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INFECTIOUS ENTERO-TOXEMIA (Milk Colic)
OF LAMBS AND KIDS

I. B. BOUGHTON and W. T. HARDY

Division of Veterinary Science



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Infectious entero-toxemia (milk colic) is an acute fatal disease of nursing lambs and kids. It occurs seasonally, principally in February, March and April, over a large part of the sheep and goat country of West Texas. While the immediate cause is the absorption of the toxin produced in the digestive tract by the spore forming anaerobe, *Clostridium Welchii*, Type D, the contributory predisposing causes are not yet thoroughly known. Healthy, vigorous lambs and kids between the ages of four to ten weeks are most commonly attacked. The mortality averages from 95 per cent to 100 per cent.

At present the disease is best controlled by penning the animals overnight in a dry lot as soon as the first case occurs. They should then be removed to rough, hilly, sparsely grassed pastures if possible. Passive immunization of susceptible lambs by injection of the specific antitoxin is possible but not practical under West Texas conditions. Passive immunization of the nursing lamb through the antibodies contained in the milk of the actively immunized mother ewe offers encouraging possibilities but is still in the experimental stage.

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INFECTIOUS ENTERO-TOXEMIA (Milk Colic) OF LAMBS AND KIDS

I. B. Boughton and W. T. Hardy, Veterinarians,
Substation No. 14, Sonora

An acute, usually fatal disease of suckling lambs and kids between the ages of four to twelve weeks and affecting principally the largest and growthiest animals was found to be an entero-toxemia caused by the pathogenic, toxin-secreting anaerobe, *Clostridium welchii*, type D. The malady is variously known as milk colic or pulpy kidney and occurs principally during the spring months. The symptoms manifested by sick animals are those of an acute toxemia; finding of the carcasses of fat, growthy lambs is usually the first announcement of an outbreak.

In freshly dead animals the kidneys are deeply congested, the liver is congested and extremely friable, the small intestines are very easily torn and splotches of blood are seen scattered along the serosa of their entire length. If autopsied three or four hours after death the kidneys are extremely soft (pulpy) and the liver is spotted with tawny-colored areas.

During the past four or five years cases of the disease have been found in increasing numbers throughout the sheep and goat ranching area of West Texas. It occurs most extensively in the regions where turf grasses predominate but outbreaks in the rougher, bunch grass areas of the region are seen occasionally. The economic importance of the disease is hard to estimate since the losses on individual ranches vary widely from year to year. There is little question, however, that the incidence of the disease is increasing.

REVIEW OF LITERATURE

Entero-toxemia in suckling lambs and isolation of *Cl. welchii*, type D, was reported in the United States for the first time by Boughton and Hardy (5) in 1936. Shaw (14) in 1937 and Shaw, Muth and Seghetti (15) in 1939 reported the disease among Oregon lambs.

The term "braxy-like" was commonly applied to acute diseases of lambs and sheep by the earlier Australian and New Zealand workers. The mortality in such diseases was extremely high. This term was adopted from the "braxy" or "bradrot" of sheep reported from England, Scotland, Germany and the Scandinavian countries in the early part of the present century.

There can be little question that the acute renal congestion (pulpy kidney) in plethoric lambs reported by Gilruth (8) in 1907 from New Zealand was a true entero-toxemia. About the year 1915 Bennetts (2) reported that a similar acutely fatal condition appeared in the Beverly-

York region of Western Australia and came to be known as Beverly disease. Bull (6) reported apparently the same disease among lambs in South Australia in 1924. Belschner (1) referred to outbreaks of a similar disease among lambs in New South Wales in 1926. There are several other references to pulpy kidney disease among lambs of all ages in both Australian and New Zealand Journals and Reports between 1915-1932, but not until the latter year was the nature of the disease discovered.

Bennetts (2) in 1932 described the disease, emphasized its seasonal sporadic occurrence, isolated a toxin-producing anaerobe which he called *Bacillus ovitoxicus* from the small intestines of typical cases in lambs, demonstrated the presence of an extremely potent toxin in sterile filtrates of the intestinal contents of these lambs, reproduced the disease by drenching susceptible lambs with cultures of the microorganism and recommended preventive measures. He suggested the name of infectious enterotoxemia for the disease.

This investigator proved by toxin-antitoxin neutralization typing tests that his organism was different from any of the types of *Clostridium welchii* known at the time of his work. But Wilsdon (17) studied many strains of *Clostridium welchii* from animal sources and classified them into four types—A, B, C, D—according to their toxin content. Working with Bennett's *B. ovitoxicus* he found that in reality it was Type D of the Welchii group. In addition, toxin-antitoxin cross neutralization tests showed:

1. Type A antitoxin neutralizes A toxin only.
2. Type B antitoxin neutralizes A, B, C, D.
3. Type C antitoxin neutralizes A, B, C.
4. Type D antitoxin neutralizes A and D.

