STUDIES ON SHIPPING FEVER

by

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INTRODUCTION

Shipping fever, stockyards pneumonia and hemorrhagic septicemia are terms used synomously to describe a diseased condition of cattle incident to shipping. Animals when being shipped are subjected to changes of weather and irregularities of feeding and watering. The febrile septicemic disease known as shipping fever or hemorrhagic septicemia is the most serious of a group of maladies which commonly result from neglect or exposure of animals in transit or shortly after reaching their destination. Losses from this source vary from year to year, but are heaviest during fall and winter months. Young animals are more susceptible to disease incident to shipping than are older animals.

Shipping fever is a disease characterized by loss of appetite, lacrimal and nasal discharges, a painful cough, diarrhea, and a tendency to lag behind the herd.

HISTORICAL

The disease known as hemorrhagic septicemia was first described in Germany by Bollinger in 1878 (Fitch, 1921) under the name Wild-und Rinderseuche, an acute disease in deer, wild boars and cattle. Kitt (1886) is credited with having isolated Bacterium bovisepticum from a disease of cattle

similar to that described by Bollinger. Hueppe (1886) isolated Bacillus bipolaris septicus from chicken cholera, swine plague, rabbit septicemia and influenza of horses.

Pasteur (1880) was the first to describe the bacillus of fowl cholera. It was largely for this research that the name Pasteurella was given to the group of organisms which produce Pasteurellosis of wild and domesticated animals. Pasteur found that cultures of the chicken cholera organism when attenuated by keeping at room temperature, produced a suitable vaccine for the immunization of chickens against artificial infection. Similar results were obtained when cultures were attenuated at high temperatures, or by the addition of disinfectants. Smith (1896) states that Pasteurella organisms present in diseased lungs are not infrequently found in the mouth and upper respiratory passages of healthy cattle. These organisms usually occur in pure culture in broncho-pneumonia. In 1892 Smith isolated bacteria from secretions covering the posterior nares, larynx, and pharynx of certain healthy pigs, that could not be differentiated from the swine plague germ.

Moore (1893) found that bacteria resembling those of the Pasteurella group were present in secretions covering the upper air passages of cattle, dogs and cats.

Outbreaks of hemorrhagic septicemia among cattle which were kept on low marshy land in Minnesota were reported by Brimhall and Wilson in (1900). This was an acute disease and affected animals died within 24 hours. Baldry (1907) obtained a toxin from a ten day culture of P. boviseptica. A filtrate of this material when inoculated under the skin of rabbits produced death in one hour. He suggests that the toxicity of Pasteurella cultures apparently plays the principle role in the rapid death of animals. This author (1907 b) while studying aggressins found that it was easy to obtain aggressins of Pasteurella of Barbone by inoculating rabbits intraperitoneally with bouillon cultures. He also found that P. boviseptica when inoculated into rabbits tends to lose its virulency for bovines. Immunization of horses by administering large intravenous doses of virulent filtered blood from swine plague was attempted by Koops (1908). Ward and Beebe (1911) isolated B. bipolaris ovi-septicus from the spleen of sheep. These organisms were highly pathogenic for sheep but less so for calves.

Mohler and Eichhorn (1912) were the first in the United States to call attention to the practical inoculation against hemorrhagic septicemia, as it appeared among buffalo in the Yellow Stone National Park. An acute form of hemorrhagic septicemia in cattle shipped from the stockyards at

Chicago, illinois was described by Fitzgerald (1914). P. boviseptica was isolated from the lung tissue in these cattle. Hadley (1914) discovered that female rabbits immunized by inoculation with an avirulent culture of the fowl cholera bacterium, are able to transmit a high degree of resistance to virulent cultures to their offspring.

Clark (1914) described an acute highly fatal form of hemorrhagic septicemia of cattle in which a saffron colored udder, bleached mucous membranes, bloody urine and subnormal temperature were the outstanding symptoms. An acute nervous type of hemorrhagic septicemia was observed in Jersey cattle by White (1914). Walker (1914) attempted to infect susceptible goats by rubbing the nostrils with nasal discharges from animals infected with pleuro-pneumonia. This material was also injected into the lung, intratracheally and subcutaneously. All attempts were negative. M'Gowan and Wang (1915) demonstrated that organisms of the hemorrhagic septicemia group could be increased in virulence by animal passage. They also increased the biological activity on special artificial media. Organisms were caused to produce acid and gas on some carbohydrate media where they had previously produced acid but no gas.

Fitch (1915) states that symptoms of hemorrhagic septicemia might be easily confused with those of certain non-

specific infections. Kinsley (1915) believes that hemorrhagic septicemia organisms seldom produce disease except in those cases where the resistance of animals has been diminished. An acute or spontaneous form of hemorrhagic septicemia among cattle in Iowa was described by Murray (1916). Hardenbergh and Boerner (1916) found that 48 hour bouillon cultures of B. bovisepticus inoculated subcutaneously in doses of 0.5 cc and 1 cc were harmless to cattle. In 1916 Gallagher found that noticeable resistance is conferred to fowls by the use of killed fowl cholera bacilli, but that immunity was not absolute since doses of 0.01 cc and 1 cc of virulent culture proved fatal to treated fowls, rabbits, and guinea pigs. Mack and Records (1916) reported favorable results with the use of bacterins in checking field outbreaks of fowl cholera. There was no apparent difference in results from the use of homologous or heterologous strains of B. avisepticus.

Hardenbergh and Boerner (1917) observed an acute outbreak of hemorrhagic septicemia in mules. They based their diagnosis on findings on typical lesions of hemorrhagic septicemia as seen in the acute form in cattle. In (1917 b) Hardenbergh and Boerner stated that living hemorrhagic septicemia vaccine, had not in any way proved harmful for field work. They also stated that living vaccines provided they

did not produce the disease, set up new centers of infection, or establish "carriers" are obviously better than dead bacterins. Besemer (1917) studied five strains of the hemorrhagic septicemia group of organisms with special reference to their action on carbohydrates. He found that the numbers of the group studied were practically uniform in their biological actions, and that passage through rabbits did not change their biochemical character. Brandenburg (1917) reports favorable results in the treatment of hemorrhagic septicemia by the administration of 2 cc of vaccine hypodermically.

Smith (1918) mentions B. bovisepticus as a secondary invader with B. actinoides. B. actinoids is definitely associated with broncho-pneumonia of calves. B. bovisepticus appears later in affected lungs together with staphylococci and streptococci. In (1918) Jorgenson observed an acute bacteremic type of hemorrhagic septicemia among cattle in Iowa, in which a single injection of killed culture vaccine (1 cc. subcutaneously) checked the outbreak. Ferrand (1918) reports success with the administration of anti-hemorrhagic septicemia serum in animals having high temperatures. He used vaccines for apparently healthy animals that had been recently shipped through large public stockyards. Jensen (1918) refers to hemorrhagic septicemia as an infectious

disease in which the mortality rate is high. This disease attacks cattle, sheep and swine. He finds that it occurs in the pectoral, intestinal and subcutaneous forms. In 1918 Smith observed an acute intestinal form of hemorrhagic septicemia in a bull which had been shipped. A very virulent strain of P. boviseptieus was isolated from the spleen, heart blood and lung tissue. Hock (1918) reported the nervous form of hemorrhagic septicemia in a sow. On autopsy lesions characteristic of hemorrhagic septicemia were disclosed. Vaccination of the remaining herd apparently prevented further losses. Tiffany (1918) mentions the acute form of hemorrhagic septicemia as the type most commonly encountered in Illinois.

Hardenbergh and Boerner (1918) summarized statistics obtained from vaccinating two groups (4000 head) of cattle against hemorrhagic septicemia. In the first group there were 11.1 per cent deaths in 1831 vaccinated animals. In the second group 1584 healthy animals and 46 sick animals received vaccine. Twenty-two head or 51.1 per cent of the sick animals died. Twenty-four or 1.5 per cent of the 1584 healthy animals died following vaccination. The difficulty of reproducing the disease by injecting infected tissue exudates into susceptible animals was discussed by Washburn (1918).

