or crepitate; no gases are developed. The disease is less fatal than blackleg. In anthrax seventy to ninety per cent. of the attacked animals die; in blackleg practically all die. In anthrax the spleen is always enormously enlarged; in blackleg this organ is not affected in any striking manner.

In blackleg the blood coagulates after death; in anthrax it has a tarry appearance. Guinea-pigs die when inoculated with either anthrax or blackleg virus. Rabbits die from anthrax but are immune to blackleg. Hence, when in doubt, a rabbit and a guinea-pig may be inoculated with blood or juice from a tumor of the suspected animal. If both the animals die, the conclusion reached is that the animals had anthrax; if the Guinea-pig alone dies, the disease was blackleg. A bacteriological examination would also serve for diagnostic purposes and, when practicable, would be preferred.

The germs of the two diseases are quite characteristic under the circumstances. The bacilli of blackleg are club- or spindle-shaped, vary considerably in length, nearly always bear spores, have rounded ends, and are actively motile. The bacilli of anthrax are somewhat larger, more uniform in length, have square ends, usually no spores when taken from fresh cadavers, and are non-motile.

Malignant Œdema. This disease has a still more striking similarity to blackleg. It cannot be expected that any one possessing no knowledge of pathology could recognize the difference between these two diseases. It may be said, however, that malignant œdema may occur anywhere, while blackleg is restricted in its occurrence to so-called blackleg areas or districts. Blackleg usually claims a large number of victims, malignant œdema only isolated ones. The latter disease is usually not as fatal as blackleg. Malignant œdema and anthrax both occur in human beings.

A bacteriological examination will determine the difference between anthrax and malignant œdema. Here artificial cultures are of especial value. Malignant œdema usually results from the infection of large wounds, blackleg from infection of small wounds. The treatment of castration, dehorning and other wounds with antiseptics is the best precaution against malignant œdema infection.

Septic Metritis (inflammation of the womb), caused by certain bacteria, may be mistaken for blackleg. The fact that this usually occurs in older animals, in females only, and as a rule after calving, will serve as a guide to a diagnosis.

Treatment. Treatment for a disease like blackleg, with its rapid and almost invariably fatal course, is practically out of the question.

It would be impossible to record here a list of the remedies and the methods of treatment that have been tried, and even recommended as infallible in producing the desired effects. Some of these methods are barbaric, and the confidence placed in the action and in the effects supposed to result from other methods borders on the pathetic. A common practice in this state is to drive or run an animal until it is completely tired out and refuses to go further, and then let the case take care of itself. Cases of cures brought about in this manner are frequently reported. However, reports of recovery without any treatment whatever are quite as common. The mere circumstance that driving a sick animal almost to death does not actually kill the animal is no proof that it cured it. The theory upon which this treatment is based is the popular supposition that the real trouble lies in the fact that the blood in the blackleg tumors is clotted and cannot circulate properly. Now, in the first place, the blood in those places is not clotted; and, secondly, if it were clotted, it is very doubtful whether running an animal, and thus accelerating its pulse and respiration, would have any tendency to unclot it. Finally, we know that blackleg is not a disease of the circulatory apparatus, but a disease due to the presence of a living germ in the animal tissues, and that death results from poisoning by the products excreted by these germs. It is difficult to see how exercising the animal could have any effect on the chemical composition of the toxin excreted by the blackleg bacillus.

Some stockmen resort to bleeding, and report cures as results. Bleeding could not possibly have any beneficial effect in such cases unless a large quantity of blood were lost, and in any case this would affect the future well-being of the animal. Since animals purported to have been saved by bleeding rarely amount to anything afterward, we must conclude that this is an unsatisfactory method of treatment. As a rule it is carried on in a barbaric fashion, and should therefore be discouraged.

