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PREVALENCE OF LISTERIA MONOCYTOGENES IN A BOVINE ASSOCIATED ENVIRONMENT.

BY

VERNE D. BRAKKE, JR.

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Microbiology, South Dakota
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1975

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PREVALENCE OF *LISTERIA MONOCYTOGENES*

IN A BOVINE ASSOCIATED ENVIRONMENT.

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Microbiology Dept. Date

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INTRODUCTION

Listeria monocytogenes has emerged into the realm of disease causing bacteria as a pathogen of public health importance. Its opportunistic and ubiquitous nature has led to an ever increasing number of case reports implicating this organism in human and animal disease.

Bojsen-Moller (6) in a historical review points out that in 1926 Murray et. al. made the first description of the organism. Murray's observation of a septicemic and monocytotic condition developing during the course of the disease in rabbits and guinea pigs led him to name this unidentified organism, *Bacterium monocytogenes*. The following year Pirie (1927) isolated a Gram positive rod from the gerbile, a rodent of South Africa. Pirie then proposed the taxonomic name, *Listerella hepatolytica*. After it was discovered that the strains from Africa and England were identical, the name was designated *Listerella monocytogenes*. This choice proved unfortunate, however, as a mycetozoan parasite was found to have the same designation. Finally, in 1940, Pirie renamed the organism *Listeria monocytogenes*, which is presently the accepted name.

Following its discovery Listeria has been observed in many countries from the tropics to the artic (6). In addition to human subjects, Listeria has been found with a very wide host range which includes 37 mammals, 17 fowls, ticks, fish, crustaceans, silage, soil, dust, slaughter house waste, stream water, sewage, and mud (13).

It was not until the mid 1950's that *Listeria monocytogenes*

really passed from the awkward and unnoticed adolescent among the pathogenic bacteria into an exciting, sometimes coy, almost flirtatious bacterium - one that enticed and captivated the investigator by seeming to be everywhere. Yet when the bacteriologist reached out to isolate it, it often eluded his culture (17). Consequently, the true significance of this bacterium in human and veterinary medicine has been questioned by many investigators. With the increased awareness of the disease stimulated by the evermounting number of case reports, Listeria monocytogenes may eventually be found to be of far greater importance than previously suspected.

Human Listeriosis

It was not until 1929 that Listeria monocytogenes was reported as a cause of disease in man (38). Infection caused by this bacterium has been recognized as a significant problem in animals (32), subsequently it was first thought that no human hosts were principal sources of human infection. However, many cases have recently pointed out that no animal contact was present in urban residences that contracted the disease (30).

Since its recognition, listeriosis in humans has been considered rare. Within the past decade it has been reported with increasing frequency, probably due to increased awareness and description rather than a real increase in incidence (5). Now it appears that listeriosis in man is not rare, but rarely recognized (10, 35). The highest precentage of listeriosis cases in humans are caused by

serotypes 4b and 1b which occur predominately in the summer and fall months (31, 34).

Many human subjects may be carriers and when physical and/or physiological stress undermines the host's resistance, active infection may result (13). Asymptomatic human and animal carriers of Listeria monocytogenes probably play a primary role in perpetuating and transmitting listeriosis (17). Medoff (30) substantiates this with the results from his studies. Medoff found that five out of eleven patients had an underlying disease and, this emphasized that listeric infections occur as frequently in previously healthy people.

Transmission to man may take place by ingesting foods of animal origin, such as unpasteurized milk products and infected meat and game (38). Listeria monocytogenes appears to be transmitted from contaminated human feces to soil, to fresh vegetables, and thence by ingestion to man (17). Often direct contact is a mode of spreading listeric infections. Listeric lesions may arise on the arms of farmers and veterinarians after delivering infected livestock (9). Infection by inhalation is more difficult to prove but was the probable method of spread when a Norwegian farmer contracted pneumonia and died of meningitis shortly after sweeping out his sheep stable. Listeria monocytogenes was recovered in cultures of pus from the patient's lung and of stable dust inhaled which contained dried listeria organisms (38).

Following a case of listeria meningitis in a man working in a

factory for egg products, feces of people working in this factory were examined for Listeria monocytogenes. Included in this investigation was a similar group of people working in an office and without direct contact with animals and also a group of slaughter house personnel. The results from these and other studies indicated that Listeria is a ubiquitous organism and that direct contact with animals is not a prime factor in the genesis of a listeric infection (21, 36).

Although many infections occur in infants and in patients with an underlying disease, most occur in previously healthy people of all age groups and are unrelated to a rural setting or animal exposure (35). As a potential menace, an indiscriminate killer of young and old alike, and until the advent of sulfa drugs and antibiotics, individuals known to survive its attacks were usually left with permanent physical or mental defects. Today the most common and successfully used antibiotics are ampicillin, penicillin, and tetracyclines (17).

Listeria in Feral Animals

Listeriosis has been reported in 26 countries and five continents ranging from the artic to the tropics (32). This wide distribution includes such hosts as the gerbil, mouse, vole, lemming, hare, raccoon, skunk, rat, shrew, sable fox, deer, moose, grouse, partridge, ptarmigan, pheasant, wild duck, crane, house sparrow, starling, white-throat, magpie, snowy owl, coyote, and squirrel (14).

That certain hosts are abundant wild species of which some are predatory and others migratory indicates that the disease should be

more widely sought in mortalities of wild fauna (32). It is suggested that many feral hosts may harbor Listeria monocytogenes, but the notion is not supported that listeric infection is widespread among them. Although the disease appears to be self-limiting among feral animals, if infected carcasses or carriers are eaten by carnivorous birds or animals, these may constitute a further spread for the bacterium (14).

McCrum (33) in his studies showed that five of 32 apparently healthy deer, Odocoileus virginianus, were shown to harbor Listeria in their alimentary tracts. Their occurrence in the alimentary canal indicated that the listeria organisms were ingested with food or water.

Following McCrum's study of the isolation of Listeria from the feces of apparently healthy white-tailed deer, Botzler (8) studied the role of the aquatic ecosystem in the long term maintenance of Listeria monocytogenes. It was found that Listeria was more prevalent in the aquatic fauna from a pond site used most frequently by deer and the aquatic animals. Listeria was isolated from seven leopard frogs, three bullfrogs, a painted turtle, a leech commonly found on turtles, and three species of snail. Most listeric isolations were made from leopard frogs collected from pond sites used frequently by deer, shortly after a period of heavy rain (8).

Listeria monocytogenes has been isolated from at least eleven different avian species. These include chicken, goose, duck, turkey, pigeon, canary, parrot, eagle, wood grouse, partridge, and snowy owl.

There are no pathognomonic symptoms or lesions for this infection, since the bacterium is often associated with some other disorder in the bird (16). Gray contends that there is strong circumstantial evidence that birds may play an important role in the transmission of the bacterium to other animals and man (13). However, other investigators contend that contact plays only a small part, if any, in this transmission (13, 21).

Listeria in Domestic Animals

It is difficult to determine the exact incidence of a sporadic, still somewhat unknown disease such as listeriosis. However, it is believed that all domestic animals are susceptible to listeric infections. Sheep and cattle are most often attacked, followed by goats, pigs, and fowl. Horses are only attacked sporadically (38).

MacDonald (29) points out that listeria infections in domestic animals are manifested by three distinct syndromes which are: a) infection of the pregnant uterus resulting in abortion, b) septicemia with visceral miliary abscesses,and, c) encephalitis. In general the clinical symptoms in cattle, sheep and goats show great resemblance, differing only in severity.

In cattle listeriosis is considered a highly chronic disease, since most animals survive only from 4-14 days after the first signs appear. Spontaneous recovery has been frequently observed however. Acute outbreaks in which deaths were sudden and a high percentage of the herd was involved were rare. Usually no more than 8 to 10%

of a herd are affected (17). Jensen contends that listeriosis occurs mainly in confined or semiconfined cattle with the six to 24 month age group most commonly affected (20).

At the onset of the disease, the infected animal separates itself from the rest of the herd. It appears depressed, confused, and indifferent to its surroundings. Incoordination and torticollis follow. Often intermittent twitching and paralysis of the facial and throat muscles and the tongue, which usually protrudes, interfere with swallowing, resulting in marked salivation. One or both the ears may be drooped. In the early stages, the animal tends to crowd in corners or lean against stationary objects as if unable to stand unsupported. If the animal walks it often moves in a circle, always in the same direction. In terminal stages the animals fall and cannot get up without assistance. When it is down, there are generally rapid and deep abdominal breathing, involuntary, aimless running motions. At this stage the animal attempts to eat or at least make chewing motions until the moment of death. Viciousness is not seen except occasionally in cattle (17).

Geographically speaking, listeriosis affects cattle in all major cattle producing regions of the world (20, 4). In a Northern Great Plains region study a highly significant increase in abortions due to Listeria monocytogenes was observed over a four-year period. Kirkbride et al. (24) attributed this increase to epizootiological circumstances.

Sheep listeriosis is characterized by either abortion or

encephalitis (2). This disease in sheep and goats has been extremely acute and death may occur within 4-48 hours after first signs appear. A few animals have survived for several days, however, recovery has been rare among those animals that exhibit definite signs of infection (17).

There are apparently nonclinical carriers among sheep. Eveleth noticed that when these animals are introduced into a new flock the disease becomes established although,it may be several months before symptoms of the disease may occur (12). Several epizootiological cycles are feasible, since the organism is able to survive for long periods in carrier animals or in the environment. Sheep manure and spoiled silage are spread on the land that is subsequently used for corn or sorghum silage or pasture (23). It is believed that feeding of spoiled silage is a very frequent cause of listeric infections in sheep (17). Opportunities also exist for direct contact transmission in closely confined sheep (23).

Listeriosis of swine is a sporadic and rare disease that occurs as an encephalitis or septicemia. Piglets about one week of age are involved in most reports,although some are as old as four weeks of age (28). This age group seems to be the most susceptible age group for acquiring this infection. There have been several reports of listeric infection and hog cholera occurring simultaneously in the same animal. Listeria monocytogenes has also been isolated from pigs with swine erysipelas or swine influenza. These mixed infections appear to be common (17).

Although the disease may be relatively rare in pigs, the somewhat frequent isolation of the bacterium from apparently normal pigs or from those which obviously died from some other cause, strongly suggests that they may play an important part in transmission of the disease or that swine may be an important reservoir of the bacterium (17). The source of the infection is unclear but Long and Duke suggest that rodents and wildlife may play a role in the epizootiology of this disease (28).

Listeric infections do not seem to be a serious problem among house pets, but it has been observed in dogs, cats and a pet squirrel (17). Although the number of reports of listeric infections in domestic pets is relatively low, the importance of investigating these animals as a potential carrier of Listeria merits further study.