Carter (1918) autopsied swine dead of hemorrhagic septicemia and found B. suisepticus in 50 per cent; B. suipestifer, 25 per cent; B. coli, 88 per cent; B. pyocyaneus, 13 per cent; streptococcus 38 per cent; and staphylococcus areus and albus in 50 per cent.

Knapp (1919) lists two instances where recently shipped cattle developed clinical symptoms of hemorrhagic septicemia following long distance transportation and exposure. Each affected animal received 1 cc of bacterin subcutaneously. The next day the treated animals showed considerable improvement. A second injection brought about complete recovery. Hoskins (1919) states that hemorrhagic septicemia seems to be associated directly with residence in certain of our large public stockyards, and that this disease has a rather definite period of incubation.

In 1919, Glover, Newsom and Alkire isolated a bipolar organism from sheep, which was pathogenic for rabbits. Hadley (1919) in studies on immunization against B. avisepticus found that avirulent strains produced a more satisfactory immunity in fowls than did treatment with killed cultures of the virulent strains. Haring (1919) reported success in controlling an outbreak of hemorrhagic septicemia in sheep by the administration of a vaccine prepared from the causative organism (B. avisepticus). Patterson (1919)

in discussing hemorrhagic septicemia of horses expresses belief that numerous petechia on the atria of the heart, large blotchy hemorrhages beneath the epicardium, and subendocardial hemorrhages are lesions pathognomonic of this disease.

In 1920, Van Es and Martin administered 0.2 and 5 cc of vaccine and virus, at six day intervals for three successive periods. After two weeks they administered 0.002 cc of B. bipolaris avisepticus virus. All rabbits including controls died within 72 hours. Van Es and Martin (1920 b) found that hemorrhagic septicemia vaccines and bacterins apparently did not have any antigenic value. They encountered uniform negative results, no matter if these products were injected once or a dozen times. Van Es and Martin (1920 c) made a very complete study of fowl cholera vaccines and bacterins. In interpreting their results they state that no reliance can be placed on vaccines and bacterins against fowl cholera, which they were able to find on the market and subject to definite tests. These authors (1922) as a result of their experiments state that Pasteurella organisms apparently plays an unimportant part in the production of hemorrhagic septicemia in cattle, sheep and swine. The use of hemorrhagic septicemia vaccines and bacterins as an effective method for the active and passive immunization a-

gainst hemorrhagic septicemia was recommended by Eichhorn (1921). King (1921) states that as far as experimentation is concerned, no data have been advanced which conclusively prove the value or worthelessness of hemorrhagic septicemia vaccines.

Fitch (1921) suggests that the natural habitat for the organisms (B. bipolaris) has not been found. Because of insufficient research it is not known what influence the presence of these germs, in the upper respiratory tract of healthy animals, has in the production of disease. Favorable results with the use of an attenuated vaccine in the vaccination of both healthy and sick cattle that had been recently shipped was reported by Bard in (1921). D'Herelle and LeLouet (1921) presented data showing that the virus of hemorrhagic septicemia attenuated by repeated passage through rabbits is capable of producing a lasting immunity in cattle and buffalos. This virus can be preserved unchanged in macerated rabbit tissues, but if kept in macerated beef tissue it apparently regains its virulence.

DeKruif (1921) isolated two types of organisms from spontaneous infections of rabbit septicemia, a type D and a type G strain. Inoculations with cultures of type G protects rabbits against artificial infection with type D or the more virulent type.

In 1921 Jones and Little observed an outbreak of hemorrhagic septicemia among recently shipped dairy cattle. Native cattle contracted the disease from these cows. Jones (1921) separated Pasteurella organisms into three groups. Group I fermented dextrose, lactose, saccharose, maltose and mannite. They were hemolytic, but did not produce indol. Group II produced acid in dextrose, and saccharose. All produced indol and were hemolytic. Group III, two strains fermented dextrose, saccharose and mannite, but did not produce acid in lactose, maltose, and salicin. In the main these groupings were retained when agglutination tests were applied. This author (1922) studied sixteen strains of Pasteurella organisms isolated from cows and calves. He found that all strains resembled each other antigenically. Serological tests showed these strains to belong to three groups. Serum prepared by immunizing a rabbit with a single Group I strain, agglutinated only Group I cultures. Cross agglutination was not observed in either Group I, II, or III.

Glynn (1921) described an occurrence of hemorrhagic septicemia among cows and calves, in which hemorrhagic diarrhea, watery discharge from the eyes, and subnormal temperature were the principle clinical symptoms. Immunological studies on the value of heat killed hemorrhagic septicemia bacterins were conducted by Graham and Swartz (1921). It

was noted that heat killed cultures of P. suiseptica possessed no advantage over commercially prepared bacterins. In fact, neither one protected guinea pigs or rabbits against artificial exposure. Dorset, McBride and Niles (1922) isolated B. bovisepticus from 50 per cent of swine affected with "Hog Flu". They were unable to reproduce the disease in experimental animals with this organism.

Newsom and Cross (1922) found that vaccines prepared from heat killed B. ovisepticus gave little, if any, protection in either rabbits or sheep. Live cultures administered in large amounts produced some protection. These authors (1922 b) observed a severe outbreak of hemorrhagic septicemia among 3000 sheep that had been recently shipped. P. ovisepticus was isolated from the spleen and lung tissue. This organism was pathogenic for sheep in large doses. In 1922 Murray and McNutt reported the isolation of Pasteurella from localized abscesses in chickens, horses, and sheep that had been previously treated with anti-hemorrhagic septicemia serum, or had been used in the preparation of anti-hemorrhagic septicemia serum. It was suggested by Moore and McAuliff (1922) that the acute form of hemorrhagic septicemia, like hog cholera may be caused by a filterable virus, and that B. bovisepticus may be a secondary invader. Hoskins (1922) isolated two strains of P. bovisepticus a virulent and non-virulent strain from the same animal.

Schalk and Roderick (1922) subjected seven groups of hogs to adverse conditions in an effort to determine whether starvation, exhaustion, parasitic infestation or improper feeding were important predisposing factors in hemorrhagic septicemia. In none of the groups were they able to detect the slightest rise in temperature, or other clinical symptoms which might suggest hemorrhagic septicemia. By means of complement fixation tests Roderick (1922) was able to differentiate a bovine, a swine and an ovine-avian-rabbit-cavia group. A toxic form of swine plague which occurred in the peracute form causing death in 48 hours was reported by Birch and Benner (1923). Cahill (1923) isolated B. paratyphi from both cattle and swine that were suffering from an apparent attack of hemorrhagic septicemia. P. oviseptica could not be demonstrated in these cases.

Benner (1923) states that the prevention of hemorrhagic septicemia consists of keeping a widely distributed microorganism from becoming pathogenic. He suggested that this is accomplished by keeping down devitalizing influences. An acute form of hemorrhagic septicemia in mules which caused death within 24 hours was observed by Church and Stubbs in (1923). Bacillus bipolaris septicus was isolated but was non-pathogenic for rabbits. Kelser (1923) mentions Bacillus bipolaris septicus along with streptococci, staphy-

lococci, and pneumonococci as secondary invaders occurring in contagious pleuro-pneumonia and equine influenca. The vaccination of cattle at the stockyards against hemorrhagic septicemia before shipping to their final destinations was proposed by Mohler (1923). Unfavorable results with the use of hemorrhagic septicemia bacterin in animals showing symptoms of illness was reported by Bossenberger in (1923).

Fitch and Nelson (1923) were unable to show any striking fermentation differences in the organisms with which they worked, but separated out four well defined groups on the basis of agglutination tests. Hemorrhagic septicemia among dairy cattle on the high plateau of Bogota was reported by Noback (1924). The disease was more prevalent after sudden changes of temperature from very warm days to cold frosty nights. Buckley and Gochenour (1924) claim to have successfully immunized a six months old heifer against hemorrhagic septicemia by one injection of 3 cc of a 24 hour bouillon culture of P. boviseptica. In 1924 Hadley reported the occurrence of hemorrhagic septicemia among 9,500 lambs following rail transportation. It was observed that this disease was apparently not transmitted by contact and was self limited.