Some cattlemen *cure* blackleg by cutting off the tips of the tails of the affected animals. Cases of recovery have been reported when the tumors had been deeply incised vertically, large quantities of frothy liquid allowed to escape, and then strong solutions of carbolic acid or other disinfectants introduced. Although this method of treatment is not irrational, it is not a safe plan, by any means, to ascribe to the treatment every case of recovery following it. Be this as it may, however, we know that it is not an infallible remedy nor anything approaching it. We know that such animals, even when they recover, are usually worthless; and, lastly, the incision of the tumors allows blood loaded with blackleg germs to escape, drop to the ground, and create new sources and opportunities of infection. Much more could be said about other methods of treatment which are equally or more unsatisfactory. But time and space are too valuable. We must conclude that there is no satisfactory treatment for blackleg.

PREVENTION.

Since therapeutic treatment for blackleg is of practically no value whatever our only hope lies in prevention. As is the case with human ills, many of the so-called preventive measures that are taken to prevent blackleg are based on a lack of knowledge of pathology, and on superstition. All sorts of preventives are used by various cattle men and all of them are supposed by those who have used them to be infallible if, by chance, no cattle happened to die after their application. The popular preventives in common use are starving, bleeding, so-called nerving, setoning or roweling, the introduction of all sorts of substances and preparations like elder pith, onions, salves, ointments, etc., under the skin, the feeding of salts and sulphur, etc., etc. It is not necessary to consume time and space to describe these operations; those who originated and those who practice them are familiar with them. Very careful and extensive observations by reliable men in different countries show conclusively that these operations have little or no value and in many cases are positively harmful. Hence the less this sort of information is disseminated the better. The mere fact that animals do not die after receiving these treatments is not sufficient evidence to prove that they would have died had they not been treated, Besides this, our records at the Experiment Station show so many contradictory reports regarding the value of these methods of treatment that when the results are averaged up there remains absolutely nothing in favor of them.

It is no doubt true that starving an animal will to a certain extent prevent the appearance of the disease, even if we base this statement on nothing else than the fact that all well-fed animals usually suffer most. But be that as it may, no one will insist that it pays to feed an animal and then starve it to keep it alive. There can be no profit in this practice.

There is only one method of preventing blackleg that is founded on scientific principles and has resulted from carefully conducted researches and experiments; it has stood the test of time and of practical application. This is preventive inoculation or vaccination.

Preventive Inoculation. Preventive inoculation is nothing new. It was practiced by the Chinese a thousand years before the birth of Christ. The Chinese resorted to preventive inoculation to protect themselves against smallpox. At the close of the last century, Jenner, an English physician, discovered the value and introduced the practice of *inoculating with cowpox* (vaccination) for the prevention of a related disease, smallpox. The Chinese used attenuated smallpox virus for protective inoculation. They exposed themselves to mild cases of smallpox and thus became immune to further attacks.

It was a matter of common observation that when animals or persons recovered from a contagious or infectious disease they would rarely take the disease a second time. They became immune. Whether the attack from which they recovered was very severe or extremely mild, the immunity, within limits, was the same. In other words, a mild case was just as effective as a severe case in preventing a second attack. The duration of the immunity depends on various factors, on the animal itself, and above all on the particular disease. Under certain conditions every infectious disease produces immunity to a second attack for a certain time. The mere fact that the patient recovers makes this necessary. For some diseases the immunity lasts only a short time— a few weeks or a few months; in other cases it lasts one or two years or even a lifetime. Protective inoculation against blackleg consists in the artificial production of a mild case of blackleg from which the animal recovers and then becomes immune. The material used for inoculation is an artificially attenuated culture of blackleg germs. This method, with various modifications, was first studied and practically applied in France by Arloing, Cornevin, and Thomas. A brief outline of their work is as follows:

Work of Arloing, Cornevin, and Thomas. In their inoculation experiments, these three investigators found that when active, unattenuated blackleg virus (blood or tissue juice containing blackleg bacilli or pure cultures of these germs) was inoculated subcutaneously or intramuscularly the inoculated animals always died of blackleg. They found that when the virus was introduced directly into a blood-vessel a mild general attack of the disease was produced, the animal recovered, and was immune against the effects of subsequent inoculations. Intratracheal inoculation produced similar effects. On the other hand, inoculations by way of the digestive tract (feeding) had no effect whatever.