Listeria in Avian Species

Listeria monocytogenes has been isolated not only from a large number of mammalian species, but also from at least eleven different avian species. Listeriosis in birds has been reported from all continents except Africa and Antartica. In general the disease is reported most commonly from the temperate zone of both hemispheres (16).

As in mammalian species the young fowl appears to be more susceptible to listeria infection than older birds. There are no pathognomonic symptoms or lesions in birds with listeriosis, since the bacterium is often associated with some other disorder in the bird.

Listeriosis is most commonly manifested by a septicemia; however, there are a few reports in which the predominant symptoms and lesions were related to the central nervous system (16).

As in most other forms of listeric infections the mode of spread among birds is not known. Present evidence suggests that most birds become infected by picking contaminated soil, fecal material or dead mammals (17).

Kwantes, in search for a possible source of transient carrier, found that chickens have a very high carriage rate. From 35 chickens purchased by medical practitioners for consumption in their home, 57% of the chickens were contaminated by Listeria monocytogenes. Both fresh and frozen chickens were examined and both types showed a similar high incidence (26). Since chicken is now a very common food in most homes, the possibility exists for the possible transmission of this organism to a susceptible human.

Listeria in Nature

The widespread geographical distribution of listeric infections occurring in man and more than forty species of animals (14), wild and domestic, suggest to some workers (32) that there is a possible natural reservoir of this organism common to all hosts.

An apparent relationship between silage feeding and listeric infection in ruminants has been mentioned for many years. It was found that silage contained rather large numbers of Listeria monocytogenes, and also that it was of sufficient pathogenicity to incite

active infection both in naturally exposed sheep and in artificially exposed mice. This may offer a possible explanation of the apparent higher incidence of listeric infection among ruminants fed silage than among those fed other rations (18).

It is suggested that Listeria monocytogenes persists in silage of poor quality for an unknown period and produces disease when fed to susceptible animals. The factor responsible for allowing this organism to persist may be the relatively high pH of poor quality silage (3). The isolation of Listeria is favored by a neutral to alkaline reaction. Kruger noted that the organism was isolated from poorly fermented silage low in acidity (40).

Seemingly Listeria monocytogenes is on the vegetation when it is chopped and placed in the silo. When proper fermentation occurs, the pH value rapidly decreases and the Listeria is killed or inhibited from reproduction. In areas within the silo where proper fermentation does not occur, an abnormal type of microbial flora develops that does not reduce the pH value to an inhibitory level for Listeria. It may be assumed that Listeria can easily reproduce in these pockets and become a significant portion of the microbial flora (3).

It has also been suggested that contamination possibly occurs by rodent and avian carriers once the silo is filled and fermentation has begun (3, 11). The relationship of silage and listeriosis was well summarized by the statement, "There is a definite but poorly understood relationship between silage feeding and listeric infections in ruminants" (17).

Since Listeria has been isolated from poor grade silage, the organism would be expected to be present in natural vegetation. In a rural area in which clinical listeriosis of man and animals had been rare, Welshimer (40) isolated Listeria monocytogenes from vegetation. These isolations were made from decaying moist vegetation which had dried and remained in the fields over the winter and which now favored the support of Listeria. Listeria organisms were not isolated from green or recently dead vegetation collected in early September, although the same plant growth yielded Listeria in the spring after standing over winter (42).

The dryness of the surface soil may explain the absence of organisms from that source in the autumn collection. Experimental studies (41) have shown that survival of Listeria in soil is influenced by moisture content. Clay and fertile soils, unprotected from evaporation of moisture, support viable cells for about one month longer than straw and shavings. On the other hand, fertile soil, protected from evaporation, maintained an abundance of organisms which remained at a stationary high level for a 295-day period.

In Welshimer's report (40) it is difficult to assess the role of soil since, in each sampling, part of the plant material was retrieved from the ground. Welshimer has demonstrated the ability of Listeria monocytogenes to survive in soil for long periods of time; however, he found no evidence of multiplication.

Studies on the George Reserve (8) suggest that the long term maintenance of Listeria is related to the terrestrial ecosystem,since

over a five-month period, from late fall to early spring, Listeria survived and multiplied in both the sterilized and unsterilized soil. The survival and multiplication of Listeria in soil over a period of months suggests that soil is the reservoir, and the natural habitat of Listeria on the George Reserve. It is therefore conceivable that plants, in varying stages of decomposition after remaining in the field through the winter, may in their partially decaying state support multiplication of the organism. The portion of the plants on the ground, which are protected from dessication and sunlight, may particularly support multiplication (40).

The ability of Listeria to grow at low temperatures (reaching peak of log phase of growth in ten to eleven days at 6° C) and its tolerance of high temperatures (withstanding pasteurization by the holding technique) supports the contention that the organism is endowed with properties that should favor its survival in soil and other areas outside the animal host (1).

Water has also been studied for its possible maintenance of Listeria. In an attempt to rule out water as one of the common reservoirs of Listeria monocytogenes, Botzler, Cowan, and Wetzler (7) attempted to monitor the survival rate of Listeria in sterile water. The water samples were collected from three ponds on the George Reserve and then inoculated with a Listeria concentration of 10^5 organisms per ml. Listeria monocytogenes survived over 8 weeks in unsterilized pond water with no evidence of multiplication.

In an earlier study (8) by Botzler et. al. concerning Listeria in

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aquatic animals, it was found that most Listeria strains were isolated from animals collected when the water level was at its maximum height. Periods after heavy rains were responsible for this raised water level. These observations seem to suggest that the aquatic ecosystem may be just as vital as the terrestrial ecosystem for the long term maintenance of Listeria.

Although these examples present intriguing evidence that the bacterium may persist in the soil or other contaminated material for long periods of time, the possibility exists that the disease is spread by body discharges of infected animals.

Larsen (27) was the first to present the results of systematic studies to elucidate the occurrence of Listeria in the feces from animals. He examined feces from both sick and healthy animals in a number of different species and isolated Listeria from cattle, pigs, sheep, chinchillas, dogs, sparrows, blackbirds, starlings, gulls, chickens, ducks, geese and turkeys. McCrum and co-workers (33) found in their study that five of 32 apparently healthy deer, Odocoileus virginianus, were shown to carry Listeria in their feces. Another group of investigators have isolated Listeria monocytogenes from feces of healthy animals and healthy human carriers (22).

It is doubtful that ruminants with encephalitis shed large numbers of organisms at one time, since the site of infection is so strictly confined to the central nervous system (2). On the other hand, Listeria monocytogenes is known to be shed with urine and feces from artificially infected animals with a septicemia. These periods

in which Listeria is excreted appears to be of rather short duration and usually not accompanied by gastrointestinal or other clinical symptoms. This known existence of Listeria led to the postulation that the oral route of Listeric infection is important with the animal gut acting as the reservoir of the agent (6). However, in Welshimer's studies the absence of Escherichia coli indicated that there was no marked fecal contamination of the soil at the time of sampling (42).

Rather than attribute the presence of Listeria in nature solely to past contamination with animal feces to the exclusion of an independent role as a free-living organism, one might liken Listeria to the Klebsiella - Enterobacter organism which extensively exists as free living forms on plants and soil, yet inhabit the gut of man and animals and under appropriate circumstances produce disease (42). Seelinger has commented on the resemblances of the biochemical and cultural characteristics of Listeria monocytogenes to some plant-soil inhabitants and has speculated, "that there may well be a primary saprophitic life of Listeria" (38).

In spite of considerable efforts by a number of investigators, the epidemiology of listeriosis remains perplexing. Of the several thousand confirmed cases of animals - only in a few instances has it been possible to trace the source of infection (15).

Listeria in South Dakota

Only one human case has been diagnosed in South Dakota involving Listeria monocytogenes according to the *State Health Department,

Pierre. However, the existence of Listeria as a causitive agent of listeric infections in cattle and sheep is much more pronounced.

During the period of 1970-1974, 155 animal listeriosis cases were diagnosed, of which 106 were bovine cases. Approximately two-thirds of these cases involved bovine abortions between January and May of each year. The remaining listeriosis cases diagnosed in South Dakota during this period include 48 cases of ovine listeriosis and only one porcine case. An average of 25 cases per year involving Listeria monocytogenes are diagnosed from bovine and ovine cases with the exception of 1972-1973 when 80 cases were diagnosed.

The purpose of this investigation is to study the epidemiology of Listeria in South Dakota and to provide information as to its natural habitat, mode of transmission, and factors responsible for its incidence.

*Personal communication with Ben E. Diamond, Director of the Division of Health and Ecological Laboratories.

MATERIALS AND METHODS

Stock Cultures

Listeria monocytogenes, strain 10403, serotype 1, was used in the preliminary studies. Other strains of Listeria were isolated from various Brookings County sites. Cultures of *L. monocytogenes* were maintained on Brain Heart Infusion (BHI, Difco) agar slants stored at 5° C and subcultured every two months. All cultures were grown aerobically at 30° C for 24 hours except strain 10403 which was incubated at 37° C for 24 hours.

Preliminary Studies

Sterile Environmental Samples. Five samples including soil, silage, straw, feces, and water were aseptically collected from a rural farm site. Three grams of each sample were placed in dilution blanks containing 99 ml sterile Bacto-Tryptose Phosphate Broth (TPB, Difco). This suspension was then sterilized by autoclaving at 15 pounds for 15 minutes (121° C). Listeria was added to sterile TPB to obtain a final concentration of 2.6×10^6 organisms per ml. These samples were incubated at 5° C under aerobic conditions. At 0, 3, and 7 days, 10-fold serial dilutions were made of the suspension and were plated in duplicate on Tryptose agar (Difco) by spreading 0.1 ml over the agar surface with a glass spreader. After incubating for 24 hr at 30° C, those plates having between 30 and 300 colonies were counted. The number of organisms per ml of suspended sample was recorded.

Unsterile Environmental Samples. A similar set of five rural farm samples was made by inoculating 3 g of each sample into 99 ml TPB. These samples however were not sterilized. Listeria (2.2×10^6 organism per ml) was inoculated into the five different samples and incubated at 5° C. Appropriate dilutions were made to determine the number of Listeria surviving at 0, 3, and 7 days using duplicate plating on tryptose agar. Using oblique light, plates were examined for small intensely blue colonies, which are indicative of Listeria (25).

Survival. In order to determine whether low numbers of Listeria monocytogenes would be able to multiply in contaminated samples, Listeria in concentrations of one organism per ml and one organism per 10 ml was inoculated into separate dilution blanks containing 99 ml TPB and 99 ml TPB plus 3 g fresh soil. These four samples were incubated at 5° C and the number of Listeria surviving was determined after 0, 3, and 7 days by plating dilutions of the original sample on tryptose agar.