Roberts (12) in 1938 working with Type D organisms, claims that the acidity of the stomach contents determines the development of enterotoxemia in lambs. He found that after a large feed of cow's milk modified to resemble ewe's milk the free acidity of the abomasal contents remained extremely low for three hours and that Type D growth is not even partially inhibited until the free acid is increased appreciably. He demonstrated the presence of toxin in the abomasum within four hours after drenching with washed Type D organisms. As a result he thinks that the abomasal hydrochloric acid is bound up with the casein of the milk and is thus unable to inhibit the growth of the organisms and toxin production. He claims to have reproduced enterotoxemia in a lamb by drenching with a large quantity (1500 cc.) of "modified milk" followed immediately by a large dose of toxin-free Type D organisms.

Gill (7) reported infectious enterotoxemia in New Zealand in 1932. Oxer (12) reported the disease among suckling lambs in Tasmania in 1932 and in the same report Bennetts (3) demonstrated that the kidney damage was due to the specific action of the Type D toxin. Montgomerie and Rowlands (11) found the disease in suckling lambs in North Wales in 1934.

Bosworth and Glover (4) showed the type D toxin is heat stable (60° C. for 15 minutes) and that the trypsin of the intestinal juice activates the heat stable factor in the toxin some 30 to 40 times. Mason (10) found that the minimum lethal dose of dried type D toxin for sheep is approximately 1 mgm. per kilogram of live weight.

Glenny and associates (9) described a method of securing dried toxin of *Cl. welchii* by adding 60-70% ammonium sulphate to the liquid toxin, skimming the toxin which rises to the top and drying it over sulphuric acid.

Watts (16) isolated *Cl. welchii* organisms from cultures of intestinal contents of normal sheep made immediately after slaughter. Neutralization tests of toxin produced by these strains revealed only type A toxin. Inferentially, B, C and D organisms were not found in this study.

OCCURRENCE AND ECONOMIC IMPORTANCE

During the first half of the preceding decade occasional reports of an acute, fatal disease among fat nursing lambs and kids were received from sheepmen in the more easterly parts of the West Texas sheep and goat country. Not until March, 1936, however, did the writers have an opportunity to examine and autopsy any typical cases. Results of this work proved the disease to be infectious entero-toxemia.

The disease occurs principally in nursing lambs and kids between the ages of four to twelve weeks. It occurs rarely in two and three weeks old lambs and has been encountered a few times in weaned lambs running in cultivated fields. Bennetts (2) found his first cases in this type of lamb. Predominantly animals in excellent condition are attacked, the condition being relatively rare among animals in poor condition.

The malady occurs almost entirely during the months of February, March, and April, immediately following the "greening up" of the range vegetation in this region, being most prevalent during years of good early spring range. Annual outbreaks are sporadic in nature and often do not recur on a ranch for two or more years. We have encountered several outbreaks in nursing lambs running in cultivated fields during November and December but have not seen the disease in range pastures during this time of year.

In West Texas the infection occurs most commonly in heavily turfed areas of the sheep and goat region, particularly on ranges where the "flats" are numerous and carry a good grass cover. Since this grass is succulent in the spring and heavily grazed during this period it is logical to suppose that a heavy concentration of the causative organisms is built up on these "flats" over a period of years. The carcasses of lambs dead of the disease are usually found on such areas and contribute heavily to the infection of the soil. Since many of the heavily turfed areas receive drainage from the surrounding grazing country they probably carry a heavier spore concentration than do the more thinly grassed areas.

The spores are resistant to climatic and soil conditions and remain viable indefinitely. It should be borne in mind that sheep and goats have been running on these ranges for twenty-five to fifty years thus affording ample opportunity for the soil to become heavily infected.

While outbreaks are larger and of annual though sporadic occurrence in the turf grass part of the region, it is not uncommon to find cases of the disease among lambs running on the rougher, bunch grass ranges farther west. In such localities, however, observation has shown that most of the cases occur among lambs grazing on the "flats" where there is a good grass cover.

As regards the economic importance of the disease there are no exact figures available. On individual ranches where a close count of losses from entero-toxemia has been kept for the past several years annual variations from 1 per cent to 30 per cent of the lamb crop have been found. While the losses on any individual ranch may not be great the aggregate annual loss throughout the sheep and goat raising sections is very large, with considerable variation from year to year. Since the largest losses are found in the regions where the ranches are smaller and the flocks less in numbers the disease here constitutes a very serious problem.

THE CLINICAL DISEASE

Entero-toxemia is commonly known among West Texas sheepmen as milk colic or clabber belly probably because they were impressed by the sight of the curdled milk found in the fourth stomach at autopsy.