On the strength of these observations, thirteen animals were inoculated at Chaumont with virus dissolved in distilled water, filtered, and injected into the jugular vein. Six months later these animals, with twelve check animals, were inoculated subcutaneously with blackleg virus. None of the former took the disease, but nine of the check animals died of blackleg. In 1881, 245 animals were inoculated, with the same success, in the Department de la Hautemarne; similarly, seventy-eight animals in the Department des Ain, in 1882.

Inoculation by the intravenous method required a skilful surgical operation, consisting of exposing the jugular vein, and very exact manipulation in introducing and withdrawing the needle, in order to prevent the virus coming in contact with the subcutaneous connective tissue. Then the subcutis of the tail was chosen as a point of operation. Virulent cultures inoculated at this point were found to produce only a temporary and harmless swelling, but resulted in perfect immunity. The dense connective tissue and the low temperature of the tail account for the mild character of the tumor; one checking the development, the other preventing the spread, of the blackleg bacilli. Aside from these precautions, the virus was reduced in virulence by subjecting it to the action of a certain degree of heat.

Method of Attenuating the Virus. Arloing's later method of attenuating blackleg virus is as follows: Forty grams of muscle substance from a blackleg tumor is rapidly dried at 32 degrees C. (90 degrees F.), then evenly mixed with eighty grams of water. This mixture is divided into twelve equal parts, each part spread on a flat plate and heated in an incubator for six hours. Half of the material is heated at 100 degrees C. (212 degrees F.), and half is heated at 85 degrees C. (185 degrees F.) The first produces a weak, the second a strong, virus. The inoculation is performed in two operations; first the weaker and then the stronger virus being used.

The virus is prepared for use as follows: One gram of the material is triturated with five grams of water, strained through fine linen, and the filtrate is injected into the animal by means of a hypodermic syringe. This amount of material is sufficient for ten animals. The point of inoculation is the under side of the tail, eight inches from its tip. The hair is carefully shaved off, a trocar is introduced for a distance of eight centimeters between the skin and bone; this is withdrawn, and then the hypodermic needle of the previously filled syringe is introduced, the required amount injected, needle carefully withdrawn, and then, to prevent escape of the injected fluid, a small rubber bandage is applied and allowed to remain four hours. Thus twenty to twenty-five animals can be inoculated in one hour.

Ten days later the operation is repeated, this time using the stronger virus.

Early Statistics on Blackleg Protective Inoculation. In 1883 Cornevin inoculated 125 animals in France, with favorable results. Similar results were obtained by Cornevin in 1884. At the close of the latter year Hess and Strebel inoculated 2000 cattle in Switzerland, and turned them out in infected regions of the Alps. In an equal number of check animals the mortality rate was twenty-eight times greater than in the inoculated animals.

From 1884 to 1888 Strebel inoculated 8641 cattle in Switzerland, of which fifteen died of blackleg=0.17 per cent.=1:576; of 21,000 check animals, not inoculated, kept under the same conditions, 491 died of blackleg=2.34 per cent., or fourteen times as many as in the vaccinated herds.

According to Strebel, the percentage of loss in unvaccinated animals in the Canton Freiburg, in 1899, was twelve and one-half times as great as in vaccinated herds (6616 head). In 1890 it was eight times as great. During the seven years of 1884 to 1890, the average proportion of loss in the vaccinated compared with the unvaccinated herds was 1:1l½.