Gochenour (1924) prepared an aggressin from Pasteurella bovisepticus (buffalo strain) which was used successfully

as an immunizing agent against hemorrhagic septicemia in cattle. This author (1925) isolated a strain of P. boviseptica from a buffalo. This strain was highly pathogenic for horses, mules, swine and sheep. Subcutaneous injections of 0.01 cc of a bouillon culture proved fatal within 36 hours. LeLouet (1925) found that the virulence of bacteriophage was markedly increased through successive passage on fresh cultures. He found that injections of 0.04 to 1.0 cc of bacteriolysates of P. boviseptica produced lasting immunity in steers. In 1925 Graham, Tunnicliff and Frank noted that rabbits and guinea pigs injected with artificial aggressin resist artificial infection more consistently than following bacterin injections. Tanaka (1926) studied 26 strains of hemorrhagic septicemia organisms from rabbits, cattle, sheep, swine, buffalo, guinea pig, chicken and observed a close similarity as to their biochemic, cultural and morphological characteristics. Bipolar organisms resembling Pasteurella were isolated from a case of parenchymatous mastitis by Schlotthouer and Hardenbergh (1926). This organism was pathogenic for rabbits but not for guinea pigs.

Marshall and Lee (1926) believe that certain forms of so-called shipping fever are possibly due to bipolar in-

fection and that treatment with anti-hemorrhagic septicemia serum may be biologically correct. In 1926 Hutyra and Marek mention the virus of hemorrhagic septicemia as being highly infectious in the latter stages of the disease. Blood and other body fluids or excrements may readily infect susceptible animals at this time. A satisfactory method for preparing salmonella pullorum antigens for complement fixation tests was described by Bushnell and Hudson in (1927). In discussing the report of the committee on transmissable diseases, Miller (1927) calls attention to instances where hemorrhagic septicemia has been transmitted from imported animals to native animals. In 1928 Belding found that fish septicemia was an important disease of fish in the New England States and that the causative organism was one of the Pasteurella group. Kinsley (1929) states that body temperature is an important clinical factor in the differentiation of hog cholera and hemorrhagic septicemia. Hog cholera is a rapidly spreading disease, while hemorrhagic septicemia is a slow spreading disease.

Mallmann (1930) met with a bovine serum which agglutinated bovine and porcine types of Brucella abortus and Pasteurella organisms from various animal species. This serum agglutinated Pf. mallei to a somewhat lower titre. Brucella organisms were agglutinated by Pasteurella sera. For this

reason he concluded that agglutination of Brucella abortus by a suspected serum was not conclusive evidence of infection with abortion. In 1933 Priestley did not succeed in establishing a serological relationship between the genera Brucella and Pasteurella. In 1930 Murray and Biester suggested that corynebacteria was the probable cause of pulmonary edema in both calves and swine. A mineral mixture agar for the cultivation of Pasteurella boviseptica was prepared by Scott in (1930). Favorable results in the treatment of "shipping fever" by intravenous injections of 8 and 10 per cent solutions of sodium bicarbonate was obtained by Hixon in (1930). Marshall (1930) recommended the use of anti-hemorrhagic septicemia serum if animals are to be shipped within two weeks and hemorrhagic septicemia bacterin as a preventive in large doses. Edington (1930) identified a type of bovine pneumonia which is due to infection with a Pasteurella boviseptica belonging to Jones Group I.

In 1931 Kinsley suggested that the causative organism of hemorrhagic septicemia may be already present in the animal body and not have a chance to develop until animals have had their resistance or vitality lowered. Miller (1931) lists bacterins and aggressins as preventives and anti-hemorrhagic septicemia serum as a curative. He states that animals treated with hemorrhagic septicemia bacterins

and aggressins during shipment showed greater losses than among untreated animals. The use of anti-hemorrhagic septicemia serum for affected animals was recommended by Mc Intosh (1931). Scott and Farley (1932) isolated P. bovi-septica in 80 per cent of the cases of shipping fever where autopsies were made. Farley (1932) observed greater losses among vaccinated cattle than among unvaccinated animals, and that revaccination of cattle at the farm after they had been vaccinated at the stockyards resulted in very heavy losses (10 and 11 per cent). In 1932 Topacio prepared a prophylactic whole culture vaccine against hemorrhagic septicemia (Barbone) which was very effective in the production of an active immunity. He found that intradermal and subcutaneous inoculations are superior to other routes of vaccination.

Newsom and Cross (1932) studied strains of organisms isolated from sheep and cattle on the basis of biochemical tests and agglutination tests. They found that all cultures could be separated into typical and atypical groups, except two, a cattle culture and a sheep culture by indol production, hemolysis and rabbit virulence. Serologically there was no cross agglutination between the two groups. Gaiger

and Davies (1932) described hemorrhagic septicemia (Barbone) as a spontaneous disease of cattle and buffalos which sometimes spreads to sheep and wild ruminants. They state that barbone is essentially a disease of young animals not transmissible by direct contact. They suggest that there are two distinct types of hemorrhagic septicemia, the spontaneous or acute type characterized by high mortality and the second, shipping fever or exposure disease, having slow onset and low mortality.

A FIELD STUDY OF SHIPPING FEVER

A cooperative project was started in 1929 for the purpose of obtaining information on cattle shipped into Kansas from the Kansas City and Wichita stockyards. The information on all shipments of cattle was obtained by the Honorable J. H. Mercer, State Live Stock Sanitary Commissioner. All reports from buyers concerning the outbreaks of disease among their cattle was made to the Live Stock Commissioner's Office. All herds in which disease developed a few weeks after receipt of cattle were visited as soon as possible.

Herd History

A complete herd history was obtained on all shipments. An effort was made to determine:

1. The nature of the immunizing agent used.
2. The age, weight and condition of animals when purchased at the stockyards.
3. Whether or not the cars in which animals were shipped were properly cleaned, disinfected, and bedded.
4. The condition of the weather at the loading point, during shipment and after reaching the destination.
5. The nature of the food, at the stockyards, during shipment and after arrival at the farm.
6. How many times animals were fed and watered during shipment.
7. Method of getting animals from unloading point to the farms, whether driven or trucked and the distance of the journey.
8. The length of time the animals were on the farm before disease appeared.
9. The number of animals first noticed sick and the progress of the disease through the herd following its onset.
10. The use of biologics after arrival at the farm.

11. Information relative to watering facilities, nature of food, if animals were allowed sufficient acerage for grazing.

Symptoms of Disease

In cattle the disease usually occurs in the acute or peracute form. In the acute form the disease develops very rapidly and may last from 24 hours to a week or ten days. There is usually an elevation of body temperature from 103.5°F. to 107°F. Affected animals refuse food. Edematous swellings may appear beneath the skin of the throat, brisket or other portions of the body. There may be difficulty of breathing, occasional coughing, increased salivation, lacrimal discharge and muscular weakness.

The peracute form was observed in the majority of field cases that were investigated. This form of the disease, develops among cattle within ten days to two weeks after arrival at the farm. The animals lose flesh rapidly, develop a nasal and lacrimal discharge, and a painful cough is readily observed when affected animals are caused to move about. There is usually a rise in temperature from 103.5°F. to 106°F. A profuse diarrhea was observed in a number of instances.

Autopsy

The thoracic cavity is the seat of most of the important pathological changes. The amount of pericardial and pleural fluid is increased and is usually of a sero-gelatinous consistency. The quality varies from a few ounces to several quarts. Pneumonic areas are commonly observed in the apical and cardiac lobes, less frequently in the diaphragmatic lobes, the lungs have a distinct marbled appearance due to edematous infiltration of the interlobular septa. The large bronchioles show congestion of the mucous membranes. Areas of pleurisy are frequently present. Adhesions of the pleura and pericardium are commonly observed, subendocardial and petechial hemorrhages on base of heart are frequantly noted. The bronchial and mediastinal lymph glands are congested and hemorrhagic gastro-enteritis was present in all cases.

BACTERIOLOGICAL STUDY OF MATERIALS OBTAINED FROM AUTOPSIES IN THE FIELD

Methods of Isolation

Most of the tissues examined were taken from autopsies made during a field study of 127 herds of cattle affected

with shipping fever. Autopsies were preformed in 28 of these herds. Portions of lung, liver, spleen, kidney, heart blood, pericardial and pleural exudates were packed in ice and brought to the laboratory for further study.