Growth in Enrichment Medium. A stock solution of potassium thiocyanate (Baker) was prepared as a 37.5% (w/v) solution by adding 37.5 g potassium thiocyanate(KSCN) to 100 ml sterile distilled water. A stock solution of TPB was made by adding 900 ml distilled water to 29.5 g dehydrated TPB and sterilized by autoclaving at 15 pounds for 15 minutes (121° C). One ml of the 37.5% KSCN was added to 9 ml of the TPB medium to give a final concentration of 3.75% KSCN in the

TPB (KSCN-TPB).

Three different concentrations of Listeria monocytogenes (2×10^1, 14×10^1, and 15×10^2) were inoculated in duplicate samples of TPB and KSCN-TPB. One half of the samples were incubated at 37° C and the other half at 5° C. Appropriate dilutions were made at 24 hr intervals and plated on tryptose agar in duplicate to determine the number of Listeria surviving.

Duplicate dilution blanks containing 99 ml sterile TPB were inoculated with 15 g of fresh soil. These samples were inoculated with Listeria monocytogenes to give a final concentration of 80 organisms per 100 ml in one sample and six organisms per 100 ml in the other. These samples were incubated at 5° C. One ml of the suspension was taken at appropriate intervals to determine the initial concentration of Listeria and another one ml sample was placed in 10 ml of KSCN-TPB and incubated at 37° C. After 24 hr of incubation, the KSCN-TPB suspension was streaked on duplicate plates of McBrides Listeria agar (Difco). Each plate was streaked by a different spreading pattern to ensure good distribution of colonies. Potential Listeria colonies were detected by viewing plates through an oblique lighting apparatus and looking for intensely blue colonies.

Primary Studies

Site Descriptions.

Five sampling sites (Figure 1) were chosen in Brookings County. These sites are all rural areas surrounding Brookings and each site

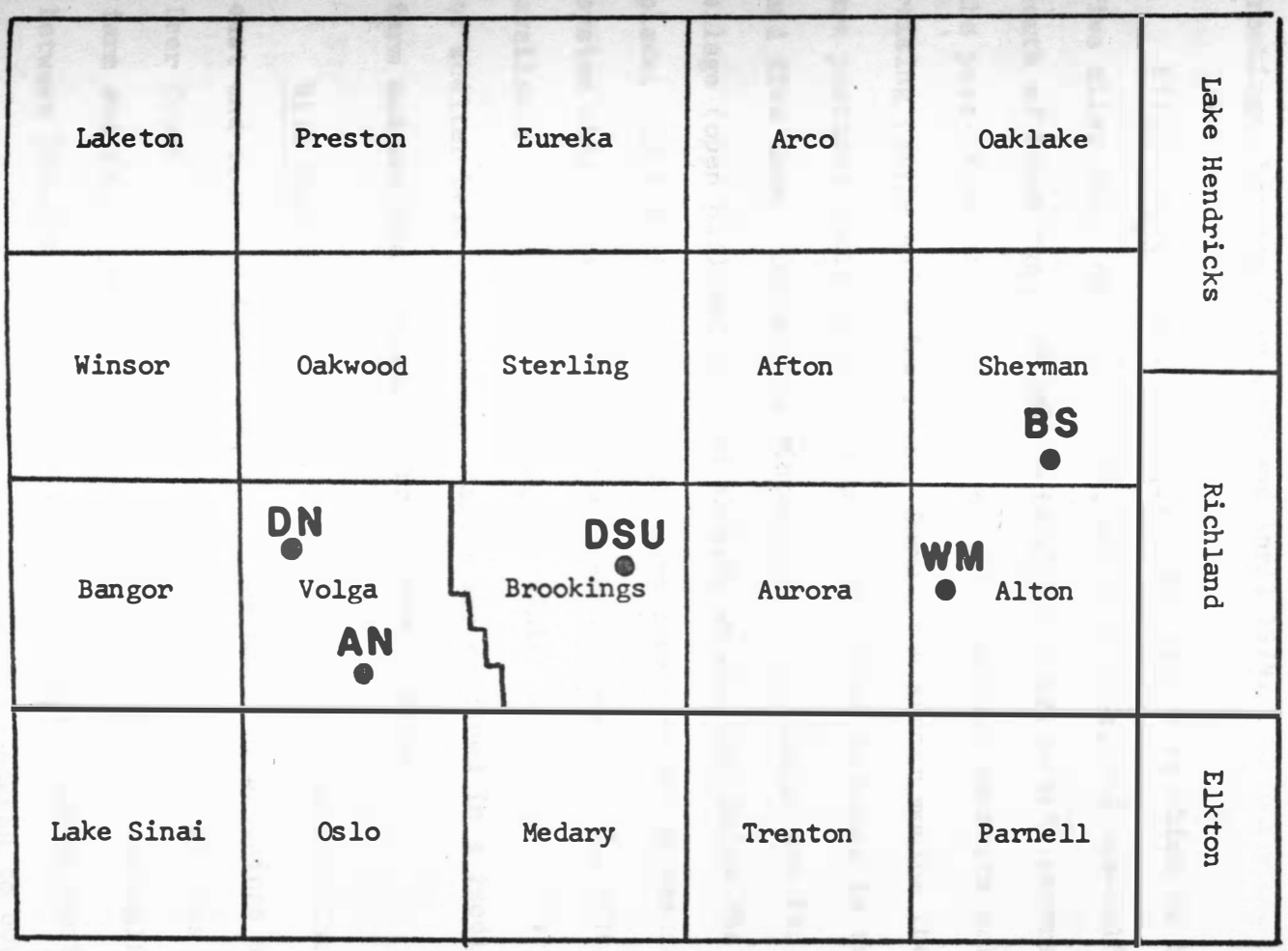

Figure 1. Location of five sampling sites in Brookings County.

has had a case involving Listeria monocytogenes diagnosed at the South Dakota Animal Disease Research and Diagnostic Laboratory, Brookings, between July 1972 and April 1974.

Willis Meyer, Bushnell (WM). This farm site which is located five miles east, one mile north, one mile east, and one-half mile north of Brookings, has been maintained under Meyer's ownership for the past 15 years. Meyer's livestock operation consists primarily of raising cattle and a few pigs. During the summer months the cattle are pastured along Deer Creek and have access to water in the creek and five dams. During the winter months the cattle are fed corn silage (open pit) and chopped alfalfa on cropland below the farm place. In the winter the cattle have access to an automatic watering system which provides water from a shallow well on the site. Shelter available for the cattle during the winter months consists primarily of shelter belt protection. Calves are confined in a feedlot on the farm and are fed corn silage from a nearby silo.

Bill Sheffeld, White (BS). This site is located nine miles east and four and three quarters miles north of Brookings along the Deer Creek bottom. In the fifth year of operation at this site, this farm and ranch operation consists of maintaining a cow-calf operation between 200-300 head of stock cows. During the summer months the cattle are pastured along Deer Creek and have access to both creek and dam water. In the fall the cows are brought in to graze the corn stalks and during the winter corn silage is fed on cropland where corn

silage had been cut from that fall. The corn silage is fed from an open concrete bunker type silo. Chopped alfalfa is also fed during the winter. The feeding area used during the winter was approximately 60 acres of cropland partially surrounded on two sides by trees. Draws, valleys and hills provide additional shelter. The cattle, during the winter months, have creek water available to them. The water in the creek is supplied by an underground spring which flows all year long.

SDSU Dairy Science Unit, Brookings (DSU). This site,located one and one half miles north and one quarter mile west of Brookings, consists of a confined dairy herd used for production testing and experimentation. Confinement occurs all year with shedded areas providing shelter during both the summer and winter months. Rations consist of silo-corn silage, alfalfa, and some grain. Automatic watering systems furnish water for the entire herd.

Two groups of cattle were involved at this location. One group of dry cows were held in a large corralled area on the site. A second group of milking cows was housed inside a large shed. Individual dual stalls with straw or chaff for bedding were provided. Access was also provided to an outdoor concrete lot.

Arne Nelson, Volga (AN). This site consisted of a large dairy operation located seven miles west, two south, and one half mile east of Brookings. Between 100 - 150 milking cows were confined in a large slatted-floor closed shed. These cows were fed silo-corn silage and

silo-alfalfa silage in the housed area by an automatic feeding system. Some grain and haylage is also fed. Individual resting stalls were provided in the housed area along with an automatic watering system which provides water from a shallow well located on the site. Other groups of dairy cattle, both dry dairy cows and heifer calves, were in separate corralled areas and fed corn silage from an open concrete bunker or trench type silo.

Dale Nelson, Volga (DN). This farm site was located eight miles west, one north, and one half mile west of Brookings and is primarily involved with dairy cattle. During the winter months the livestock is maintained in a confined situation with access to a dry lot area. On this site corn silage from three different sources were utilized. These sources included silage from an open silage pit, and silage from both closed and open topped silos. Baled alfalfa and some grain also supplemented the silage. Water was supplied to an automatic watering system from a shallow well on the site. During the summer months the dairy cows were allowed to graze in a nearby pasture.

At all sites manure which had accumulated over the winter was spread over the cropland before spring planting. This is a common practice in Brookings County on most farms where confined feeding produces large quantities of manure.

Environmental Samples

Soil, silage, feces, alfalfa, water and other miscellaneous

samples were taken at the five Brookings County sites (WM, BS, AN, DN, and DSU). Six samples and occasionally seven samples were taken at each site in November, January, March, and May 1974-1975.

Soil. Soil samples varied for each sampling site. Types of samples include the following: soil where silage fed, cropland soil, feedlot soil, and drylot soil.

Approximately 40 g of surface soil was collected at a depth of 2 to 3 cm using sterile plastic disposable gloves (Ace) and placed in 18 oz plastic Sterile Whirl Pak bags (Nasco) for transport to the lab for processing. During the two winter samplings a pick-shovel (Army surplus entrenchment tool) was used to chip pieces of soil loose. The area of the pick used for loosening the soil was sterilized with 70% ethanol before using it on another soil collection site.

Silage. Silage samples were collected on all the five sampling sites. The samples varied from one sample per site to three samples per site depending on the types of silage available at each site. The silage types available included open pit-corn silage, silo-corn silage, silo-alfalfa silage, and concrete bunker or trench-silo corn silage.

Silage from the open silage pits was sampled from the outer darker spoiled silage. Silo-silage was sampled where the silage was fed from the silo or from the silage feeding bunks. Approximately 40 g of silage was collected aseptically using sterile plastic gloves, placed in a sterile Whirl Pak bag, and sealed.

Feces. Samples (40 g) of feces were collected from all sites;

however, the type of sample varied among the different sampling sites. Concentrated feces samples were obtained from gutters, stalls, and feeding floors (DN, AN, DSU). Feces mixed with bedding or feedlot soil were collected from all sampling sites (WM, BS, AN, DN, DSU). The feces samples were collected aseptically and placed in sterile Whirl Pak bags for transport to the laboratory.

Alfalfa. One of two types of alfalfa was commonly found at collection sites for feeding purposes. Baled alfalfa was fed in bunks and chopped alfalfa was fed on the ground or in bunks. Both types of dried alfalfa (30 g) were aseptically collected and placed in sterile Whirl Pak bags for later processing.