In the Austrian Alps, Sperk inoculated 925 animals in 1885; none of these died, while of 6387 check animals 107 died of blackleg. In 1886, 2140 animals were inoculated in Salzburg, and 3820 in Tirol-Vorarlberg. In these herds the loss was four and sixteen respectively. Of 9160 check animals in Salzburg, and 17,401 in Tirol-Vorarlberg, the deaths from blackleg were 86 and 330 animals respectively. According to these figures, the mortality rate in the unprotected animals was 1 to 2 per cent.; that of the protected animals 0.2 to 0.4 per cent. The cost of the inoculation is given at from fifty to seventy pfennige or (twelve and one-half to seventeen and one-half cents) per head.

In Bavaria, 963 animals were inoculated in 1886, and no deaths occurred.

In Prussia, in 1886, sixty-four cattle were inoculated, with no losses. In 1887, 485 were inoculated, and turned out with 264 unprotected cattle. Of the former, two died; of the latter, three; nearly three times as many.

Professor Kitt, of Germany, made the observation that single vaccination, with vaccine attenuated by heating six hours at 85 to 90 degrees C., and injecting the material at the shoulder, was just as effective as and more convenient than the old method of double inoculation at the tail. The results of the first experiments seemed to be in harmony with this view; but later experience showed that a certain degree of danger attended this method. The experiments carried on at the Kansas State Agricultural College will give further light on this question.

PREPARATION OF BLACKLEG VACCINE AT THE KANSAS EXPERIMENT STATION.

The first vaccine was made in August, 1898, when 5000 doses were prepared. We followed the method of Arloing, and expected to vary it as we found necessary in the course of our experience. Our first material was obtained from a calf that had died on Mr. McDowell's farm, near Manhattan, July, 1898. We arrived at the place a very short time after the calf's death, the carcass being still warm, and removed a small amount of affected muscular substance, which was taken into the laboratory, and cut into long, thin strips, about the size of a lead-pencil. These strips were strung on a cord and then hung up in the attic to dry. It was summer, and the temperature of the attic during the day ranged between 90 degrees and 100 degrees F. In the course of a few days these strips were dry. They were then cut into pieces about a quarter of an inch long. Of the material thus prepared, we took ten grams and ground it as fine as possible with a mortar and pestle, gradually adding twenty grams of distilled water and working the mass into a thick paste. This was spread out in a round, flat dish with vertical sides (Petri dish), and then placed in a hot-air sterilizer.

This sterilizer was regulated with a thermostat of the improved Reichert pattern. It maintains a remarkably even temperature, although it requires some watching. We found that when forty grams of prepared muscle was placed in the sterilizer heated to 100 degrees C., the temperature would immediately drop fifteen or twenty degrees, the rapid evaporation of the water being the cause. With the aid of an extra Bunsen burner we could run the temperature up again in about ten minutes, and, with careful watching, keep it there for an hour, after which the thermo-regulator would maintain an even temperature for the other five hours. The dried-muscle pulp was then removed, ground into a fine powder with a mortar and pestle, divided into carefully weighed portions of one-tenth gram each, wrapped in papers marked:

First Vaccine, Ten Doses, Kas. Agr. Exp. Sta., Date,———.

A second lot was prepared in the same way, but at a temperature of eighty-five degrees C., and dispensed in packages marked:

Second Vaccine, Ten Doses, Kas. Agr. Exp. Sta., Date,———.

A number of calves belonging to the College herd were then inoculated with this material. The material was prepared according to the following directions, each animal, however, receiving twenty times the specified dose.

DIRECTIONS FOR USING BLACKLEG VACCINE.*

The vaccine is always sent in pair packages. Each package contains enough vaccine for ten calves about eight months of age, or for twice that number below this age.

Each animal must be inoculated twice: Once with the *first* vaccine and ten days later with the *second* vaccine. The operation in each instance is performed in the same way.

The instruments† required are as follows: (1) A hypodermic syringe graduated into cubic centimeters, and with a capacity of at least one (but better five) cubic centimeters; (2) a small porcelain mortar and pestle; (3) a small glass funnel; (4) a quantity of filter paper; (5) a small salt-mouthed bottle to receive the funnel; (6) a ten-cubic-centimeter graduate; (7) a quantity of thoroughly boiled clear water.