Isolation of organisms from this material was made on salts agar (Scott 1930), consisting of:

Peptone	20 grams
Agar	15-20 grams
Ammonium phosphate ,.....	0.5 grams
Potassium bicarbonate	0.5 grams
Potassium citrate	2.0 grams
Glucose	0.5 grams
Glycerol	5.0 grams
Sodium chloride	2.7 grams
Beef heart decoction	1000 cc

To maintain or increase virulence 2.5 grams of ferric ammonium citrate was added. This agar medium does not require filtration and its reaction is adjusted to about pH 7.2. Brom thymol blue was used as an indicator.

Table 1 lists the bacterial flora isolated from 26 cases of shipping fever. P. boviseptica was found in most of the cases.

Table 1. Bacterial flora found in cases of shipping fever.

Pasteurella Boviseptica	Cases	Colon Aerogenes	Cases	Alcaligenes *Bronchisepticus*	Cases
Alone	5	Alone	0	Alone	0
Associated with *E. coli*	6	Associated with *P. boviseptica*	6	Associated with *P. boviseptica*	4
A. bronchi-septicus	4	*A. bronchi-septicus*	3	*E. coli*	3
E. coli and *A. bronchisepticus*	1	*P. bovisep-tica* and *A. bronchi-septicus*	1	*P. boviseptica* and *E. coli*	2
Acid-former	1	*P. bovisep-tica* and diphtheroid	1	*P. boviseptica* and acid-former	1
E. coli and diphtheroid	1	*P. bovisep-tica* and acid-former	1	Diphtheroid, *E. coli* and *P. bovi-septica*	1
E. coli and acid-former	2			*E. coli*, acid-former and *P. boviseptica*	1

(table 1 continued)

A. bronchi-septicus and acid-former	1		
Totals	21	12	12

Direct isolations from rabbits and guinea pigs were made from the heart blood or tissue exudates. Three general groups of organisms were found in the materials obtained from autopsies. Pasteurella bovisepticus was the most constant organism found. This organism was isolated from 23 (82.10 per cent) of the 28 cases examined.

P. boviseptica was isolated alone in 7 of the 21 cases and from both heart and lung in 5 autopsies. P. boviseptica alone was isolated from the heart in 4 cases. Colon type organisms were isolated with P. boviseptica in five cases where animal inoculation was necessary.

Pasteurella cultures were also obtained from routine autopsies in four cases. P. boviseptica and Actinomyces necrophorous were both recovered from the necrotic material, of a case of necrotic laryngitis. Second from the lung of a calf, which had died of acute bloat. Third, from the lungs of a sheep which had died of overfeeding disease. Fourth, from a steer that was brought too rapidly onto full feed of concentrates. The second most common organisms isolated were of the colon-aerogenes type. These were isolated in 10 (35.71 per cent) of the cases. Colon type organisms were isolated in all materials that took 20 or more hours to reach the laboratory.

In most cases where the materials were obtained in the fresh condition only P. boviseptica were isolated. P. boviseptica was isolated most consistently from fresh lung tissue obtained from cases of shipping fever. The third class of organisms isolated were Gram negative rods which did not ferment any of the 21 carbohydrates examined. A number of these organisms were found to correspond culturally and by agglutination tests to Alcaligenes bronchisepticus.

Methods of Identification

The identification of P. bovisepticus was made by comparing morphological, fermentation and pathological characteristics of the organisms isolated, with the character of twelve Pasteurella boviseptica cultures obtained from three commercial laboratories, three experiment stations, the Bureau of Animal Industry and from India.

The fermentative characters of the Pasteurella cultures, the colon-aerogenes types and other organisms were determined on "Salts sugar free" medium, (Scott, 1930), consisting of:

Distilled water	1000.00 cc
Peptone	20.00 grams
Ammonium dihydrogen phosphate	0.50 grams
Potassium bicarbonate	0.60 grams

Ferric ammonium citrate ..... 2.5 grams

Disodium hydrogen phosphate ..... 1.0 grams

Brom thymol blue q.s. to cause a dark green color.

This medium was placed in small test tubes sterilized and incubated 12 hours. One cc of a ten per cent solution of the carbohydrates was then added. Five characteristics were considered sufficient to make an identification of Pasteurella cultures.

1. Smears from tissues showed bipolar organisms when stained with methylene blue.
2. The colonies growing on agar were small "dew drop" and were seldom more than one mm in diameter.
3. The organisms examined from agar slants and broth cultures were short non-motile Gram negative rods.
4. The cultures proved pathogenic for rabbits and guinea pigs.
5. The cultures produce acid but no gas in glucose and in sucrose but did not attack lactose and maltose.

Sources of Pasteurella Cultures Studied

The Pasteurella organismsused in this study were obtained from widely separated points. Twenty-five bovine strains, one equine, three buffalo and two avian strains

are represented.

1, 2, and 5. Bovine strains - Jensen Salsbery Laboratories.

4. Buffalo - obtained from a commercial laboratory.

6. Equine - obtained from Dr. C. A. Brandly of the Department of Bacteriology, Kansas State College.

7. Swine, Jensen-Salsbery Laboratories, Kansas City, Missouri.

8. Buffalo strain - Bureau of Animal Industry, Washington, D. C.

10. Bovine, Dr. A. T. Kinsley of Kinsley Laboratories, Kansas City, Missouri.

15. Buffalo, Bureau of Animal Industry, Washington, D. C.

17. Bovine, isolated from a field case of shipping fever in Kansas.

18. Bovine, isolated from a field case of shipping fever in Kansas.

19. Bovine, isolated from a field case of shipping fever in Kansas.

24. Bovine, Fort Dodge, Iowa.

26. Bovine, isolated from a field case of shipping fever in Kansas.

27. Bovine, isolated from a field case of shipping fever in Kansas.

28. Bovine, isolated from a field case of shipping fever in Kansas.

29. Bovine, isolated from a field case of shipping fever in Kansas.

30. Avian, Dr. C. A. Brandly, Department of Bacteriology, K. S. C.

31. Avian, Dr. C. A. Brandly, Department of Bacteriology, K. S. C.

32. Bovine, isolated from a field case of shipping fever in Kansas.

34. Bovine, isolated from a field case of shipping fever in Kansas.

36. Bovine, Dr. Johnson, Kansas City, Missouri.

43. Bovine, obtained from a field case of shipping fever in Kansas.

44. Bovine, obtained from a field case of calf diphtheria.

45. Bovine, obtained from Dr. Jungherr.

46. Bovine, obtained from Storrs Experiment Station, Storrs, Connecticut.

47. Bovine, obtained from Storrs Experiment Station, Storrs, Connecticut.

48, 49. Indian buffalo strains furnished by Dr. Cooper of Muklesar, India.

50. Bovine, obtained from a field case of shipping fever.

51. Bovine, obtained from a field case of shipping fever.

57. Bovine, obtained from a field case of shipping fever.

Biochemical Study of Pasteurella Organisms

Fermentation studies of thirteen strains of Pasteurella organisms obtained from different sources were made. These organisms were inoculated into a salts-sugar free medium to which 1 cc of a 10 per cent solution of the various carbohydrates had been added after sterilization. These carbohydrate solutions were inoculated from a 24 hour salts broth culture of the organism to be tested.

Table 2 shows that all thirteen cultures reacted identically with the thirteen carbohydrates.

A study of the Gram negative non-fermenting organisms was made. It was found as shown in table 3 that these organisms did not produce acid or gas in any of the 21 carbohydrates studied. These organisms have the following

Table 2. Fermentation Reactions of Pasteurella organisms.

Carbohydrate	Strain of Pasteurella Organisms												
	4	6	7	8	17	19	25	28	30	44	48	49	51
MONOSACCHARIDE													
Pentoses													
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexoses													
Glucose	A	A	A	A	A	A	A	A	A	A	A	A	A
Levulose	A	A	A	A	A	A	A	A	A	A	A	A	A
Galactose	A	A	A	A	A	A	A	A	A	A	A	A	A
DISACCHARIDES													
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	A	A	A	A	A	A	A	A	A	A	A	A	A
POLYSACCHARIDES													
Dextrin	-	-	-	-	-	-	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-	-	-	-	-	-	-
GLUCOSIDES													
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-
ALCOHOLS													
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannite	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Gram negative non-fermentable organisms of the Bronchisepticus group.