Water. Water samples from all site locations were obtained from automatic watering systems with the exception of creek water at one site (BS). Before sampling the water the area to be sampled was thoroughly agitated to suspend material settled on the bottom. Sterile 8 oz sampling jars were used to collect the water.

Miscellaneous Samples. Corn stalks were collected at one site (BS) by uprooting decaying corn stalks and placing them aseptically into a sterile Whirl Pak bag. A grain sample was collected at the Dale Nelson site from grain that was being fed to the cows. Approximately 30 g were aseptically collected and placed in a sterile Whirl Pak bag for processing later.

All samples were maintained at ambient temperature until processing

was available in the laboratory.

Processing Collected Samples.

All collected samples were transported to the laboratory and immediately processed (Figure 2). Each sample was weighed using a triple beam balance. Fifteen grams of each solid sample or 15 ml of water was then placed in sterile 8 oz wide mouth sampling jars (5 1/4 x 2 3/8) containing 100 ml of TPB. The sample was then thoroughly mixed by shaking, number coded, and held at 5° C for two months. All samples were screened for the presence of Listeria on days 1, 10, 20, 30, and 60 past TPB inoculation. The remains of the original samples were frozen and stored in a -20° C freezer for future reference.

Enrichment. One ml of each refrigerated sample at appropriate intervals (1, 10, 20, 30, 60 days) was inoculated into 9 ml of KSCN-TPB. This suspension was thoroughly mixed using a vortex mixer (Deluxe Mixer - S/P). After incubation at 30° C for 48 hr, one loop-ful (0.01 ml) was plated on McBride Listeria agar. Duplicate plates were streaked in different spreading patterns to ensure adequate distribution of colonies.

Isolation and Selection. After incubating the McBride Listeria agar plates at 30° C for 24-48 hr, the plates were examined by oblique lighting (Figure 3) for small intensely blue colonies (Figure 4). Typical colonies suspected of being Listeria monocytogenes were picked

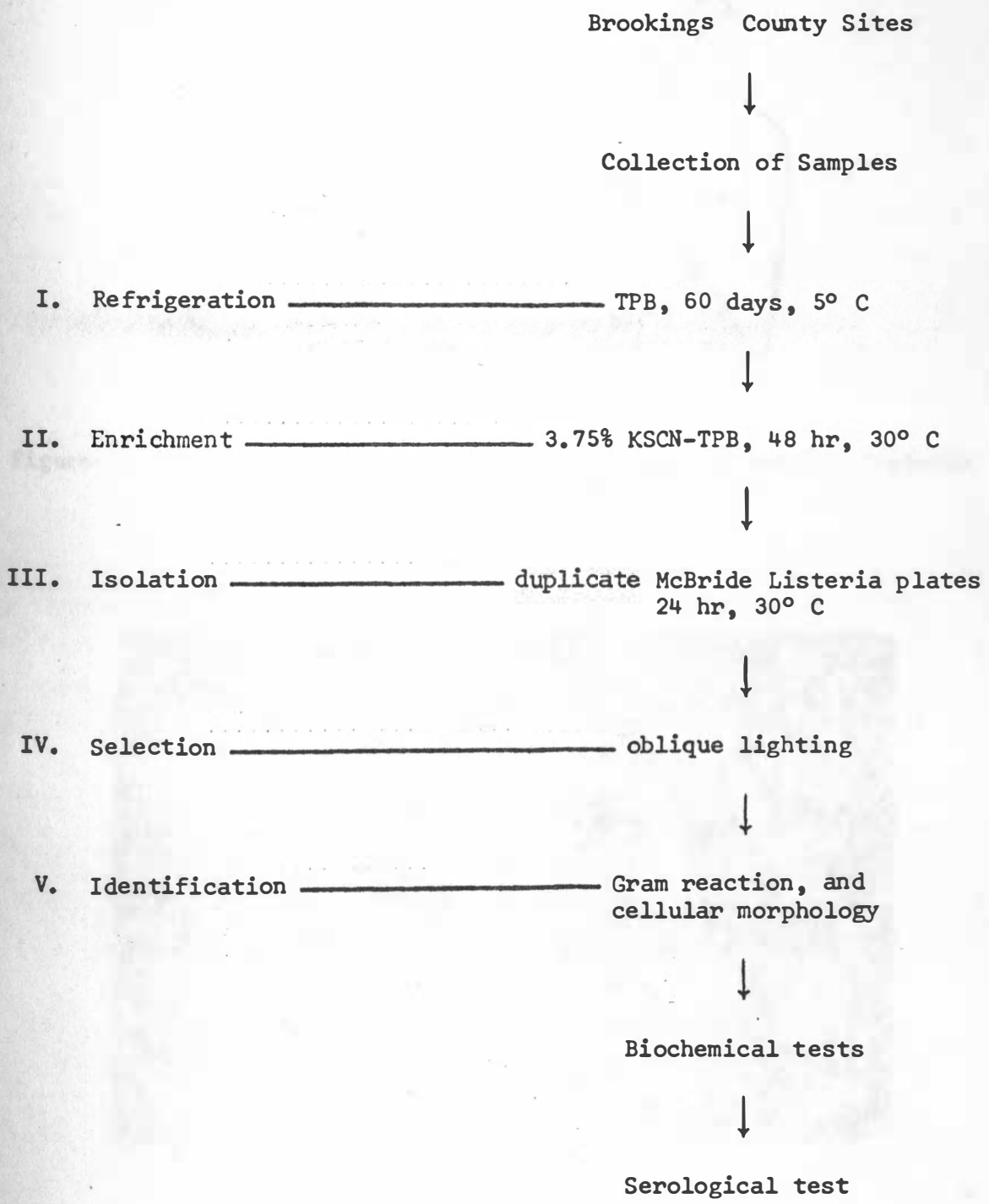

Figure 2. Isolation and identification of Listeria monocytogenes from environmental samples.

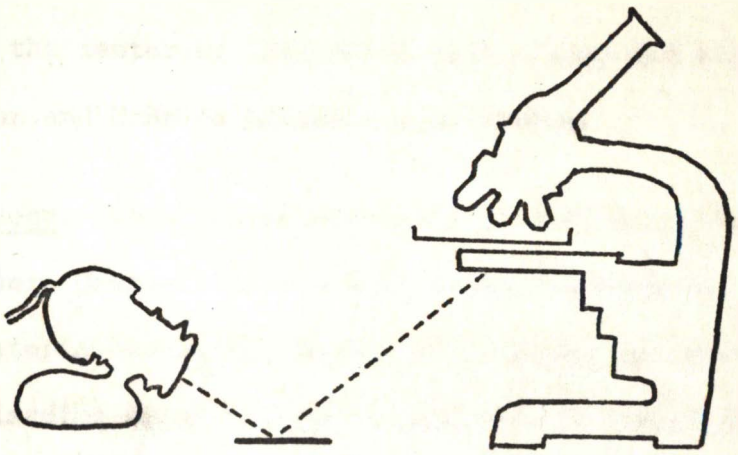

Figure 3. Method of selecting Listeria colonies on McBride Listeria agar using obliquely transmitted light (19).

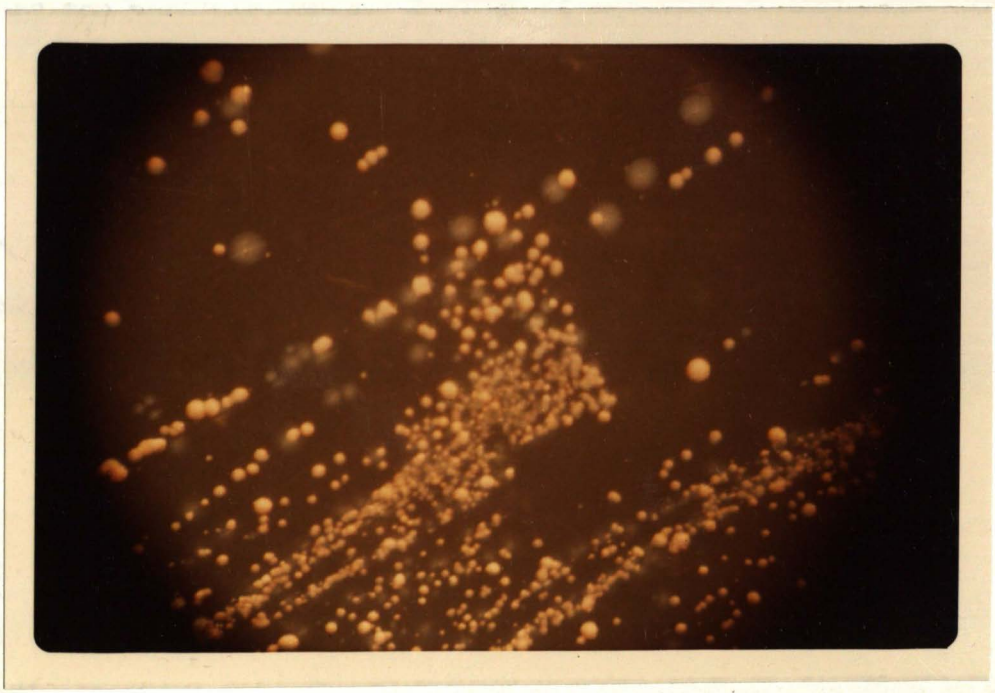

Figure 4. Intensely blue colonies of L. monocytogenes on McBrides Listeria agar, shown by oblique illumination.

by touching the center of the colony with a loop and streaking on Tryptose agar and McBride Listeria agar plates.

Morphology. Gram stains were made of each suspected Listeria isolate. Short Gram-positive almost coccoidal rods were screened as positive Listeria isolates. Smears of positive isolates showed typical palisading or diptheroid formation with some V and Y forms.

Bacto-Motility Medium (Difco) was dispensed in 5 ml quantities in 16 x 125 mm screw-cap tubes and lightly inoculated with a straight wire to a 2-3 cm depth. Duplicate sets were prepared for each isolate and incubated at 37° C and room temperature (22-26° C). Tubes were recorded for motility after two days of incubation.

Biochemical Tests. Catalase was noted by the addition of hydrogen peroxide, 3% (w/v), to the colonies on Tryptose agar plates. This was done after a gram stain was made and after a transfer of the pure culture to a BHI slant was completed.

Fermentation patterns were determined using following carbohydrates: L-arabinose, D-galactose, glycogen, mannitol, lactose, melizitose, melibiose, rhamnose, sucrose, xylose, glucose, salicin, inulin, maltose, trehalose, and esculin. These carbohydrates were prepared as 1% concentrations in Phenol Red Broth Base (Difco). All sugars except xylose were sterilized by autoclaving for not more than 15 minutes at 15 pounds pressure (121° C). Xylose was filter sterilized using a .45 μ pore size filter (Millipore).