Empty the contents of one or more of the packages marked *first vaccine* into the mortar, add a few drops of the boiled but now cooled water to this, and with the pestle work or rub it into a thin paste; then add for each package of vaccine used ten cubic centimeters of the boiled water, add the water gradually, and continue to stir the mixture with a grinding motion of the pestle in the mortar.

Fold a filter paper, doubling it twice, the second time at right angles to the first, and then place it in the funnel, with the latter in the salt-mouthed bottle. Wet the filter paper with some of the boiled water; let all visible water drain off and discard it. Stir the vaccine powder in the water thoroughly and then pour into the filter. Practically all the liquid in the mixture will pass through; the filter, having previously been saturated, will absorb none of it. The clear, somewhat straw-colored liquid that passes through is the material used for vaccination. The filter paper, after once used, and the residue of the powder, are to be destroyed by burning.

Each animal above eight months old receives one cubic centimeter of this fluid; each animal below this age receives one-half this dose. A double dose will not injure the animal, but is unnecessary. Less than a full single dose is insufficient to produce immunity. The place of inoculation is under the skin, on the lower surface of the tail, four inches from its tip. Insert the hypodermic needle of the syringe, with the point directed toward the root of the tail, and inject the required amount.

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*Prepared in the bacteriological laboratory of the veterinary department, Experiment Station, Kansas State Agricultural College.

†Any druggist can furnish these instruments and would no doubt cheerfully instruct in their use.

Some hypodermic syringes have a regulator attached to the piston-rod, by means of which the exact amount of fluid injected can be controlled. This is the preferable kind.

While withdrawing the needle of the syringe after inoculation, and for a moment longer, exert a slight pressure with the finger at the point of entrance of the needle, in order to prevent a possible escape of the inoculating fluid or virus.

Caution.—Clean with boiling water all instruments—syringe, funnel, bottle, mortar, etc.—before preparing the vaccine with them. Use the vaccine immediately after it is prepared. If kept for half an hour or an hour after this, it should be in a stoppered bottle. Do not use it after more than one hour's standing. The vaccine, when left undisturbed in the original packages, may be used until eight weeks old from the time it leaves the laboratory of the Experiment Station.

Ten days after the first vaccination, vaccinate again, this time with *second vaccine*, prepared precisely as was the first and administered in the same way, but four inches higher up on the tail.

Notice.—This vaccine will prevent the outbreak of blackleg to a great extent, but it must not be considered as an absolute preventive.

Keep a careful record of the date of vaccination; date of deaths, should any occur after vaccination; number of animals vaccinated, age of animals, as near as practicable, etc. Lastly, follow the above directions implicitly and not those of some one else. Do not try to experiment with this vaccine—the Experiment Station is doing that part of the business.

In the course of a few months we will send you a printed form to fill out as a report on the result of the work.

If you desire additional vaccine, write for an application blank.

Do not write for vaccine unless you need it and intend to use it. This vaccine costs money, and the state cannot afford to furnish it for any other purpose than that for which it is intended.

These inoculations produced no observable injurious effects on the animals treated, and we concluded that, to say the least, the material seemed to be harmless as far as this method of treatment was concerned. August 30, 1898, a short press bulletin on blackleg was issued and sent to the various newspapers of the state. The bulletin outlined briefly the character and importance of the disease, gave the symptoms by which it might be recognized, called attention to the value of protective inoculation, and concluded by stating that "the Experiment Station has manufactured vaccine which will soon be ready for free distribution among the stock-raisers of Kansas." From that time on we received almost daily inquiries regarding remedies for this disease and also applications for vaccine. The pressure of the work that always presents itself at the beginning of a college term was such that arrangements to send out vaccine could not be made until the beginning of October. By that time we had prepared about 5000 double doses of vaccine, and sent out the first order October 5, l898, to Mr. G. W. Addison, Eureka, Greenwood county, Kansas (seventy-five doses), From October 5 to November 11, 1898, we sent out our entire stock on hand, supplying seventy-eight cattlemen, in forty-one counties, with an average of sixty double doses. The smallest quantity sent was ten doses; the largest quantity, 250 doses. One stock-raiser who sent for 250 doses did not use it because he had other work to do.