Carbohydrates	Cultures					
MONOSACCHARIDES						
Pentoses	B2	B3	B4	B5	B8	B9
Arabinose	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-
Xylose	-	-	-	-	-	-
Hexoses						
Glucose	-	-	-	-	-	-
Galactose	-	-	-	-	-	-
Levulose	-	-	-	-	-	-
Mannose	-	-	-	-	-	-
DISACCHARIDES						
Lactose	-	-	-	-	-	-
Maltose	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-
Trehalose	-	-	-	-	-	-
TRISACCHARIDES						
Raffinose	-	-	-	-	-	-
POLYSACCHARIDES						
Dextrin	-	-	-	-	-	-
Inulin	-	-	-	-	-	-
GLUCOSIDES						
Salicin	-	-	-	-	-	-
ALCOHOLS						
Dulcitol	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-
Mannite	-	-	-	-	-	-
Perseitol	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-
PHENOLS						
Inositol	-	-	-	-	-	-

characteristics:

1. Short single rods, occurring singly, motile and Gram negative.
2. Gelatin not liquified.
3. Agar slant, moist, white glistening.
4. Potato; abundant growth, brownish and glistening.
5. Indol not formed.
6. No acid or gas in carbohydrate media.
7. Acetyl-methyl-carbinol test; negative.

This group of organisms proved to be non-pathogenic when inoculated into guinea pigs and rabbits.

A comparison was made with two-strains of Alcaligens bronchisepticus obtained from Park Davis Co. Fermentation reaction, agglutination, production of indol, acetyl-methyl-carbinol and pathogenicity tests showed that the strains isolated from cases of shipping fever correspond to A. bronchisepticus.

A Study of Organisms Isolated From Normal Lungs of Cattle

Portions of 83 normal lungs were obtained from a packing house in Kansas City, and were shipped to Manhattan packed in ice. These lungs were examined by cultural methods and the bacterial flora was determined. Pasteurella boviseptica

was not isolated from any of these lungs. Colon organisms were isolated in 8 cases and the A. bronchisepticus or related organisms in 8 cases.

The most constant organisms isolated were staphylococci and streptococci.

Table 4 lists the total number of times that the different organisms were isolated and the percentage. Staphylococci and Streptococci were isolated in the greatest percentage of cases.

Table 4. Bacterial flora of 83 normal lungs.

Species	Times Isolated	Lungs Containing Organisms (%)
Staphylococci Gram positive	36	43.0
Streptococci Gram positive	33	39.0
Gram positive rods, free growing	26	31.3
Diphtheroids	8	9.6
Colon-aerogenes group	8	9.6
A. bronchisepticus	8	9.6
Spore-forming organisms	7	9.0
Cocco-bacillus	4	4.9
Pasteurella	0	0

Identification of Colon-aerogenes was determined by cultural characteristics and carbohydrate fermentation. These organisms fermented dextrose, lactose, and saccharose.

Moore (1893) isolated organisms resembling those of the Pasteurella group from secretions covering the mucosae of the upper air passages in healthy cattle, dogs and cats. These organisms were found to be present apparently in normal habitat.

These isolated organisms when inoculated into rabbits varied from the rapidly fatal septicemic forms which destroyed rabbits in 16 hours when inoculated subcutaneously to those that possessed so little virulence that thirteen days were required for rabbits to die. The presence of P. bovi-septica in the respiratory tract of normal animals would indicate that conditions of handling, feeding, watering and exposure might lower the resistance of animals so that this organism could produce clinical cases of shipping fever.

Smith (1896) observed Pasteurella organisms in diseased lungs and stated that these organisms were not infrequently found in the mouth and upper respiratory passages of healthy cattle.

Smith (1892) isolated bacteria from secretions covering the posterior nares, larynx and pharynx of certain healthy pigs. These organisms could not be differentiated from the swine plague germ.

Tests For Indol Production and Hemolysis

Indol was determined in cultures grown in Dunhams Peptone solution for 48 hours, then cultures were allowed to remain at room temperature for six days after incubation before the test was applied. Thirty-two cultures were studied. The Ehrlich-Bohme technic, as described in the Manual of Methods, Society of American Bacteriologists was used. Hemolysis was measured by surface streaking on chicken blood agar plates. Three tests each were applied for the presence of indol and hemolysis. Cultures No. 18, 28, 32, 48, and 49 did not produce indol. No hemolysis was observed with the cultures streaked on rabbit blood agar.

Newsom and Cross (1932) studied cultures of Pasteurella isolated from sheep and cattle. They were able to classify these cultures into typical and atypical groups by means of fermentation reactions. Agglutination tests showed that there was no cross agglutination between the typical and atypical groups, but that the groups could be divided into at least two subgroups.

The production of indol, hemolysis of rabbit blood agar and pathogensis for rabbits showed the typical fermentation groups produced indol and were pathogenic. The atypical groups did not produce indol and were non-pathogen-

ic.

VACCINATION OF ANIMALS

Study of Vaccination As Practiced in The Field

A history of the condition of the shipped in animals was obtained and an effort was made to determine whether or not the animals were vaccinated before shipment or at the stockyards and whether any biological product had been used after arrival at the farm.

Table 5 shows a comparison of the losses from shipping fever among those animals vaccinated at the stockyards or on the farm and among those animals not treated. The nature of the product used at the stockyards could only be ascertained in a few cases, so all vaccinations, whether with bacterins, mixed vaccines or aggressin, are listed under one head.

Table 5 shows that losses were highest during October and November and were again high during March. The severe weather conditions of the fall coincide with the heaviest shipping of feeder cattle. The long rail transportation apparently had some correlation with the severity of the disease as it manifested itself on the farm. Especially heavy losses occurred among cattle shipped from Texas and from Canada during October and March. In one of these cases a

shipment from Canada was delayed. Direct shipment of cattle to the feeder from the ranch on which they were raised and without being handled by a public or local stockyards had fewer clinical cases, but even under these conditions at least one such shipment suffered a severe outbreak of shipping fever.

Table 5 shows that losses among vaccinated cattle was about three times as high as among untreated animals. Among 5661 unvaccinated cattle there was a loss of 3.58 per cent and among 4119 untreated animals the loss was 1.02 per cent. The losses were higher in the vaccinated group during every month in which outbreaks occurred and in only one month, February, when the weather was fair and cool, did the losses among the treated animals approach the losses among the vaccinated group.

Table 6 indicates the incidence of shipping fever among herds vaccinated at the stockyards, or at the farm. This table lists the vaccine used at the farm. Table 6 also shows a comparison of the losses incurred from shipping fever among animals that had been vaccinated at the yards or on the farm, and among untreated animals.

In 381 animals vaccinated at the yard and given bacterin at the farm the losses were 11.2 per cent, and among 90 head vaccinated at the yards and given aggressin at the

Table 5. Losses from shipping fever in unvaccinated and vaccinated cattle.

Month	Not Vaccinated				Vaccinated				Weather
	No.	No. Herds	Dead No.	Dead Per Cent	No.	No. Herds	Dead No.	Dead Per Cent	
1930									
October	404	3	9	2.2	718	8	19	2.6	Rain
November	963	11	11	1.1	1249	18	58	3.4	Snow - rain
December	1357	8	5	0.3	1052	17	40	3.7	Snow - rain
January	596	7	6	1.0	1232	12	39	3.1	Cold - fair
February	149	4	3	2.0	684	7	19	2.8	Fair - cool
March	217	3	2	.92	292	5	6	2.5	Changeable
April	212	1	1	.47					Mostly fair-warm
July					100	1	6	6.0	Hot - dry
August	70	1	2	2.85	133	2	6	4.5	Hot - dry
1931									
January	126	2	1	.70					Clear - cold
February	25	1	2	8.0					Mostly fair-warm
March					201	1	30	14.8	Changeable
Total	4119	41	42	1.02	5661	71	203	3.58	

Table 6. Distribution of shipping fever in animals treated and untreated in the stock yards.