The sugars were aseptically inoculated with 0.1 ml of a 24 hr

culture of the Listeria isolate grown in TPB at 30° C. At intervals of 24-48 hr, 3-7 days, 14 days, and 21 days the carbohydrates were recorded for acid production.

Serology. All strains showing typical morphological, cultural, and biochemical characteristics of Listeria monocytogenes were tested serologically by a macroscopic slide agglutination method. Concentrated Listeria O Antiserum (Difco) types 1, 4, and poly (1, 2, 3, 4) were added to sterile physiological saline (0.85% NaCl) to a final dilution of 1:20. A drop of each antiserum was thoroughly mixed with one loopful of antigen. The slides were rocked for 1-3 minutes under a bright light to optimally view the agglutination reaction.

Fluorescent Antibody (FA) staining was used in the preliminary studies. A drop of suspension was placed on a microscope slide and allowed to air dry. After fixing for one minute in 95% ethanol, several drops of Bacto-FA Listeria Type 1 (Difco) were placed over the smear. The slides were then incubated in a humidity chamber for 30 minutes at room temperature. The excess conjugate was drained off and the slides were then placed in FA Buffer (Difco) for 10 minutes with two changes of buffer and a final rinse in distilled water. A small amount of FA Mounting Fluid (Difco) was placed in the center of the stained area and mounted with a cover glass. Stained slides were examined with a Leitz Dialux microscope, equipped with a BG 38 and KP 490 exciter filters and a No. 510 barrier filter.

RESULTS

Preliminary Studies

The routine isolation of *Listeria monocytogenes* from the environment has been observed to be time consuming and unproductive. Various investigators (15, 37, 40, 42) have found it necessary to hold their samples for six months or longer before the isolation of Listeria was possible. Since the present research is attempting to establish the presence of Listeria in the local environment, preliminary studies were necessary to demonstrate the feasibility of such isolation.

Environmental Samples. Listeria (2-3 x 10^6 organisms) was inoculated into feces, water, silage, chaff, and soil with their natural microbial flora present or in a sterilized condition. In the former natural condition Listeria increased approximately three logs (Table 1) in three days at 5° C followed by a plateau over the next four days of incubation. However, seven days of incubation at 5° C was necessary for a similar three log increase when Listeria was inoculated into sterile environmental samples.

This study indicated that high numbers of Listeria (10^6) are able to increase under conditions in which the normal bacterial flora is present. No inhibition due to the presence of these organisms was observed when compared to the sterile control samples; instead, an increased multiplication rate was observed. Seelinger contends that such saprophytes as *Proteus* or enterococci likewise multiply at 5° C and could compete with or inhibit the Listeria present (38).

TABLE 1

Growth of Listeria in sterile and unsterile environmental samples at 5° C.

Sterile Sample in TPB	Organisms per ml		
	0 time	3 days	7 days
chaff	2.7×10^6	5.6×10^7	8.0×10^8
feces	3.1×10^6	1.3×10^8	2.4×10^9
silage	2.6×10^6	1.2×10^8	1.7×10^9
soil	2.4×10^6	1.1×10^8	1.3×10^9
water	2.3×10^6	1.1×10^8	1.8×10^9

Unsterile Sample in TPB	Organisms per ml		
	0 time	3 days	7 days
chaff	2.4×10^6	2.0×10^9	6.0×10^8
feces	2.1×10^6	1.1×10^9	1.3×10^9
silage	2.0×10^6	8.2×10^8	1.6×10^9
soil	2.2×10^6	7.1×10^8	1.5×10^9
water	1.9×10^6	6.7×10^8	1.6×10^9

Survival. Since Listeria would multiply in both sterile and unsterile samples when inoculated with a high concentration of Listeria, the ability of low numbers to multiply at 5° C in both sterile and unsterile samples was next investigated.

Sterile TPB (control) with a Listeria concentration of one organism per ml reached a population of 10^8 organisms after 14 days incubation. Listeria was not observable in the sample containing sterile TPB plus fresh soil (unsterile sample) until day 13 or later because of overgrowth by bacteria other than Listeria.

Another control containing a final Listeria concentration of one organism per 10 ml increased at 5° C to a concentration of 10^6 by the 14th day. The concentration of Listeria in the unsterile sample was impossible to determine because other bacteria present increased to the point where such a low inoculum of Listeria was unable to be detected due to overgrowth by bacteria from the unsterilized sample.

Since a selective media would be necessary to control the growth of unwanted organisms, McBrides Listeria agar was used instead of Tryptose agar when testing unsterile samples. Both media were observed to be equally sensitive in the recovery of Listeria, but McBride agar had the advantage of being selective. Colonies of Listeria monocytogenes growing on McBride Listeria agar appeared somewhat smaller and bluer, using oblique light than colonies growing on Tryptose agar.

Growth in Enrichment Medium. The ability of Listeria to grow in 3.75% KSCN in TPB at 37° C was compared to its growth characteristics in TPB. KSCN did inhibit the growth of Listeria in a 24 hr period by

one log, when compared to its normal growth response in TPB. All concentrations of organisms involved were inhibited similarly (Figure 5). The Listeria concentrations as low as 2×10^1 organisms per ml significantly increased over a 24 hr period to approximately 10^6 organisms per ml. The ability of Listeria to reach high concentrations is important when unsterile samples will be used, since a low number of Listeria should rapidly increase while unwanted organisms are being inhibited by the KSCN.

A similar study was run at 5° C using KSCN. This incubation temperature plus KSCN had a greater inhibition on the Listeria in that only a one log increase was observed in two separate Listeria concentrations by day nine (1.4×10^2, 1.5×10^3), and no increase was observed in the 2×10^1 concentration. Since in most environmental samples the Listeria concentration would be expected to be very small, if present at all, a cold enrichment using 3.75% KSCN-TPB was ruled out.

A larger sample of soil was used in another preliminary study to increase the level of soil organisms. Even with the increased levels of these soil organisms, final Listeria concentrations of 6 organisms per 100 ml and 80 organisms per 100 ml were found to multiply readily (Figure 6) in the 5° C incubated suspension.

By the fourth day at 5° C Listeria was detectable in the soil samples inoculated with 6 organisms per 100 ml using the KSCN-TPB enrichment isolation procedure. This isolation came 1-2 days before the Listeria could be detected by direct plating (Figure 6).

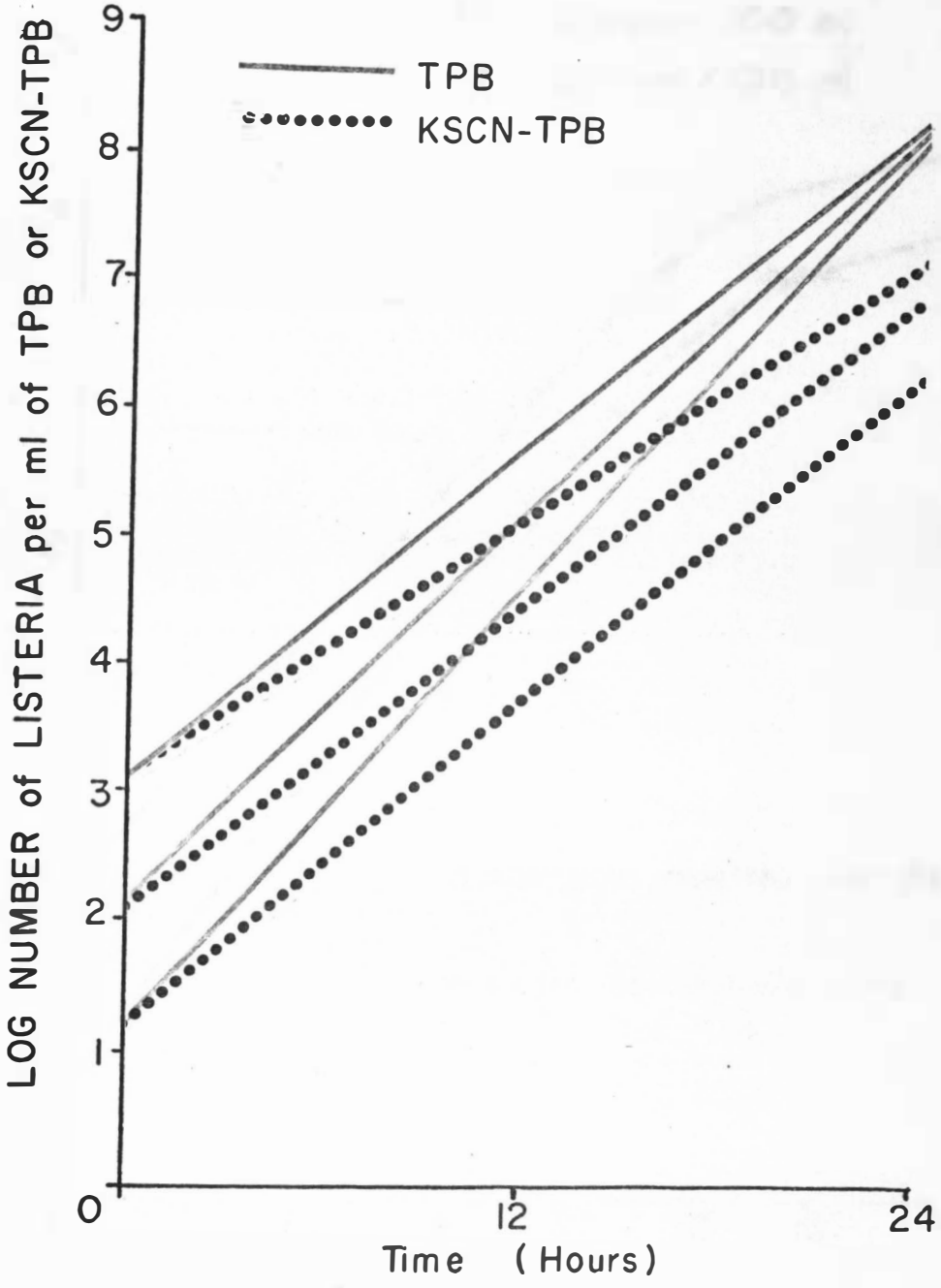

Figure 5. Growth of different concentrations of L. monocytogenes at 37° C in TPB and KSCN-TPB.