Symptoms

Generally the affected animals are visibly sick for only a short time—a few minutes to ten to twelve hours. The mortality will average 95 to 100 per cent. Extremely rapid loss of condition accompanied by a severe diarrhea are characteristic of the few animals which recover spontaneously.

Probably the disease can best be described as either convulsive or comatose depending upon the symptoms manifested during its brief course.

In the convulsive type the animal staggers, wanders aimlessly, drools saliva, breathes rapidly and shallowly and goes down within a relatively short time. Recumbent, the animal characteristically lies quietly for several minutes and then suddenly "throws a fit" during which the head is thrown back, the jaws clamp and rapid running motions are made with all four legs. During this attack the respirations are extremely rapid and the pulse is thready. Within a few moments the convulsion disappears and the animal lies quietly on its side until a succeeding attack. Invariably a last convulsive seizure occurs just before death.

The comatose type of the disease is characterized by the dull, sleepy appearance of the affected animals. They are listless, refuse to nurse,

are inclined to stand in one spot with the head hanging despondently, the lips drooping and a stupid expression on the face. The respirations are shallow and the pulse, at first bounding, gradually becomes thready and faint. Such animals soon go down, remaining prone on the side with little or no movement until death.

In both types the temperature seldom exceeds normal by more than one or two degrees and often drops below normal shortly before death. Physically the urine is unchanged but tests show heavy glycosuria. A mild bowel stasis is characteristic of the disease. The writers have never seen a fatal case in an animal suffering from diarrhea.

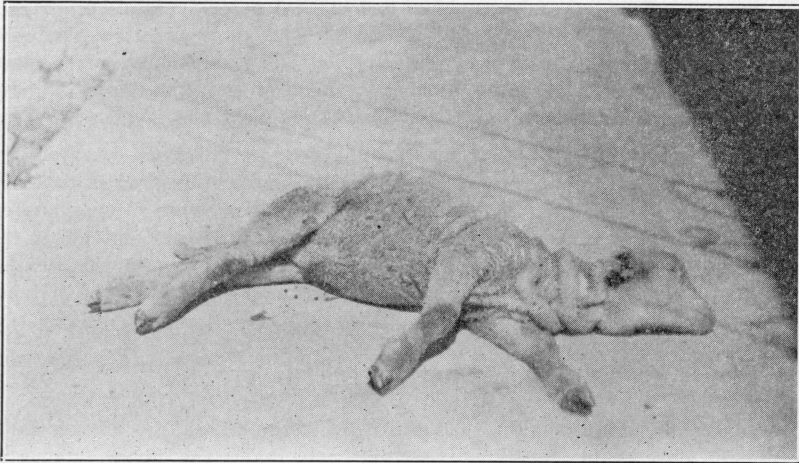


Fig. 1. Comatose Type of Infectious Entero-toxemia.

Autopsy Lesions

The abomasum is invariably distended with gas unless it has ruptured and scattered its contents over the abdominal cavity. This rupture evidently occurs either after death or very shortly before since we have never seen any evidence of peritonitis in such cases. Areas of mucosal congestion are the only gross lesions in the abomasum. Splotches of blood 2 to 4 millimeters in diameter are usually seen scattered along the serosa of the small intestine and occasionally occur on the mucosa. This part of the gut, especially the ileum, is very easily torn. Stripping the intestine from the mesentery, without tearing, is exceptionally difficult. The duodenum and jejunum appear normal and are practically empty. The wall of the ileum is thickened, has a peculiar dead-white appearance and its contents are viscid in consistency and pale yellow in color. Feces in the large intestine are firm and rather dry.

The almost pathognomonic kidney and liver changes are best seen when the animal is autopsied three to four hours after death.

The liver is extremely friable, congested and spotted with tawny-colored areas 2 mm. to 4 cm. in diameter. These tawny areas are not often seen in the freshly dead animal, the liver in such instances being pink to red in color and slightly congested.

The kidneys, in the freshly dead animal, are deeply congested and dark red in color. Autopsy several hours after death shows them to be the same color but "mushy" in consistency. When squeezed lightly in the hand the parenchyma oozes between the fingers.

Generally the spleen is softer than normal but otherwise no gross change is visible.

Numerous epicardial and occasional endocardial petechiae and ecchymoses are seen. The lungs show only a slight passive congestion. All the abdominal lymph glands are swollen and oedematous, sometimes spotted with small hemorrhages. The bladder is usually empty. Gross changes in the brain are confined to engorgement of some of the superficial blood vessels.

Microscopically the changes observed are those resulting from a toxemia. The liver cells are granular, show cloudy swelling and fatty changes. Congestion of the medulla of the kidney and, to a lesser extent of the cortex, is marked. Denuded patches are seen occasionally in the tubular epithelium. The mucous membrane of the ileum may show large patches of desquamation.