Month	Not Vaccinated at Yards									Vaccinated at Yards						
	Not treated at farm		Number of Herds	Treated at farm						Not treated at farm		Number of Herds	Treated at farm			
				Bacterin		Aggressin		Serum					Bacterin		Aggressin	
	No.	Dead		No.	Dead	No.	Dead	No.	Dead	No.	Dead		No.	Dead	No.	Dead
1930																
October	401	8	3					3	1	649	15	8	69	4		
November	842	17	10	180	7			121	0	1021	28	18	48	3		
December	899	5	7	540	29	93	6			325	14	17			90	9
January	591	5	7	260	3					939	16	12				
February	149	3	4	44	0					640	33	7				
March	217	2	3	182	5					110	1	4				
April	212	1	1													
July			1	100	6											
August	70	2								70	1	2	63	5		
1931																
January	124	1	2					2	0							
February	25	2	1													
March												1	201	30		
Total	3530	46		1306	50	93	6	126	1	3754	108		381	42	90	9
Percentage		1.30	39		3.82		6.46		.71		2.87	69		11.2		10

farm the loss was 10 per cent. The losses among the cattle vaccinated at the yards and untreated at the farm were 2.87 per cent in 3754 head. Animals not vaccinated at the yards and treated with aggressin at the farm showed a loss of 6.46 per cent in 93 head. Of 1306 cattle given bacterin at the farm the loss was 3.82 per cent. The losses among animals not vaccinated at the yards or treated at the farm showed a loss of 1.30 per cent in 3530 head studied.

It was seen that vaccination of animals at the farm after they had been subjected to the trials and exposures of rail transportation was followed by a slightly greater proportion of loss than where vaccination was performed at the yards. Double vaccination at the yards and again at the farm, proved very costly in the comparatively few cases in which this was practiced.

In two cases anti-hemorrhagic septicemia serum was given to very sick animals at the farm. In one herd three animals were treated with the loss of one, in a second two sick animals were treated, with no subsequent deaths and both recovered. One herd of 121 head, which showed evidence of shipping fever in 20 per cent of the animals, was treated with serum and some of this herd died.

A herd of thirty cattle including ten head of well conditioned calves, ten of medium grade and ten of poor quality, were purchased at the stockyards in Kansas City, February 16 and trucked to a farm near Manhattan, Kansas. These cattle were unloaded at the farm and were immediately allowed to fill themselves with cold water. A few hours after arrival there was a severe snow storm and a drop in temperature from a little above freezing to sub-zero.

Five days later several calves were noticed to be coughing and to have lacrimal and nasal discharges. Temperatures on the entire herd ranged from 103.5°F. to 106.5°F. A severe diarrhea was observed in three cases.

The herd was divided into two groups of 12 and 18 animals including as nearly as possible an equal number of calves showing most severe infection. Group I, 12 head were allowed to remain in the feed sheds, and were given 30 cc of anti-hemorrhagic septicemia serum, subcutaneously. One calf in Group I was in the advanced stages of pneumonia when treated. Group II, the remaining 18 head, were used as controls and were allowed the freedom of a large feed lot and stalk field.

The calf in advanced stages of pneumonia died three days after treatment and was autopsied. Pasteurella bovi-

septica was isolated in pure culture from the lung tissue.

Van Es and Martin (1922) reported some protective qualities of sera used against hemorrhagic septicemia of cattle and swine examined by preventing death of a considerable number of experimental animals when the latter were injected with virulent cultures. Also by a lengthening in the surviving period of those which succumb to the infection. However, the passive immunity conferred lasts less than one week.

Table 7 classifies the cattle according to weight. In this table as in the two previous tables, the losses among the vaccinated group are higher than those with the untreated animals. The losses are higher for the vaccinated groups in each weight classification. For example, considering animals between 200 and 300 pounds, there were no deaths among 184 untreated animals and 5.8 per cent in 293 vaccinated cattle. In the case of 500 and 600 pound animals, 496 untreated cattle showed a loss of 0.6 per cent. The number of deaths in the increasing weight classifications shows only a very slight decrease and it would seem that the size of the calves has only a very slight correlation with the incidence of shipping fever.

Buckley and Gochenour (1924) believe that the use of aggressin and bacterin are counter-indicated in infected herds, also that for the first few days following vaccina-

Table 7. Losses from shipping fever in cattle of different weights.

Weight	Not Vaccinated			Vaccinated		
	No.	Dead		No.	Dead	
		No.	Per cent		No.	Per cent
200 - 300	184	0	0	293	17	5.8
300 - 400	919	16	1.7	2034	106	5.2
400 - 500	441	7	1.58	749	26	3.5
500 - 600	496	4	0.8	749	42	5.6
600 - 700	1113	7	0.6	176	10	5.7
700 - 800	192	4	2.0	76	3	3.9
800 -	255	4	1.5			
Total	3600	42	1.16	4077	204	5

tion animals are more susceptible to disease than are non-vaccinated animals.

Hadley (1924) emphasizes the importance of exposure as a predisposing factor in shipping fever in cattle.

Newsom and Cross (1934) state that since hemorrhagic septicemia is apparently self limited, the use of vaccine during an outbreak is questionable as to value. They also emphasize the importance of exposure, a contributing cause in the production of hemorrhagic septicemia in sheep.

The Bureau of Animal Industry (1934) started an investigation in 1932 on the value of anti-hemorrhagic septicemia serum, hemorrhagic septicemia bacterin and aggressin in the control of hemorrhagic septicemia. Four methods of vaccination was employed and approximately 8000 cattle were used. Of these 4364 were vaccinated and the remainder were controls. The almost complete absence of shipping fever in the animals under test prevented the investigation from yielding much information.

Calf Infection Experiments

Seven calves were inoculated with 48 hour agar culture emulsions corresponding to tube No. 4 of McFarland's nephelometer.

Calf 1: Injected subcutaneously with 20 cc of P. bovisseptica, strain No. 8. This calf showed a rapid rise in temperature, bloating, and was dead in 18 hours. Autopsy showed extensive subcutaneous hemorrhages petechiae on serous surfaces, and the lungs were injected and edematous. The rumen, abomasom and intestines showed extensive areas of inflammation and were extremely friable throughout.

Calf 2: Injected intravenously with 22 cc of P. bovisseptica, strain No. 28. This animal died in 15 hours. The symptoms and lesions were identical with those of calf 1, with the exception that petechiation of serous membranes was absent.

Calf 3: Injected intravenously with 20 cc of Escherichia coli. There was a rapid rise in temperature accompanied by acute bloat. The animal died in 6 hours. Autopsy revealed a few subcutaneous hemorrhages, petechia on serous surfaces, congestion of the lungs and extensive gastroenteritis.

Calf 4: Injected subcutaneously with 12 cc of P. boviseptica, strain 28. The temperature increased from 101.4°F. to 105.2°F. in five hours, accompanied by stiffness and inappetence. The animal was almost normal within 24 hours. A second injection of 20 cc of emulsion of P. boviseptica was given at this time. This injection was followed by a slight rise in temperature and a complete recovery within three days.

Calf 5: Animal fed ordinary hay and grain ration for three weeks and kept in the Veterinary Hosptial. This animal was injected with 15 cc of Pasteurella boviseptica culture. The temperature increased from 101.3°F. to 105.8°F. in five hours followed by stiffness and loss of appetite. Death occurred 15 hours after injection. The lesions found on autopsy were subcutaneous hemorrhages, petechia of serous surfaces, injection and edema of lungs. There was an extensive gastro-enteritis. The rumen content was dry.

Calves 6 and 7: Grain and hay ration was withheld from these animals for 24 and 48 hours previous to injecting 15 cc of P. boviseptica emulsion. The increase in temperature of calf 6 was 101.6°F. to 105.2°F., and in calf 7, 101.6°F. to 102.2°F. The temperature was normal in 24 hours and both animals made a rapid recovery.

Ten days after the first injection, calf 6 was injected with 20 cc of a culture of E. coli. The temperature increased 101.2°F. to 102°F. in four hours. This animal was in an apparently normal condition in 24 hours.