At this stage our time and funds were becoming cramped, and we discontinued the manufacture and distribution of vaccine and notified all applicants to this effect. Applications, however, came in more frequently than before. We had as yet received no unfavorable reports on the use of our vaccine, but a number of gratifying reports were received. This, with the encouragement received from the Board of Regents at their next meeting, in the form of additional funds, induced us to take up this work again and conduct it on a larger scale. In January, 1899, we began sending out vaccine the second time. During the remainder of that month we sent out double vaccine sufficient to inoculate 630 calves over eight months of age, or twice that number below this age. In March 8265 doses and in April 9050 doses were distributed. April 17 we sent requests for reports on the use of the vaccine sent out between October 5 and November 11 of the previous year. Each recipient of vaccine was asked to notify the department immediately if at any time in the future deaths should take place among the vaccinated cattle.

The total number of calves and yearlings for which vaccine was sent out was 4735—3420 calves below six months of age and 1315 yearlings. Of the seventy-eight cattlemen supplied with vaccine, sixty-seven sent reports on the use or non-use of the vaccine, by filling out blanks sent with a stamped and addressed envelope. The other eleven did not reply after receiving a second request. Of the 4735 doses sent out to seventy-eight cattle owners, we received reports on the use or non-use of 2770 doses sent to sixty-seven cattlemen. Of the sixty-seven cattlemen, thirty-eight received 1630 doses, with which they inoculated 1599 animals. Twenty-eight cattlemen received 1140 doses, but did not use them for various reasons. Thus, out of a total of 4735 doses sent out between October 5 and November 11, only 1599 animals were inoculated. According to this, only 33¾ per cent. of the material sent out was used. Those who did not use the vaccine were applicants for the largest quantities, or they sent for ten doses only and did not use it because they had no instruments for its application.

RESULTS OF THE USE OF VACCINE.

Number inoculated.	Number died before inoculation.	Number died between inoculations.	Number died after inoculations.	Place inoculated.
30*	0	0	0	Tail.
15*	0	0	0	Neck.
18	0	0	0	"
14	0	0	0	"
80*	1	0	0	"
5*	1	0	0	Shoulder.
50	0	0	0	Tail.
8*	0	0	0	"
20	0	0	1**	"
10	1	0	0	Shoulder.
40	1	0	0	Tail.
100*	0	0	0	"
20*	0	0	0	"
68*	0	0	0	Neck.
10	0	0	0	Tail.
16*	2	0	0	"
8*	0	0	0	"
40	0	0	0	Shoulder.
71*	0	0	0	Tail.
50	1	0	0	"
12*	0	0	0	Shoulder.
60*	3	0	0	"
20*	0	0	0	Tail.
10*	....	0	0	....
50	0	0	0	Shoulder.
10*	0	0	0	Tail.
50*	3	0	0	"
54*	6	0	0	Shoulder.
40*	4	0	0	Tail.
40*	13	0	0	"
20*	0	0	0	Shoulder.
25*	....	0	0	Tail.
66*	7	0	0	"
122*	6	1	0	Shoulder.
20*	15	0	0	"
33*	4	....	2†	Tail.
160*	3	0	3‡	"
80	....	....	0	"
1545	71	1	6	....

*Owners of these cattle had never before used vaccine.

†"When these died a number of others were lame. I think they had blackleg, and the inoculation saved them."—F. W. K.

‡Ascribes loss to imperfect inoculation. Has used United States single vaccine since using this, with slight loss thereafter.