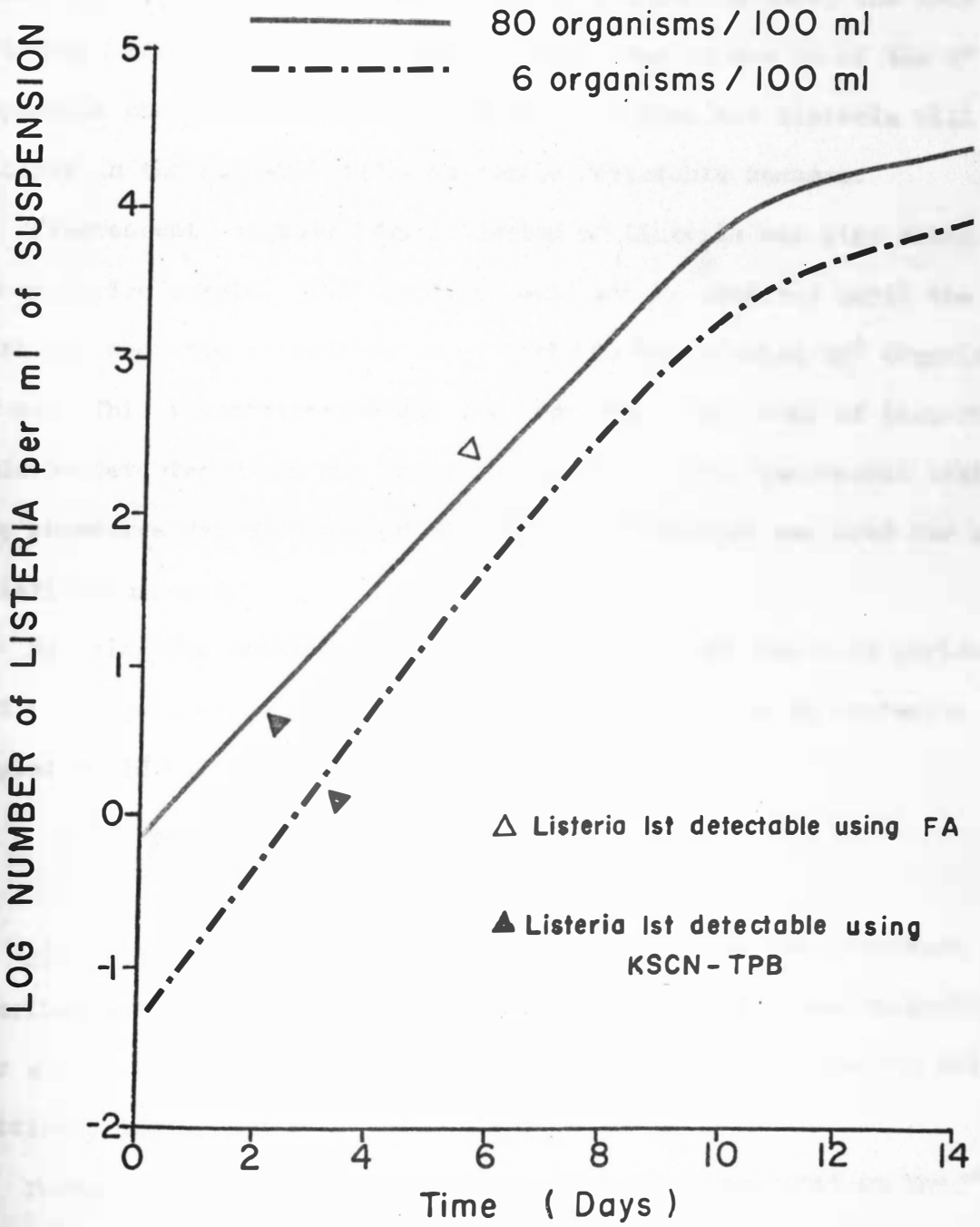

Figure 6. Growth of low levels of Listeria in an unsterile soil suspension at 5° C.

Listeria was detected in the soil sample inoculated with 80 organisms per 100 ml by the second day of incubation using the KSCN enrichment technique. This indicated that even if one ml of the 5° C suspension contains one Listeria organism or more the Listeria will multiply in the KSCN-TPB media to easily detectable numbers.

Fluorescent antibody identification of Listeria was also tried on the unsterile sample. The Listeria could not be detected until the sixth day when the concentration of Listeria had reached 10^2 organisms per ml. This identification was 3-4 days after the level of Listeria could be detected using the enrichment medium. The fluorescent antibody procedure was dropped and the KSCN-TPB technique was used for all isolation purposes.

By using the combination of cold enrichment and the KSCN enrichment technique, extremely small quantities of Listeria in unsterile samples could be isolated quickly.

Primary Studies

Listeria monocytogenes was isolated according to the procedure described in the methods and materials. Of the 123 samples collected over a seven-month period, 76 isolates of Listeria were obtained and positively identified as *L. monocytogenes*.

Forty-three of the 76 positive isolates were recovered on the first day of 5° C incubation using the 3.75% KSCN-TPB enrichment method for isolation. Nineteen more isolates were obtained ten days after refrigeration at 5° C. Other isolations of Listeria include five

isolates after 20 days of incubation, six isolates after 30 days, and three after 60 days incubation at 5° C. Negative samples were held up to two months; however, it is possible that more Listeria isolates could have been obtained by incubating the negative samples for periods longer than two months.

The ability to isolate Listeria monocytogenes from samples at each site is recorded in Tables 2-6. A negative indicates that no Listeria was isolated after 60 days incubation at 5° C. A positive indicated that a Listeria isolate was obtained within the 60 days incubation at 5° C. Isolation of Listeria from a sample on the first day would indicate that high levels of Listeria that could be readily detected, existed in the sample. However, a sample that required 60 days of incubation for the Listeria to attain recognition, existed in the sample at a much lower level. Therefore, the amount of time required to isolate the Listeria is a rough estimate of the number of organisms present in the original sample.

Listeria monocytogenes was isolated at every site during all four sampling periods. Sixty-two percent of the samples collected (76 of 122) were positive for the presence of Listeria. However, the incidence from site to site varied from a high of 79.2% (AN) to a low of 36% (DSU).

At the Willis Meyer site (Table 2) Listeria was isolated from 18 out of 25 samples collected (72% incidence). No significant increase or decrease in the incidence of isolation was observed among the four sampling periods.

The presence of Listeria in the silage was most striking, since Listeria was isolated on the first day for all sampling periods. Silo silage was sampled in the spring sampling, after the feeding of pit silage to the stock cows was discontinued. This silage sample possessed high levels of Listeria which allowed for its isolation after the first day of 5° C incubation.

Cropland soil where silage was fed appeared to be positive for Listeria as a direct result of the feeding of the silage on the ground. Only one isolation of Listeria was made at this site in soil located at a distance from where silage was fed. Twenty days were required to isolate the Listeria, indicating that a much lower level of Listeria had been persisting where the soil sample was taken.

TABLE 2

The isolation of Listeria monocytogenes from samples collected at the Willis Meyer site.

Sample Type	Fall	Early Winter	Late Winter	Spring
Cropland Soil	-	-	-	+(1)
Soil-silage Fed	+(1)*	+(1)	+(1)	+(1)
Feedlot Soil	+(1)	+(1)	+(10)	+(1)
Pit Silage	+(1)	+(1)	+(1)	+(1)
Chopped Alfalfa	-	+(20)	-	-
Water	-	+(10)	+(10)	+(1)
Silo Silage	ND**	ND	ND	+(1)

* Number of incubation days required for isolation
** Sample not collected

Feedlot soil possessed high levels of Listeria which was indicated by the early isolation of Listeria. This feedlot soil possessed high levels of organic material due to fecal contamination. Also this soil appeared to possess a higher level of moisture than did the cropland soil.

Chopped alfalfa that was fed to the stock cows was very dry and loosely packed. The only positive isolate from this type of sample was obtained in the early winter sample when moisture (snow) on the outer surface of the chopped alfalfa stack was present.

The water from automatic watering systems was negative for Listeria only in the fall, just prior to the feeding of silage. Once silage feeding had begun, the water was contaminated with high levels of Listeria. This relationship was more evident in the spring when the stock cows were moved to another pasture and another group of livestock were fed silo-silage in bunks just 15 to 20 feet from the water. Contamination of the water appeared to be due to the silage, since pieces of silage were floating in the water. This type of direct silage contamination was not observed at the earlier samplings. The stock cows were fed silage in cropland below the farm site and had to walk 1/4 mile to get to the automatic watering system; therefore, the contamination in the water was not as heavy. These findings were also substanciated by taking ten days to isolate Listeria in the two winter samples.

Samples collected at the Bill Sheffeld (Table 3) site were positive for Listeria in 12 of the 24 samples (50%). Silage again

appeared to be directly responsible for the isolation of Listeria from soil where silage had been fed on the ground. The early detection of Listeria from these samples indicated the high levels of Listeria in these samples.

Soil away from silage contamination was negative at all sampling periods except in the early winter. In some of the negative samples the presence of fungi in the sample had a detrimental effect on the recovery of Listeria.

TABLE 3

The isolation of Listeria monocytogenes from samples collected at the Bill Sheffeld site.

Sample Type	Fall	Early Winter	Late Winter	Spring
Cropland Soil	-	+(10)*	-	-
Cropland Soil	-	+(10)	-	-
Cropland Soil Silage Fed	ND**	+(10)	+(1)	-
Corn Stalks	+(10)	ND	ND	+(20)
Pit Silage	-	+(1)	+(1)	-
Chopped Alfalfa	-	+(30)	ND	ND
Creek Water	+(10)	+(10)	-	+(20)

* Number of incubation days required for isolation
** Samples not collected

Corn stalks were sampled in the fall and spring with the isolation of Listeria from both samples. Incubation from 10 and 20 days were

isolation indicates a generally high level of Listeria in all soil samples collected at this site.

TABLE 4

The isolation of Listeria monocytogenes from samples collected at the Arne Nelson site.

Sample Type	Fall	Early Winter	Late Winter	Spring
Drylot Soil	+(1)*	+(30)	+(1)	+(1),+(1)
Feces	+(10)	+(30),-	+(1),+(30)	+(1),+(10)
Pit Silage	+(1)	ND**	ND	ND
Silo Silage	-	-	+(1)	+(1)
Silo Alfalfa	-	-	+(1)	ND
Water	+(1)	+(10)	+(1)	+(1)

* Number of incubation days required for isolation
** Sample not collected

A similar high level of Listeria existed in feces samples. The time required for isolating Listeria ranged from 1 to 30 days. This range indicated various levels of Listeria existing in the different feces samples. These samples varied from concentrated feces to feces plus straw.

Pit silage was sampled only during the fall, since by the time of the early winter sampling all the pit silage had been fed. This silage was found to contain high levels of Listeria, requiring only one day of incubation for its isolation.

The corn and alfalfa silage samples were generally negative; however, in the late winter sampling both types of samples were positive on the first day of incubation. Both silos at the late winter sampling were nearly empty. In the spring an alfalfa sample was not available, but a silage sample was obtained from a third silo that had not previously been sampled. This silage sample also contained a high level of Listeria.

Water samples obtained from an automatic watering system yielded an almost pure culture of Listeria after the first day of incubation. All sampling periods yielded Listeria isolates by the end of the first day, with the exception of the early winter sample which required ten days of incubation. The silage may have been responsible for this contamination, since the water was located next to the silage feed bunks.