These experiments indicate that calves can be killed by intravenous injections of normally pathogenic and non-pathogenic microorganisms, and that P. boviseptica can cause death in calves on moderate feed, but that calves on full feed consisting of grain, hay, and plenty of water, readily resist cultures of P. boviseptica given subcutaneously. E. coli failed to kill a calf that had received a previous injection of P. boviseptica.

Washburn, (1918) was unable to reproduce shipping fever by the administration of nasal secretions and blood from affected animals.

Immunization of Rabbits

Rabbits were injected five to ten times with cultures of P. boviseptica in saline suspensions. Each of the ten rabbits received separate injection of one of the ten strains. The sera from these rabbits was used to determine the agglutination reaction of 32 cultures of P. boviseptica in dilutions of 1:40 to 1:1600. The mixture of antigen and serum was incubated for 12 to 15 hours and then left at

room temperature for 12 hours before readings were made. It was necessary in a few cases to prolong incubation before obtaining agglutination.

Table 8 shows that there was no direct relationship between the degree of agglutination developed by the rabbit and its resistance to subsequent test doses of virulent cultures. Rabbits 10 and 897 showing agglutination titres of 1:1600 died within five days, whereas, rabbit 898 had a titre of 1:80 and survived.

Twenty-four rabbits weighing 1600 to 2000 grams were obtained.

Three rabbits were injected with heat killed emulsions of culture No. 8 and three with culture No. 17 standardized to 10 times, 100 times, and 1000 times tube 1 of McFarland's nephelometer.

Three rabbits were injected with formalin killed emulsion of culture No. 8 and three with culture No. 17 standardized to 10 times, 100 times, and 1000 times tube 1 of McFarland's nephelometer.

Three rabbits were injected with acetaldehyde killed cultures of No. 8 and three with cultures No. 17 standardized to 10 times, 100 times, and 1000 times tube 1 of McFarland's nephelometer.

Table 8. A comparison of the agglutination titres obtained in rabbits by injections of *P. boviseptica* and their survival following an injection of *P. boviseptica* cultures.

Rabbit	Culture	Immunization			Agglutination Titre for Culture 17	Test Injection			Result
		Injection No.	Dose (cc)	Last Injection date		Date	Culture	Dose (cc)	Date	
724	18	8			1:320					D. 5 days
10	17	5			1:1600					D. 5 days
897	17	5			1:1600					D. 5 days
898	17	1	10	4-4	1:80	5-1	17	0.12	5-1	Lived
829	1	1			1:160					D. 4 days
895	1	1			0					D. 3 days
839										D. 2 days

Three rabbits were injected with merthiolate killed emulsions of culture No. 8 and three with culture No. 17 standardized to 10 times, 100 times, and 1000 times tube 1 of McFarland's nephelometer.

Difficulty was at first encountered in preserving the different concentrations of antigen with 1:10,000 mertheolate until a 1:1000 dilution was used.

The antigens used in the immunization of the rabbits were prepared by growing the cultures on salts agar for 48 hours and then washing off the surface growth with Tyrodes solution.

Tyrodes Solution

NaCl	8.0 grams
KCl	0.2 grams
$CaCl_2$	0.2 grams
$MgCl_2$	0.01 grams
Na_2HPO_4	0.05 grams
$NaHCO_3$	0.2 grams
Glucose	0.8 grams
Distilled water	1000.0 cc

The concentrations of 10 times tube 1 were determined by comparing with tube 10 of McFarland's nephelometer. Concentrations of 100 and 1000 times tube 1 were prepared by centrifugation and removing the clear supernatent fluid

until the required concentrations were determined volumetrically.

All rabbits were inoculated subcutaneously at regular intervals of one week, with 1 cc of the concentrations of antigens described above until ten injections were made. Three weeks after receiving the tenth injection subcutaneously the inoculations were continued, only intraperitoneal injections were used but in the same concentration. Six of these injections were made.

All rabbits were bled and their sera tested for the presence of antibodies by agglutination reactions of 32 cultures of P. boviseptica in dilution of 1:200 to 1:1600. The serum antigen mixtures were incubated for 12 hours and then placed at room temperature for 12 hours. It was necessary in a few cases to incubate the tubes a second time before readings could be made.

All sera were tested against antigens prepared from 32 cultures of P. boviseptica but only those antigens which showed positive results were listed.

Table 9 lists the maximum agglutination reactions in the dilutions as observed in rabbits immunized against culture No. 8. No appreciable difference was noted in the agglutination titre for rabbits immunized with the concentrated or the more dilute antigens.

Table 9. Rabbits immunized against culture No. 8.

Antigens	Concentrations of antigen compared to McFarland No. 1								
	10	100				1000			
	H	H	F	A	M	H	F	A	M
1	800	400	400	400	200	800	400	1600	1600
2	400	400	1600	800	400	400	1200	400	1200
4	800	200	400	200	1200	800	1600	800	800
5	400	200	400	800	200	400	800	1600	1600
6	400	400	200	200	200	400	400	200	400
7	400	400	200	400	400	400	800	400	200
8	1600	800	800	1600	800	1600	800	800	1600
10	1200	400	800	800	400	400	400	1600	1600
14	200	400	400	200	400	200	200	1200	200
15	400	200	200	400	1200	400	-	1200	-
17	400	200	400	200	-	200	200	400	800
18	200	1200	400	-	200	800	800	400	400
19	800	400	1200	400	1200	-	200	400	1600
24	400	200	800	800	400	200	-	1200	1200
26	200	1200	400	400	-	1600	1200	1200	1200

H = heat killed 60°C., 1 hour. 1200 = incomplete at 1:200 dilution.
F = formaldehyde 1 per cent.
A = acetaldehyde 1 per cent
M = merthiolate 1 - 1000.
10, 100, and 1000 represents the concentrations of antigens comparable to tube No. 1 of McFarland's nephelometer.

There is an indication that culture 5, 10 and 19 might be grouped with No. 8 strain.

Table 10 lists the agglutination reactions of rabbits immunized against culture No. 17. There is an indication that cultures 26, 14, 7, 6, 4, and 2 belong to an agglutination group. Culture 10 and possibly No. 8 may belong to the same group as No. 17.

Table 11 lists the number of complete agglutination reactions in dilutions of 1:400, 1:800, and 1:1600 found in table 8 and 9. This table also includes the sera from animals immunized with antigens treated with the four preservatives. This table shows that sera from animals immunized by antigens preserved in acetaldehyde gave 73 per cent high titres; mertheolate 63 per cent; formaldehyde 54 per cent and heat 52.7 per cent. This would indicate on the limited number of sera tested, that possibly acetaldehyde is the more efficient preservative for the preparation of Pasteurella boviseptica antigens.

Table 11 includes complement fixation of sera immunized against culture No. 17. Sera from rabbits immunized by the administration of heat and acetaldehyde preserved antigens gave complete complement fixation in dilutions of 1:40. For sera obtained from rabbits immunized with heat and actealdehyde prepared antigens gave 75 per cent complete comple-

Table 10. Rabbits immunized against culture No. 17.

Antigens	Concentration of antigen compared to McFarland No. I.								
	10	100				1000			
	H	H	F	A	M	H	F	A	M
1	200	400	400	200	400	i200	200	400	800
2	800	i200	-	400	200	200	200	400	200
4	200	-	200	400	i200	200	i200	1600	400
5	i200	i200	i200	400	200	i200	800	800	400
6	800	200	200	400	400	200	400	200	200
7	1600	i200	i200	200	400	400	i200	400	200
8	1600	800	400	400	400	i200	400	1600	400
10	200	800	400	400	400	200	800	1600	800
14	800	i200	200	i200	200	-	200	800	200
15	400	200	i200	400	400	i200	200	200	-
17	800	800	1600	800	1600	800	800	1600	800
18	200	400	i200	200	800	200	200	800	400
19	i200	i200	-	400	400	-	400	400	200
24	400	i200	800	400	800	-	200	400	400
26	400	1600	-	-	i200	400	i200	-	i200

H = Heat killed 60°C. 1 hour
F = Formaldehyde 1 per cent
A = Acetaldehyde 1 per cent
M = Merthiolate 1 - 1000

i200 = incomplete at 1:200 dilution

10, 100 and 1000 represent the concentrations of antigen comparable to tube No. 1, McFarland's nephelometer.