**Dishorned.

Thirty-six owners had 1545 cattle.

Sixteen owners, or 44.44 per cent. of total number heard from, lost cattle from blackleg. These sixteen owners of 846 cattle lost seventy-one head from blackleg immediately before vaccination; nearly 8.4 per cent.

Loss ranges from one out of a herd of eighty (=1.25 per cent.) to fifteen out of a herd of thirty-five (=42.85+ per cent.), as the following table shows:

Number in herd.	Deaths before.	Deaths after.
80	1	0
5	1	0
10	1	0
40	1	0
16	2	0
50	1	0
60	3	0
50	3	0
54	6	0
40	4	0
40	13	0
66	7	0
122	6	0
20	15	0
33	4	2†
160	3	3‡
846	71	....

Per cent. of loss before =8.4.

Per cent. of loss after =0.23+ = less than ¼ per cent. = 1/36 of the rate before vaccination.

In one herd in which no blackleg existed before vaccination, one animal died of blackleg on second day after second vaccination. This animal was dishorned at the time of vaccination—a practice that must always be regarded as attended with much risk.

From January 17, 1899, to October 1, 1900, 106,840 doses of double vaccine were distributed. The reports covering this entire period, the last of which were received in January, 1901, may be briefly summarized as follows: A total of 1270 stockmen, representing practically every county in Kansas, were supplied with 106,840 doses of double vaccine. Until January 19, 1900, this vaccine was distributed entirely free of charge; after that date, ten cents per ten-dose package was charged, in order to pay the cost of manufacture, distribution, etc. Of the 1270 stockmen who received vaccine, reports were sent in by 814. Of these only seventeen made no use of the vaccine received. This favorable showing, compared with that made by the vaccine sent out during the first period, is no doubt due, in a great measure at least, to the fact that the vaccine was not given away entirely free, as was done at first. These 814 cattlemen and farmers owned or otherwise controlled 104,269 head of cattle. At the time these reports were made 55,723 head of these cattle were under two years old, and consequently of a susceptible age; 54,393 of these susceptible cattle were inoculated with Experiment Station vaccine.

Total number inoculated.	54,393.
Loss after inoculation.	323=less than 0.6%.
Loss in an equal length of time before inoculation,	2301=more than 4.23%.

Of the 814 cattle owners who sent reports, 408 (over half) sustained losses previous to inoculating with protective virus. Only 157 cattle owners out of 814 had any losses whatever from blackleg after vaccination. Of the 814 cattle owners, 634 followed directions and inoculated at the tip of the tail; 180 inoculated either in the shoulder, flank, dewlap, or at the base of the ears. Of the 634 who inoculated at the tail, 121 (over nineteen per cent.) sustained losses from blackleg after inoculation. Of the 180 who inoculated in the soft tissues (shoulder, flank, dewlap, and base of ear), thirty-six (or twenty per cent.) sustained losses from blackleg after inoculation—a showing not much in favor of either method. A careful study of the itemized tables gathered from the reports, but which on account of their great bulk cannot be published in this bulletin, shows that although on the average the losses from both methods are the same, they seem to be due to different causes. Losses resulting after tail inoculation usually result at a later date than those following inoculation at other points; in the latter case they frequently follow within a very few days, or even sooner, after inoculation. This points to the possibility of death from the effects of the inoculation. That this may occur, even with the most carefully prepared vaccine, is demonstrated by the history of Mr. F. M. Wagoner's herd of forty-seven head of yearlings. Mr. Wagoner had had no blackleg in his herd for over twelve months when he vaccinated his cattle with Experiment Station vaccine, April 3, 1899. April 4 ten took sick; April 5 all affected animals died, from the effects of blackleg produced by inoculation. The following copy of a report made to Mr. Wagoner, after a careful personal investigation of the matter, will explain itself:

MANHATTAN, KAN., April 24, 1899.