EXPERIMENTAL INVESTIGATIONS

In our bacteriologic work with lambs dead of the disease we have isolated organisms only from the digestive tract, principally the ileum. Repeated culturing of blood, liver, kidney, spleen and bone marrow of typical cases have been uniformly negative. Similar results are reported by other workers.

The Causative Organism

Smears from the mucous membrane of the ileum of our first case, a lamb dead about six hours, showed enormous numbers of Gram positive encapsulated rods, morphologically indistinguishable from *Clostridium welchii*, and a very few Gram negative organisms. It was noted that some of these rods carried an outer faintly pink envelope, probably the capsule. Bennetts (2) mentioned noting this same characteristic in his original work. Smears made from the duodenum of this lamb showed an average of less than one such organism per field while jejunal smears average three to the field. Similar smears from subsequent cases showed approximately the same average.

Sporulation was occasionally seen in ileum smears, was constant in cecal and large intestine smears and never seen in duodenal and jejunal smears.

Bennetts (2) found that the type D *welchii* grew better and produced a more potent toxin when grown in horse meat. Since this type of meat was not available at the time of our first work with this organism we used beef meat mash to which glucose in .2 per cent concentration was added. We found this medium entirely satisfactory for primary growth and for the production of the toxin used in typing tests. The dried toxin secured with this beef meat mash consistently showed a mouse minimum lethal dose of approximately .045 milligrams.

The microbe is usually difficult to isolate in pure culture because of the other organisms present in the gut but occasionally can be obtained by direct inoculation of a loopful of the contents of the ileum into beef meat mash. Our best toxin producing strain, No. 422, was isolated in this way. We isolated the organism, *Cl. welchii*, type D, from four typical cases of the disease during the first month of the season in 1936. Since that time we have isolated and typed the organism many times.

Routinely, isolation of pure cultures were obtained by inoculating ileum contents into beef meat mash and litmus milk tubes which were incubated anaerobically in sealed Mason jars (pyrogallic acid method) for 24 hours. Characteristic "stormy" fermentation and acidification of the litmus milk and digestion and reddening of the meat accompanied by strong gas production demonstrated the presence of *Cl. welchii*. Inoculum from the meat mash was seeded into dextrose-agar shakes and poured into dextrose-agar plates and incubated anaerobically.

After twenty-four hours typical, small, dull-white discrete colonies were picked and planted in dextrose agar. Colonies were again fished and inoculated into meat mash, containing .2 per cent dextrose and in litmus milk. At the same time smears were stained by Gram's method to confirm the morphology of the organisms.

Small quantities of the meat mash culture, .5 cc. or less, were injected intramuscularly into guinea pigs to test pathogenicity. Usually a 48-72 hour culture produced a gaseous swelling and spreading oedematous infiltration within 12 hours and death about six to twelve hours later. Autopsy findings in such animals consisted essentially of a gaseous hemorrhagic gelatinous infiltration extending from the point of injection along the belly almost to the brisket and reddening and digestion of the adjacent muscles. The organism was recovered in pure culture from the digested muscles and occasionally from the abdominal fluid but never from the body organs, blood or bone marrow.

In cases where the first cultures showed many contaminants it was necessary to inoculate alkaline egg medium, incubate until sporulation occurred (6-14 days), heat at 80° C. for twenty minutes to destroy vegetative organisms and then inoculate tubes of meat mash. Inoculum from these tubes were seeded in dextrose agar and typical colonies picked and plated.