The Dale Nelson site (Table 5) yielded a similar high incidence of isolation (72%) of Listeria monocytogenes. Listeria was isolated from the eight types of samples collected. The early and late winter sample produced the largest number of Listeria isolates.

Drylot soil and feces were sampled from the same corralled areas. The soil sample was taken from areas where fecal contamination was minimal, and the feces sample was taken where fecal matter was maximally present. Drylot soil and feces samples that yielded positive isolates possessed high levels of Listeria, since isolations were obtained from all samples after the first day of incubation at 5° C.

Concentrated feces samples obtained from the milking parlor gutter

also contained high levels of Listeria. Listeria was not isolated in the fall from a fresh feces sample taken from a cow that had aborted a calf a year earlier. However, Listeria was isolated from grain that the cow had contaminated with salival secretions.

TABLE 5

The isolation of Listeria monocytogenes from samples collected at the Dale Nelson site.

Sample Type	Fall	Early Winter	Late Winter	Spring
Drylot Soil	+(1)*	+(1)	-	-
Drylot Feces	ND**	+(1)	+(1)	+(1)
Feces	-	+(1)	+(1)	+(10)
Pit Silage	-	ND	+(10)	ND
Silo Silage	ND	+(1)	+(1)	+(1)
Alfalfa	-	+(60)	+(60)	-
Water	-	+(10)	+(10)	+(1)
Grain	+(20)	ND	ND	ND

* Number of incubation days required for isolation
** Sample not collected

Three different silage sources were sampled through the four sampling periods. Pit silage was negative in the fall and found positive at the second winter sampling. No pit silage was available for sampling in the spring. Silo silage yielded first day isolations of Listeria from all silo silage samples collected. These isolations

were made from good quality silage.

Baled alfalfa yielded two Listeria isolates from the two winter samples. Sixty days were required for the Listeria to be isolated, indicating that a low level of the Listeria organisms existed. The fall and spring alfalfa samples were negative; however, further incubation beyond the two month incubation used may have yielded positive isolates.

Water from an automatic watering system was negative for the fall sampling but positive in subsequent water samples. The short incubation period is indicative of a substantial number of Listeria in the water.

The Dairy Science site (Table 6) was found to have the lowest level of Listeria isolates (36%). However, at least one Listeria isolation was made in each of the four sampling periods. The late winter produced the largest number of Listeria isolates (five). This isolation rate was markedly higher than the other sampling periods in which only one or two Listeria could be isolated. Listeria was found in all samples of drylot soil. An early winter soil sample was unable to be collected due to a thick layer of ice and snow covering the sample area.

Bedding which included chaff or straw plus fecal contamination also produced three Listeria isolates. The highest level of organism appeared to exist in the first winter sample, since the Listeria isolates were recovered after the first day of incubation at 5° C. The fall and the late winter bedding sample required 10 and 30 days,

respectively,to isolate the Listeria, indicating a possible rise and fall in the number of Listeria present over a five-month period.

TABLE 6

The isolation of Listeria monocytogenes from samples collected at the SDSU Dairy Science Unit.

Sample Type	Fall	Early Winter	Late Winter	Spring
Drylot Soil	+(1)*	ND**	+(20)	+(1)
Feces	-	-	+(10)	-
Bedding	+(10)	+(1)	+(30)	-
Silo Silage	-	-,-	+(60)	-
Alfalfa	-	-	-	-
Water	-	-	+(30)	-

* Number of incubation days required for isolation
** Sample not collected

Silo-corn silage samples were negative with the exception of the late winter sample. Sixty days were required to isolate Listeria from that silage sample,indicating a low level of Listeria in the sample. Incubation of the negative silage samples for longer than two months may have,however, allowed for the isolation of Listeria.

All baled alfalfa samples that were collected at this site were negative for Listeria after two months of 5° C incubation.

The only water sample that was positive for Listeria was the late winter sample. The late winter sampling was also the period with the

highest rate of isolation of all four sampling periods. The isolation of Listeria from feces, silage and water, which all had previously produced negative isolations of Listeria, may have been related to an increase in the numbers of Listeria prior to that sampling period.

All isolates were identified as Gram-positive non-sporeforming short rods, catalase positive, and motile at 22-26° C. However, variation in motility among the 76 Listeria isolates was evident when incubated at 37° C (Tables 7-11).

Biochemical differentiation of the 76 Listeria isolates was carried out using 16 carbohydrates. An attempt was made to determine if all the isolates identified as Listeria were in fact Listeria monocytogenes and not other species of the genus Listeria (L. dentrificans, L. grayii, L. murrayi). Therefore a series of carbohydrates (39) were employed which would separate the various species of the genus Listeria.

All carbohydrates were read for the production of acid at 1-2 days, 3-7 days, 14 days, and 21 days post inoculum. Glucose, maltose, salicin, esculin produced acid in 1-2 days in all 76 Listeria isolates without any exceptions. Lactose was positive after 3-7 days incubation for all Listeria isolates.

Carbohydrates that were negative in 21 days for all 76 Listeria isolates include the following: L arabinose, glycogen, mannitol, melibiose and inulin. Carbohydrates that varied as being either positive for some isolates or negative for others are listed, according to sample location, in Tables 7-11.

All the isolates were determined to be Listeria monocytogenes. This biochemical identification was substantiated through serological slide agglutination reactions. Of the total 76 Listeria isolates, 65 were type 4, two were type 1, and nine were type 2,3. The serotype of each isolate is recorded in Tables 7-11.

TABLE 7

Differential biochemical and physiological characteristics of Listeria *isolates from the Willis Meyer (WM) site.

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
WM 117741	cropland soil	+**	[+]	+	-	(+)	+	+	-	4
WM 117743	feedlot soil	-	(+)	-	(+)	-	+	+	+	4
WM 117744	pit silage	+	-	-	[+]	(+)	+	+	-	4
WM 129752	soil-silage fed	-	(+)	-	[+]	-	+	+	+	4
WM 129753	feedlot soil	-	-	-	[+]	-	+	+	+	4
WM 129754	pit silage	-	(+)	+	[+]	-	+	+	+	4
WM 129755	chopped alfalfa	+	(+)	+	[+]	+	+	+	-	1
WM 129756	water	-	-	+	[+]	+	+	+	-	4
WM 36752	soil-silage fed	-	(+)	+	[+]	-	-	+	+	4
WM 36753	feedlot soil	(+)	[+]	+	-	+	+	+	-	4
WM 36754	pit silage	(+)	(+)	+	[+]	+	+	+	-	4
WM 36756	water	+	(+)	+	[+]	+	+	+	-	4

Table 7 (continued)

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
WM 55752	soil-silage fed	-	-	-	[+]	-	+	+	+	4
WM 55751	cropland soil	-	+	+	(+)	-	+	+	+	4
WM 55754	pit silage	-	(+)	+	(+)	-	-	+	+	4
WM 55755	silo silage	-	[+]	+	[+]	-	+	+	+	4
WM 55756	water	(+)	(+)	+	[+]	+	+	+	-	4
WM 55757	feedlot soil	-	[+]	+	[+]	-	+	+	+	4

* All isolates produced acid but no gas in 24-48 hours from: glucose, salicin, maltose, esculin; 3-7 days from lactose. No acid in 21 days from: L Arabinose, glycogen, mannitol, melibiose, inulin.

** Key: + = acid produced 24-48 hours; (+) = acid produced 3-7 days; [+] = acid produced 14 days; - = no acid produced 21 days.

TABLE 8

Differential biochemical and physiological characteristics of Listeria *isolates from the Bill Sheffeld (BS) site.

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
BS 117743	corn stalks	-**	-	+	[+]	-	+	+	+	4
BS 117746	creek water	+	-	+	-	+	+	+	-	4
BS 129751	cropland soil	-	-	+	[+]	-	+	+	+	4
BS 129752	cropland soil	-	-	+	[+]	-	+	+	+	4
BS 129753	soil-silage fed	+	(+)	+	[+]	+	+	+	-	1
BS 129754	pit silage	-	(+)	+	(+)	-	+	+	+	4
BS 129755	chopped alfalfa	+	(+)	+	[+]	+	+	+	-	4
BS 129756	creek water	-	-	+	[+]	-	+	+	+	4
BS 36753	soil-silage fed	(+)	-	+	-	+	+	+	-	4
BS 36754	pit silage	+	(+)	+	[+]	-	+	+	+	4
BS 55755	corn stalks	-	-	+	-	(+)	+	+	+	4

Table 8 (continued)

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
BS 55756	creek water	+	-	+	-	+	+	+	+	4

* All isolates produced acid but no gas in 24-48 hours from: glucose, salicin, maltose, esculin; 3-7 days from lactose. No acid in 21 days from: L Arabinose, glycogen, mannitol, melibiose, inulin.

** Key: + = acid produced 24-48 hours; (+) = acid produced 3-7 days; [+] = acid produced 14 days; - = no acid produced 21 days.

TABLE 9

Differential biochemical and physiological characteristics of Listeria *isolates from the Arne Nelson (AN) site.

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
AN 1125741	drylot soil	-**	[+]	+	[+]	-	+	+	+	2,3
AN 1125742	feces-stall	-	-	+	[+]	-	-	+	+	4
AN 1125743	pit silage	-	[+]	-	[+]	-	+	+	+	2,3
AN 1125746	water	-	-	+	[+]	-	-	+	+	4
AN 129751	drylot soil	-	(+)	-	[+]	-	+	+	+	2,3
AN 129752	feces-stall	-	+	+	(+)	-	+	+	+	2,3
AN 129756	water	-	(+)	-	[+]	-	+	+	+	2,3
AN 36751	drylot soil	-	[+]	+	[+]	-	+	+	+	4
AN 36752	feces-stall	-	(+)	-	[+]	-	+	+	+	2,3
AN 36753	feces-floor	-	+	+	(+)	-	+	+	+	4
AN 36754	silo silage	-	+	-	(+)	-	[+]	+	+	4

Table 9 (continued)

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
AN 36755	silo alfalfa	-	[+]	+	[+]	-	[+]	+	+	4
AN 36756	water	-	(+)	-	(+)	-	+	+	+	4
AN 55751	drylot soil	-	[+]	+	[+]	-	+	+	+	2,3
AN 55752	drylot soil	-	+	-	(+)	-	+	+	+	4
AN 55753	silo silage	-	[+]	+	[+]	-	[+]	+	+	4
AN 55754	feces-stall	-	+	-	[+]	-	+	+	+	2,3
AN 55755	feces-floor	-	+	-	(+)	-	+	+	+	4
AN 55756	water	-	+	-	[+]	-	+	+	+	4

* All isolates produced acid but no gas in 24-48 hours from: glucose, salicin, maltose, esculin; 3-7 days from lactose. No acid in 21 days from: L Arabinose, glycogen, mannitol, melibiose, inulin.