Table 11. Showing high dilution in sera from animals immunized with antigens treated by heat, formaldehyde, acidaldehyde and mertheolate.

Concentration of antigen comparable to McFarland's tube No. 1	Antigen	H	No. Tests	F	No. Tests	A	No. Tests	M	No. Tests
100	17	6	14	8	15	10	14	9	14
1000		5	12	4	12	12	14	9	15
100	8	9	15	10	15	10	14	6	13
1000		11	14	8	13	10	15	11	14
No. tests		29	55	30	55	42	57	35	56
Per cent		52.7		54.0		73.0		63.0	
Complement fixation showing per cent high dilution 4 tests									
No. Tests		3		2		3		2	
Per cent		75.0		50.0		75.0		50.0	

H = heat killed at 60°C. for 1 hour.
F = formaldehyde 1 per cent.
A = acetaldehyde 1 per cent.
M = merthiolate 1:1000.

ment fixation, and 50 per cent complete complement fixation for sera obtained from rabbits immunized by the use of formaldehyde and mertheolate prepared antigens.

For the application of complement fixation tests all sera were tested against an ecto-antigen prepared from culture No. 17. Bushnell and Hudson, (1927) showed that ecto-antigen are water clear and while they are not suitable for the agglutination test, they have many advantages for complement fixation.

Preparation: The mass of cells obtained by centrifugation of a saline suspension of a culture grown for 48 hours on agar media. The suspension was washed twice with salt solution and the supernatent fluid discarded. Seven cc of saline solution are added to the sediment (about 1 cc) This sediment of cells is thoroughly mixed with the salt solution with a pipette, then thirteen cc of a salt solution are added and the washing process continued by inspirating into a pipette and blowing out at least fifty times. The suspension is again centrifugated at high speed and the supernatent fluid, which is practically cell free and water clear, is used as the antigen.

This process gives an antigen which is highly antigenic and not anticomplementary in 1.5 cc amounts. It is especially valuable because it is very clear and does not mask the reading of the tests.

Table 12 shows the results of complement fixation of sera against culture No. 17. It is seen that complement fixation in dilutions of 1:40 was complete for those antigens preserved with acetaldehyde and merthiolate in antigens 1000 x tube 1 of McFarland's nephelometer.

Tanaka, A., 1926, found that the Pasteurella organisms which he studied were only very slightly antigenic either for the agglutination, complement-fixing substances or for the immunization of animals.

A study of housing conditions on farms where shipping fever occurred was made.

It was noted that the herds were handled under a variety of conditions. Less than half of the farms visited were equipped with adequate facilities for taking care of shipped in cattle. In some of these instances individuals were attempting to feed cattle as a sideline without equipment.

Some farmers purchased from 50 to 150 head of cattle during adverse weather conditions and placed them in pastures which were not equipped with sheds or barns. It was noticed that the greater number of deaths were observed among those herds which were not properly cared for during the first week or ten days after arrival at the farm.

Table 12. Complement fixation of sera against culture No. 17.

Concentration of antigen used to immunize rabbits	Antigen used to immunize rabbits		Complement fixation		
			Dilutions		
	Antigen No.	Treatment	1-40	1-20	1-10
10 x 1	8	H	4	4	4
10 x 1		F	2	4	4
10 x 1		A	0	2	4
10 x 1		M	2	2	4
10 x 1	17	H	2	2	4
10 x 1		F	0	0	0
10 x 1		A	4	4	4
10 x 1		M	4	4	4
100 x 1	8	H	4	4	4
100 x 1		F	4	4	4
100 x 1		A	4	4	4
100 x 1		M	0	0	1
100 x 1	17	H	4	4	4
100 x 1		F	0	2	4
100 x 1		A	4	4	4
100 x 1		M	-	-	-

(Table 12 continued)

1000 x 1		H	-	-	-
1000 x 1	8	F	0	0	1
1000 x 1		A	4	4	4
1000 x 1		M	4	4	4
1000 x 1		H	4	4	4
1000 x 1	17	F	4	4	4
1000 x 1		A	4	4	4
1000 x 1		M	4	4	4

- = not tested.

The cattle that were properly fed and watered before shipment, in the stockyards, and after arrival at the farm showed very few cases of shipping fever.

SUMMARY

Shipping fever, stockyards pneumonia, and hemorrhagic septicemia are terms used synomously to describe a diseased condition of cattle incident to shipping. Animals when being shipped are subjected to changes of weather and irregularities of feeding and watering. The febrile septicemic disease known as shipping fever or hemorrhagic septicemia is the most serious of a group of maladies which commonly result from neglect or exposure of animals in transit or shortly after reaching their destination. Losses from this source vary from year to year, but are heaviest during fall and winter months. Young animals are more susceptible to disease incident to shipping than are older animals.

A hundred references were studied starting with early references pertaining to the work of Bollinger 1878 (Fitch 1921) and including the latest results obtained by the Bureau of Animal Industry (1934) on the controlled experiments pertaining to the value of biologics in the control of shipping fever in the field.

A field study of cases of shipping fever among herds of cattle shipped into Kansas from the stockyards at Kansas City and Wichita was made. This study extended over a two year period from 1929 to 1930. During this period 127 herds including approximately 7000 cattle of the stocker and feeder class were visited and examined.

Autopsies were made in 28 cases and materials were collected for laboratory study. Bacteriological and serological studies were made of the organisms isolated from these autopsies.

Three general groups of organisms were found in examining material from these cases. The most constant organism found was Pasteurella boviseptica which was isolated in 23 (82.10 per cent) of the cases.

The second most common organism isolated was of the colon type. These organisms were isolated in all materials which took more than 20 hours to reach the laboratory.

The third class of organisms isolated were Gram negative rods which did not ferment any of the 21 carbohydrates examined. A number of these organisms were found to correspond culturally and by agglutination tests to Alcaligenes bronchisepticus.

Five characteristics were considered sufficient to make an identification of Pasteurella cultures.

1. Smears from tissues showed bipolar organisms when stained with methylene blue.
2. The colonies growing on agar were small "dew drop" and were seldom more than 1 mm in diameter.
3. The organisms examined from agar slants and broth cultures were short non-motile Gram negative rods.
4. The cultures proved pathogenic for rabbits and guinea pigs.
5. The cultures produced acid but no gas in glucose and sucrose, but did not attack lactose and maltose.

The bacterial flora of 83 normal lungs of cattle, was made. Staphylococci and streptococci were isolated from the larger percentage of these lungs. The colon-aerogenes group, A. bronchisepticus and other organisms were also isolated. Pasteurella boviseptica was not isolated from any of these lungs.

It was noticed that losses were higher among those animals vaccinated at the stockyards or at the farm. Losses were approximately three times as high among vaccinated animals for the number of animals studied, as among the non-vaccinated animals.

Weight apparently had no effect in the resistance to shipping fever as losses were equally as high for the different weight classifications.

Seven calves were inoculated with 48 hour agar culture emulsions of P. boviseptica and Escherichia coli. It was found that calves could be killed as readily with intravenous injections of E. coli as with P. boviseptica. Subcutaneous inoculation of calves with these organisms was followed by a slight rise in temperature and recovery within 24 hours.

Agglutination titre and the survival of animals following inoculation with P. boviseptica was apparently of no significance as rabbits with a titre of 1:1600 died just as readily following artifical inoculations as those rabbits showing a titre of 1:80.

Immunological studies of different concentrations of P. antigens preserved by Heat 60°F. for one hour, formaldehyde one per cent, acetaldehyde 1 per cent and merthiolate 1:1000 was made. Agglutination and complement fixation studies were made of all sera. It was noted that agglutination titres of 1:1600 were attained in a number of cases. Acetaldehyde was apparently the better preservative for the production of P. antigens used in this study.

Similar results were obtained by complement fixation. Mertheolate was apparently the more efficient preservative in antigens 1000 x tube 1 of McFarland's nephelometer, in that complement fixation was complete in dilutions of 1:40.

A study of housing conditions on farms where shipping fever occurred, showed that when proper methods of watering, feeding, and housing were observed for the first week or ten days after arrival at the farm losses from shipping fever were very low.

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