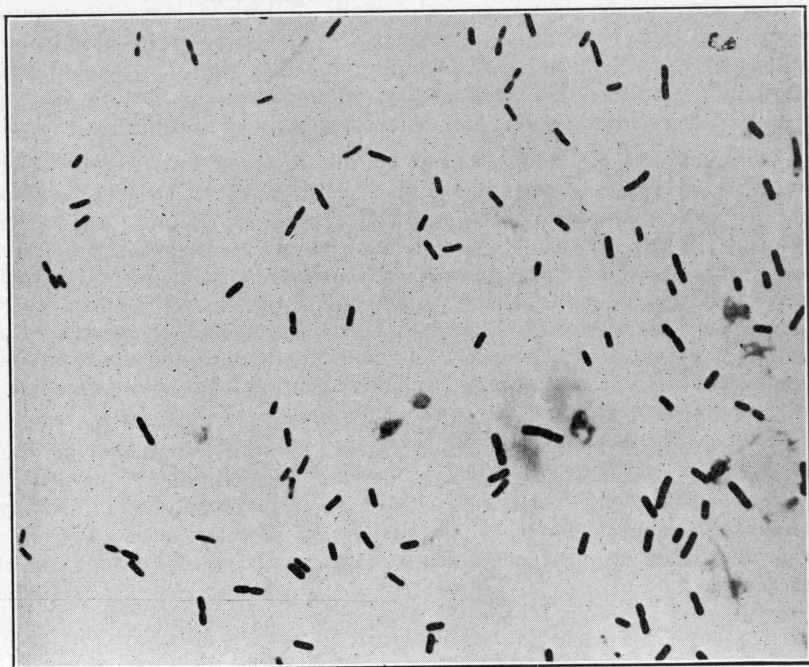
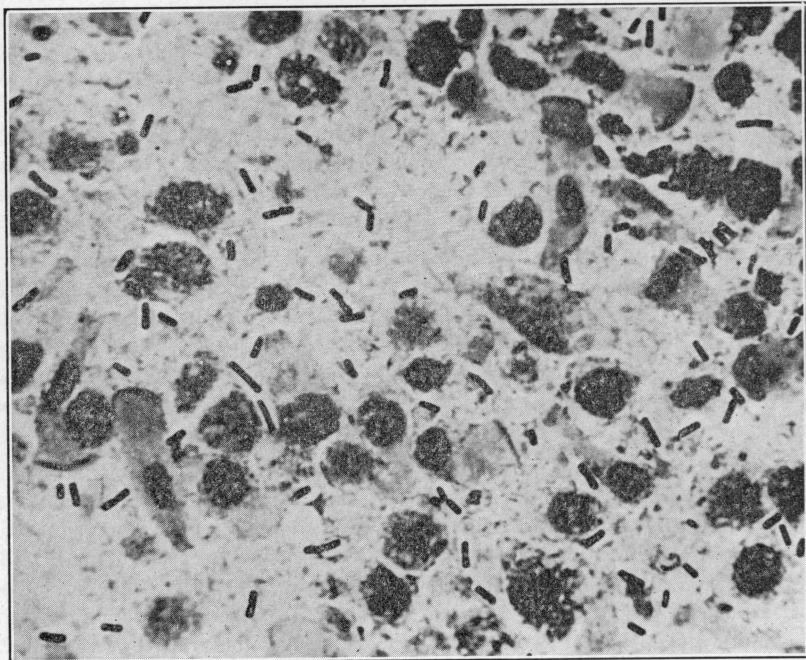


Fig. 2. *Clostridium Welchii*, Type D.
(a) Direct smear from small intestine.
(b) Smear from meat mash culture.

Cl. welchii, type D, is an anaerobic, non-motile rod, with rounded ends, averaging 2.6-4 microns x .9-1.2 microns. Some extremely long forms, up to 30 microns, were seen in young meat mash cultures. It is Gram positive, but red staining organisms are not uncommon in four or five day cultures. A capsule is demonstrable with suitable staining. Sporulation, central or sub-terminal, occurs six to fourteen days after inoculation into alkaline egg medium and also in glucose agar enriched with horse or sheep blood serum.

The organism is not a strict anaerobe, growing well in meat mash from which the air was expelled by boiling and rapid chilling just before inoculation, when incubated aerobically.

Pure cultures (four lamb strains) produced acid and gas in glucose, lactose (acid after 4 days), dextrose, levulose, glycerol, xylose, raffinose, galactose, mannose, sorbitol, acid only in salicin and arabinose and saccharin were not fermented.

Our studies confirmed the findings of practically all investigators who have worked with the *welchii* group that the sugar reactions cannot be depended upon to differentiate the types within this group. Consequently toxin-antitoxin tests in which serum and toxin produced by known pure strains of each type (A, B, C, D)* were made, using mice as the test animals, in order to identify our organism definitely.

These cultures were grown in flasks of meat mash, heated and chilled just before inoculation, and incubated aerobically for 24 hours for Types A, B and C and sixty hours for type D. Our investigations, like those of other workers show that type D is a slow toxin-producer, the peak of toxicity being reached only sixty or more hours after inoculation. The supernatant fluid was decanted, filtered through paper pulp in a Buechner filter and then drawn through a Mandler candle. To this fluid toxin 67% ammonium sulphate was added. After standing about six hours the toxin which rose to the surface was skimmed off, pressed in filter paper, and then dried over H_2SO_4 for twenty-four hours. It was then removed, powdered in sterile mortars, and stored in the refrigerator. The minimum lethal dose of each type toxin (dissolved in sterile distilled water) was determined by intraperitoneal injection of mice, the MLD being the smallest quantity of toxin required to kill at least two of three mice injected.

Antitoxins of types B and D were secured from sheep immunized by increasing weekly subcutaneous injections of meat mash cultures of the respective organisms. The initial doses were 5 cc. of anaculture; weekly doses thereafter were increased until a potent antitoxin was secured (approximately three months). Antitoxic potency was determined by mixing varying quantities of serum with 10 MLD of the dried toxin and injecting intraperitoneally into white mice. The serum was considered

*Cultures of pure strains of *Cl. Welchii* were secured from:

Type A—Dr. J. P. Scott, Kansas State College, Manhattan, Kansas.