Mr. F. M. Wagoner, Ellis, Ellis county, Kan.:

DEAR SIR—Regarding the cause of death of the ten animals in your bunch of forty-seven cattle, several days after vaccinating with vaccine furnished by this Station, I would say that on March 13 (the day we sent you some vaccine) this department sent out 1520 full doses of vaccine to fifteen different cattle owners, in quantities ranging from 10 to 400 doses. We have sent circulars of inquiry to all of these parties. From none of them have we, as yet, received an unfavorable report following the use of the vaccine. Aside from this, some of the check vaccine which we had preserved was used to inoculate an experiment animal belonging to the farm department of the Experiment Station. This animal received forty full doses of second vaccine at one application, with no apparent injurious results whatever.

Upon examining the tails you sent us, I find that every one of them contained two inoculation marks within four inches from the root of the tail. These inoculation marks led into wounds, indicating that the needle was inserted inwards, towards, and in some cases into, the bone. This alone is sufficient to account for the death of the animals. You will notice that the directions say that the animal should be inoculated four and eight inches from the end of the tail—the first time four inches from the end; the second time, four inches higher up. In the case of the tails examined, we find that the inoculation wounds were, in some cases, an inch and a half apart; in no case was the distance greater; in one case the inoculation wounds were less than an eighth of an inch apart.

I regret exceedingly that you should lose your cattle through such a sad mistake; but, as you see, the mistake is entirely on your side.

I remain, very truly yours, PAUL FISCHER.

Deaths following tail inoculation usually occur at a later date than those following inoculation into the soft tissues. This may be due to the circumstance that very frequently the virus injected into the tough subcutaneous tissue of the tail runs out again, and thus the animal remains practically *not inoculated,* and of course does not become immune.

Summarizing briefly, we may say that protective inoculation is the only successful method of combating the ravages of blackleg. Of a total of 55,938 animals inoculated, and among which a loss of 2372 animals previous to inoculation had occurred, the loss for an equal length of time after inoculation was reduced to 325—or a reduction from 4.24+ per cent. to 0.58+ per cent. In other words, the death-rate was more than seven times as great before as after inoculation. There is no doubt whatever that careful attention to the details of inoculation will still further reduce the rate of mortality.

Single Vaccine. To determine whether it was necessary to follow the old method of double inoculation, or whether a single vaccine, stronger than the first but weaker than the second vaccine used in these experiments, might be equally effective and be applied without too much danger, we undertook the preparation of such a virus. It was prepared precisely as the double vaccine, only that the temperature at which it was attenuated was kept at 92 degrees C. instead of 85 degrees C. and 100 degrees C., respectively.

The following summarized results of the reports received will speak for themselves: 11,268 animals were inoculated. These were owned or otherwise controlled by 111 different stockmen and farmers. Of this number of cattle, 308 (over 2.75 per cent.) died of blackleg previous to inoculation; 34 (slightly more than .3 per cent.) died in an equal length of time after inoculation. The period covered varied from three months to about one year. This shows a reduction in the death-rate from 2.75 per cent. to 0.3 per cent.; or, in other words, before vaccination the death-rate was more than eight times as great as after vaccination.

This is an exceedingly favorable showing when compared with the effects of the double vaccine, with which the rate was reduced only to one-seventh of that before inoculation. It must be remembered, of course, that the results of the single-vaccine experiments are based on 11,268 animals, whereas those of the double-vaccine experiments are based on nearly five times that number, viz., 54,393. In each case we must consider the possibility, or rather the probability, of errors, since none of the inoculations were carried on by experienced men. The large majority of the cattle vaccinated were treated by men who had had no previous experience whatever, and in many cases where losses after vaccination were severe the owners admitted that these losses were probably due to their own carelessness. Taking this into consideration, the result of the experiment must be looked upon as favorable in every way.

In spite of the numerical results quoted above, it stands to reason that double vaccine is safer and more effective than single vaccine, and further more extensive tests may prove this. On the other hand, there is room for an error in this statement.