Type B—Dr. Ivan Hall, University of Colorado Medical School.

Type C—Dr. Ivan Hall, University of Colorado Medical School.

Type D—Dr. H. W. Bennetts, Dept. of Stock, Perth, Western Australia.

sufficiently potent when .5 cc. or less would protect two mice against 10 MLD of the toxin for at least 24 hours. Type A antitoxin (perfringens) was secured from Parke, Davis & Co. and from Lederle, Inc.

Each toxin was tested against a potent antitoxin from the blood of a sheep immunized against the organism isolated from a typical case of lamb entero-toxemia. In addition cross-immunization tests were made except for Type C antitoxin which was not available.

A typical test to type the organism, No. 422, isolated from a fatal field case of entero-toxemia in a lamb, is illustrated in Table 1. In setting up the test each toxin was dissolved in sterile distilled water in convenient dilution. Measured amounts of toxin and anti-toxin were mixed in the syringes and left for 45 minutes at room temperature prior to intraperitoneal injection into white mice. The total amount injected—toxin and anti-toxin—never exceeded .6 cubic centimeters.

Table 1. Typing Test of Organism No. 422*

Mouse	Material Injected	Result
709	1½ MLD toxin Type A + .3 cc antitoxin from #422*	Lived
710	do .3 cc do	Lived
711	do Control	Died—6 hrs.
630	2½ MLD toxin #422* + .2 cc antitoxin Type A	Died—3½ hrs.
631	do do	do
636	do Control	Died—1 hr.
913	3 MLD toxin Type B + .2 cc antitoxin #422*	Died—6 hrs.
914	do + .2 cc do	do
915	do Control	Died—4 hrs.
634	2½ MLD toxin #422* + 2 cc antitoxin Type B	Lived
635	do + .2 cc do	do
637	do Control	Died—3 hrs.
809	3 MLD toxin Type C + .2 cc antitoxin #422*	Died overnight
810	do + do	do
811	do Control	do
839	2 MLD toxin Type D + .2 cc antitoxin #422*	Lived
840	do + do	do
841	do Control	Died 7¾ hrs.
7	10 MLD toxin #422* + .1 cc antitoxin Type D	Lived
8	do + .1 cc do	do
9	do Control	Died—3 hrs.

*Isolated from ileum of lamb dead of entero-toxemia.

It has been shown by Wilsdon (9) that each antitoxin protects against its specific toxin and that type D protects against both types A (per-

fringens) and D (ovitoxic) toxins but not against types B (lamb dysentery) or type C (paludis) toxins. Contrariwise, type D toxin will kill type A antitoxin-injected mice but not types B, C or D antitoxin-protected mice.

Perusal of the table shows that the antitoxin No. 422 (serum from a sheep immunized against the organism isolated from a fatal, natural case of lamb entero-toxemia) protected mice against types A and D toxins but not against types B and C toxins. Further, No. 422 toxin killed mice injected with type A antitoxin but did not kill mice injected with types B and D antitoxins. Type C antitoxin was not available but since type C toxin killed No. 422 antitoxin-injected mice it is clear that No. 422 antitoxin was not C type because all *welchii* antitoxins protect against their specific toxins.

Results of the test in this table prove conclusively that the organism No. 422 is Type D.

We were able to isolate only Type A organisms from the intestines of 6 lambs dead from various other causes. This result agrees with Watts' (16) findings.

Toxicity of Intestinal Filtrates

Filtrates were prepared by stripping the contents of the ileum into a graduated beaker, diluting with equal parts of normal saline, mixing thoroughly, centrifuging for 15 minutes, filtering the supernatant fluid through paper pulp and then through a Mandler filter and culturing for sterility. No typing tests were made of intestinal filtrates from natural cases during the 1936 outbreak because the small quantity of each filtrate secured was used up in testing its toxicity for experimental animals. The toxicity tests of intestinal filtrates made during the 1936 outbreak are seen in Table 2.

Table 2. Toxicity of Intestinal Filtrates from Lambs Dead of Entero-Toxemia
1936

Filtrate	Quantity	Injection	Animal	Result
No. 393.....	3 cc 8 cc	Intravenously do	Adult rabbit Lamb (12 lbs.)	Died 15 minutes Died 6 hours
No. 422.....	2 cc	do	Adult rabbit	Died 15 minutes
No. 423.....	3 cc	do	do	Died 20 minutes

The organism isolated in each case was *Cl. Welchii*, Type D.

Toxicity tests of intestinal filtrates from two lambs dead of entero-toxemia in 1937 showed a MLD of .0125 cc. for one (No. 158) and .75 cc. for the other (No. 227). Typing tests of these filtrates are shown in Table 3.

Table 3. Typing Tests of Intestinal Filtrates of Lambs 158 and 227
1937

Animal	Quantity Filtrate	Quantity Antitoxin	Result
Mouse.....	.05 cc. 158	+ .1 cc. Type A	Died during night
do	do	do	Died 8 hours
do	do	+ .1 cc. Type B	Lived
do	do	+ do	Lived
do	do	+ .1 cc. Type D	Lived
do	do	+ .1 cc. Type D	Lived
do	1 cc. filtrate 227	+ .1 cc. Type A	Died during night
do	do	+ .1 cc. Type D	Lived

Type D organisms were later isolated from each lamb.

The toxicity of the intestinal filtrates with which we have worked remained constant for weeks when stored in the refrigerator. This is in contrast to liquid toxins from cultures which we found to lose toxicity rather rapidly even when stored under similar conditions.

Dried toxins maintain their toxicity for extended periods of time. The mouse MLD of the dried toxin of No. 422, for example, was found to be .05 mgms.; after one year's storage in the refrigerator the mouse MLD remained the same. The dried toxin of No. 422, redissolved in sterile water, although weakened, still remained pathogenic for mice after heating for 15 minutes at 60° C.

Artificial Reproduction of the Disease

Intravenous injections of susceptible lambs with either liquid or dried toxin of type D organisms and of intestinal filtrates reproduce the natural disease both in symptomatology and pathology.

Bennetts (2) was able to reproduce the disease in susceptible lambs by administering small doses of tincture of belladonna and opium, followed shortly by large doses of meat mash culture in milk. Roberts (15) reproduced the disease by administering a large dose, 1500 cc., of "modified milk" (cow's milk with casein added) followed immediately by a large dose of washed type D organisms. We tried Bennett's method on three lambs and Roberts' on two lambs with negative results. No explanation for this failure is offered.

Antitoxin Immunization

A healthy horse was immunized by subcutaneous injections of 72-hour old meat mash cultures of Type D (No. 422). The initial dose, 12 cc., caused a severe reaction which subsided after three weeks. Increasing doses, up to 500 cc., were then injected at five-day intervals and the

serum tested for antitoxin at various periods. Four months after the first injection the serum showed 75 units* per cubic centimeter and the animal was bled, the serum was separated and filtered, tested for sterility, preserved with "merthiolate" 1-5000 and stored in the refrigerator. After six months' treatment the antitoxin titer reached 100-125 units per cc. which we were not able to increase during several months of repeated injections.

Field immunization experiments were conducted in three flocks of nursing range lambs on ranches where the disease was prevalent. On each ranch the lambs were rounded up, injected subcutaneously with antitoxin in the axillary region and returned immediately to the same pastures. An equal number of control lambs was left in each pasture except on Ranch 3 where lambs in an adjoining, similar pasture were used as controls.

Table 4. Field Test of Type D Antitoxin*

Ranch	Preceding History	No. Lambs	Antitoxin	Management During Test	Result
1	22 lambs died ent.-tox. in preceding 3 days	100	5 cc	Left in Pasture	Lived
		100	Controls		4 died (4%)
2	35 lambs died ent.-tox. in preceding 10 days	177	5 cc	do	Lived
		150	Controls		10 died (6.66%)
3	21 lambs died ent.-tox. in preceding 8 days	441	5 cc	do	Lived (1 died 1 hour after treatment)
		420	Controls		12 died (2.85%)

*Antitoxin #911—contained 75 units per cc.

These field trials demonstrate that the specific antitoxin will protect grazing susceptible lambs against the disease. Shaw, Muth and Seghetti (14) report similar results in field tests among lambs injected with Type D antitoxin furnished by the writers.

The short period of passive immunity (2-3 weeks) following antitoxin injection coupled with the cost of such treatment, precludes the adoption of this type of prevention except perhaps in small, valuable breeding flocks. Certainly such treatment is not practical in a range flock where the outbreaks are sporadic in annual occurrence and the loss from "cut off" lambs may be greater than the loss from the disease.

PREVENTION AND CONTROL

Penning the lambs and their mothers in dry lots overnight will usually arrest an outbreak of entero-toxemia abruptly. Presumably this practice, common in West Texas, enables the lambs to evacuate the organisms

*The unit was established arbitrarily as that quantity of serum which would protect mice against 10 MLD dried toxin for 24 hours.

from the intestines. A single penning, after the disease has appeared in a pasture, is usually sufficient but additional penning may be required some ten days to two weeks later if the disease reappears. "Marking" the lambs in itself does not arrest the outbreak (as some ranchmen believe) but it does remove the lambs from the infected range and thus allows them to eliminate the organism from the intestines. A small percentage of the lambs may be "dogied" during such pennings but the loss is negligible compared to the loss from the disease in dangerous pastures.

Removal of the lambs, when gathered with their mothers to arrest an outbreak, to rough, hilly pastures is a good practice, as in such a case there is less chance that the animals will pick up any large numbers of the organisms.

Specific antitoxin immunization of the susceptible lambs is costly and not practical except in the case of small valuable breeding flocks. If performed it should be done when the lambs are between three and four weeks old.

Injection of the nursing lambs with either anaculture or toxoid at about ten days of age produces some immunity, as judged by the antitoxic content of the blood serum, in approximately three weeks. Since lambs are usually dropped during a thirty-day period in each pasture and since such treatment would mean gathering the ewes and lambs several times during this period, it is obvious that such a procedure would not be practical under West Texas conditions.

Several investigators, including the writers, have shown that the milk of actively immunized ewes contains appreciable amounts of specific antitoxin. And the blood serum of lambs nursing such ewes contain demonstrable amounts of antitoxin. Prevention, by immunization of the pregnant ewe shortly before lambing with anaculture or toxoid made from *Cl. welchii*, type D, has been tried experimentally by the writers and offers encouraging results. Field trials, using a toxoid produced by the addition of 2 per cent zinc chloride to meat mash cultures of the organism for immunization of the ewes, have been conducted for the past two years with fairly satisfactory results. This work is to be reported in a later publication.

DISCUSSION

While there is no question that death in cases of entero-toxemia is directly due to the toxin of *Cl. welchii*, type D, found in the abomasum and small intestine of lambs dead of the disease, the predisposing factors, nutritional and physiological, are not clearly understood.

Entero-toxemia in West Texas is most prevalent in healthy, vigorous suckling lambs and kids in excellent condition which are running in pastures carrying a heavy turf grass cover, especially when the grass is succulent. The disease is also common in fall and winter lambs on cultivated fields. Occasionally it does occur among lambs in fair condi-

tion running in sparsely grassed pastures. Probably the thickly grassed areas are more heavily infected with type D organisms, (vide Bennetts (2)) but the fact that large outbreaks are seen in sparsely-grassed, rough regions also indicates that the condition of the animals rather than the density of the soil infection is probably the determining factor.

Bennetts noted the bowel stasis in typical cases and argued that such stasis was essential in order to let the type D organisms proliferate and secrete toxin. Further, he was able to reproduce the disease by slowing the bowel movements artificially before drenching with toxic cultures of the organism. The enhancement of the toxin by the trypsin of the succus entericus noted by Glover helps to strengthen the bowel stasis hypothesis.

On the other hand Roberts (13) found that the free acid of the abomasum was bound up with the casein of the milk and, from this, argued that proliferation of the organisms and subsequent elaboration of the toxin occurred in this stomach, the toxic contents gradually being emptied into the intestine where its toxicity is enhanced by the trypsin of the succus entericus.

The fact that we were unable to reproduce the disease in susceptible lambs by either Bennetts' or Roberts' method, however, indicates that the exact mechanics of the disease are still obscure. In addition Shaw and associates were unable to produce the disease by injection of a potent D type toxin directly into a loop of the small intestine.

The original theory of Bennetts that the high protein-low fiber diet of the lambs sensitized the intestinal mucosa in some way and rendered it more permeable to the toxin cannot stand in view of the fact that the disease does occur at times among lambs when the milk supply of the ewes is not abundant and the grass is far from succulent. This fact also weakens the theory of Roberts.

The reproduction of the disease symptomatically and pathologically in susceptible animals by the intravenous injection of either the liquid or dried toxins of the D type organism, however, is convincing evidence that essentially the disease is a toxemia resulting from absorption of the toxin present in the intestine.

Observations in dangerous areas seemingly indicate that the lamb flock gradually acquires an immunity through constant contact with the organism on the range. At least, the disease does not appear naturally in nursing lambs more than twelve weeks old in the West Texas region. The question of age susceptibility may be raised but a few tests of blood serum from lambs older than this have shown that at least some of such animals carry an appreciable amount ($\frac{1}{8}$ to $\frac{1}{4}$ unit) of antitoxin in their blood stream. Further, there is the probability that the ewes in such flocks acquire an immunity and, since the milk of such ewes contains a very small amount of antitoxin, there is the additional probability that such small amounts of antitoxin are sufficient to protect the lamb through

the dangerous period. That such immunity is not absolute however is shown by the fact that the disease has been diagnosed in weaned lambs in the feedlots.

SUMMARY

(1) Enterotoxemia (milk colic) in nursing lambs in West Texas is described.

(2) *Clostridium welchii*, Type D, an anaerobic, spore-forming, toxin-producing microorganism is established as the basic cause. This organism has been isolated and identified by cultural characteristics and toxin-antitoxin typing tests from several clinical cases of the disease.

(3) The fact is brought out that the contributing factors and the exact manner in which the disease is caused are not definitely known.

(4) Specific antitoxin has been produced and found to protect susceptible lambs in infected pastures.

(5) The economic importance and prevention of the disease is discussed.

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