** Key: + = acid produced 24-48 hours; (+) = acid produced 3-7 days; [+] = acid produced 14 days; - = no acid produced 21 days.

TABLE 10

Differential biochemical and physiological characteristics of Listeria *isolates from the Dale Nelson (DN) site.

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
DN 1125741	drylot soil	-**	-	+	[+]	-	+	+	+	4
DN 1125743	grain	-	[+]	+	[+]	-	+	+	+	4
DN 129751	drylot soil	-	[+]	[+]	[+]	-	+	+	+	4
DN 129752	drylot feces	-	-	+	[+]	-	+	+	+	4
DN 129753	feces-gutter	-	-	+	[+]	-	[+]	+	+	4
DN 129754	silo silage	-	+	+	(+)	-	+	+	+	4
DN 129755	baled alfalfa	-	(+)	+	[+]	-	-	+	+	4
DN 129756	water	-	[+]	+	[+]	-	[+]	+	+	4
DN 36752	drylot feces	-	(+)	+	(+)	-	-	+	+	4
DN 36753	feces-cow	-	+	+	(+)	-	+	+	+	4
DN 36754	silo silage	-	(+)	-	[+]	-	+	+	+	4

Table 10 (continued)

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
DN 36755	baled alfalfa	-	+	+	(+)	-	+	+	+	4
DN 36756	water	-	[+]	+	[+]	-	[+]	+	+	4
DN 36757	pit silage	-	[+]	(+)	[+]	-	+	+	+	4
DN 55752	drylot feces	-	-	+	[+]	-	+	+	+	4
DN 55753	feces-gutter	-	(+)	(+)	[+]	-	[+]	+	+	4
DN 55754	silo silage	-	-	+	[+]	-	+	+	+	4
DN 55756	water	-	-	+	[+]	-	+	+	+	4

* All isolates produced acid but no gas in 24-48 hours from: glucose, salicin, maltose, esculin; 3-7 days from lactose. No acid in 21 days from: L Arabinose, glycogen, mannitol, melibiose, inulin.

** Key: + = acid produced 24-48 hours; (+) = acid produced 3-7 days; [+] = acid produced 14 days; - = no acid produced 21 days.

TABLE 11

Differential biochemical and physiological characteristics of Listeria *isolates from the Dairy Science Unit (DSU) site.

Sample No.	Sample Description	D Galactose	Melezitose	Rhamnose	Sucrose	Xylose	Trehalose	Motility 22° C	Motility 37° C	Serotype
DSU 1115741	drylot soil	-**	(+)	-	[+]	-	+	+	+	2,3
DSU 1115743	feces-bedding	-	[+]	+	[+]	-	+	+	+	4
DSU 129755	feces-bedding	-	(+)	+	[+]	-	+	+	+	4
DSU 36751	drylot soil	-	(+)	+	(+)	-	+	+	+	4
DSU 36752	feces-straw	-	[+]	+	[+]	-	[+]	+	+	4
DSU 36753	silo silage	+	-	+	[+]	+	+	+	+	4
DSU 36755	feces-bedding	-	(+)	+	(+)	-	+	+	+	4
DSU 36756	water	-	+	+	[+]	-	+	+	+	4
DSU 55751	drylot soil	-	(+)	+	(+)	-	[+]	+	+	4

* All isolates produced acid but no gas in 24-48 hours from: glucose, salicin, maltose, esculin; 3-7 days from lactose. No acid in 21 days from: L Arabinose, glycogen, mannitol, melibiose, inulin.

** Key: + = acid produced 24-48 hours; (+) = acid produced 3-7 days; [+] = acid produced 14 days; - = no acid produced 21 days.

DISCUSSION

The preliminary studies brought out a unique characteristic of Listeria which is its ability to survive and multiply in both sterile and unsterile suspensions incubated at 5° C. An increased rate of growth observed in unsterile samples was apparently due to the effect of having the normal flora of the sample present. The greater rate of increase indicated that possibly Listeria could be detected in the presence of large numbers of normal flora. Both high and low concentrations of Listeria, amongst high levels of additional organism, multiplied at 5° C allowing for the increase of Listeria to detectable levels.

Detecting the usually low numbers of Listeria required not only the cold enrichment technique but also another means of allowing Listeria to multiply. Therefore, KSCN-TPB was used as an enrichment media. This media allowed for the rapid multiplication of Listeria at 37° C while inhibiting other competing organisms. The use of 30° C, instead of 37° C, as an incubation temperature was used in the primary studies, because the samples that were to be collected would be more adaptive of a lower temperature.

The preliminary results indicated that, if one Listeria organism was placed in the KSCN-TPB enrichment media, Listeria could be detected after 24-48 hr incubation at 30° C. This enrichment media allowed for the multiplication of Listeria to a level where individual colonies on McBride Listeria agar could be picked out using an oblique light procedure. This procedure detected Listeria earlier than direct

plating or direct FA procedures.

In the primary studies the incidence of Listeria monocytogenes at the five sampling sites was brought out. The striking prevalence of Listeria was revealed by using the combination of cold enrichment and KSCN-TPB incubation. The 43 isolates that were isolated one day after incubation were not due to an increase in number but rather due to the existence of detectable numbers in the original sample. Those samples that were negative after the first day were allowed to incubate at 5° C. This allowed for the eventual emergence of Listeria to levels which could be detected,and thirty-three Listeria isolates were recovered as a direct result. The samples were held for two months. However, the possibility exists that more samples could have been positive for Listeria upon further incubation at 5° C.

Various studies have utilized potassium thiocyanate as a selective media for isolating L. monocytogenes from contaminated material (7, 8, 25, 33). The biochemical basis for the inhibiting action of potassium thiocyanate is not understood. It is known to chelate molybdenum and iron, and it possibly exerts its inhibitory action on the iron containing proteins, e.g. cytochromes. L. monocytogenes in the presence of KSCN can be successfully isolated due to the total inhibition of most bacteria and only the partial inhibition of Listeria, the cytochrome content which is low in deep culture (25). In this study the KSCN-TPB was found to not totally inhibit the population of naturally existing organisms. In fact it merely seemed to hold organisms at

stationary levels and allowed the Listeria to multiply.

Streaking one loopful from the KSCN medium which had been incubated at 30° C for 48 hr, revealed between 5-100 colonies of Listeria. These colonies were easily picked out using the oblique lighting technique. The colonies appeared as small smooth entire bright blue colonies in 24 hr. After 48 hr the colonies became lighter blue with a slightly rough margin. It was important to view all plates within 24 hr after removing the plate from the 30° C incubator. Otherwise bacterial and fungal overgrowth of Listeria colonies eliminated the ability to pick out the blue colonies.

The use of the obliquely transmitted light proved to be an essential tool for the selection of Listeria colonies. Ability to pick out suspected colonies of Listeria increased to the point that only 1-2 colonies out of the total suspect Listeria isolates proved not to be Listeria. Colonies other than Listeria possessed no similarity to these intensely blue Listeria colonies. Other bacteria present appeared as either yellow, white, red, brown or green colonies.

Various types of soil samples (Table 12) were collected to observe similarities or differences in the number of isolates recovered. Cropland soil was sampled only at the Willis Meyer and Bill Sheffeld sites and initially for the purpose of comparing this type of soil to soil where silage was fed. This relationship became quite evident with the presence of Listeria where silage was fed due mainly to the direct contamination by the silage.

However, it was also noticed that one-fourth of the cropland soil

which had no silage or fecal contamination yielded Listeria isolates. Relatively poor ability to isolate the Listeria was in part due to an abnormal flora of bacteria and fungi developing in the cold enrichment sample. This was made evident by the ability of Listeria to be easily isolated the first day and thereafter decrease in number until it no longer could be isolated. The dryness of the soil sample was due to lack of top soil moisture which would hamper the ability of Listeria to survive in such an environment.

TABLE 12

Isolation of Listeria from soil samples collected in the Fall (F), Early Winter (W1), Late Winter (W2), and Spring (S).

Sample Type	WM	BS	AN	DN	DSU	Time
Cropland soil	-	-,-				F
	-	+,+				W1
	-	-,-	ND*	ND	ND	W2
	+	-,-				S
Cropland soil (silage fed)	+	ND				F
	+	+				W1
	+	+	ND	ND	ND	W2
	+	-				S
Feedlot or drylot soil	+		+	+	+	F
	+		+	+,+	ND	W1
	+	ND	+	-,+	+	W2
	+		+,+	-,+	+	S

* Samples not collected

In comparison, feedlot or drylot soils contained a large amount of organic material and moisture. This soil type also provided the highest percentage of Listeria isolates (17/19) when compared to other

soil types. The maintenance of Listeria is clearly favored by this type of soil. The role that fecal contamination plays in this high incidence is not certain. The fecal material may not only be the source of Listeria but also a source of organic material and moisture to this soil environment.

Concentrated fecal samples (AN,DN) possessed a very high level of Listeria since seven samples out of eight were positive for Listeria (Table 13). These feces samples were taken out of a gutter, off a slatted feeding floor, and therefore were fresh samples of feces. Similar samples were not collected at the Willis Meyer site and the Bill Sheffeld site since areas of concentrated feces were not available for sampling. Feces was present, to some degree, in the feedlot soil and the soil where the silage was fed. At the Dairy Science Unit, Listeria was isolated from feces sampled at all sampling periods except the spring. An increase in isolates from feces during the early winter may have been directly related to the finding of Listeria in the early winter silage sample.

A second type of feces samples included large amounts of straw or chaff used for bedding purposes. Listeria was isolated from more than one-half of these samples (6/11). This incidence was well below that of the concentrated fecal samples; this may be due to the dryness and lesser amounts of feces present in these samples, as compared to the concentrated feces.

Listeria was found to be excreted in samples taken of fresh feces. Whether these cows were harboring the organism or whether the organisms were just being passed through from silage was not possible to be

determined.

TABLE 13

Isolation of Listeria from feces samples collected in the Fall (F), Early Winter (W1), Late Winter (W2),and Spring (S).

Sample Type	WM	BS	AN	DN	DSU	Time
Concentrated feces			+	-		F
			+	+		W1
	ND*	ND	+	+	ND	W2
			+	+		S
Feces & bedding (straw or chaff)			ND		+,-	F
			-		+,-	W1
	ND	ND	+	ND	+,+	W2
			+		-,-	S

* Samples not collected

Silage samples were collected from all five Brookings County sites (Table 14). The type of silage samples taken (pit-silage, silo-silage) varied for each site; however, each site produced an isolate of Listeria. Pit-silage was sampled at four sites (WM, BS, DN, AN), and Listeria was isolated from this type of silage at a high incidence (8/11). This incidence is not an indication of the presence of Listeria in the good silage portion of pit-silage, since Listeria was isolated in samples obtained from the spoiled portion of pit-silage. Pit-silage samples were not collected at DSU, because this type of silage was not fed. Only one sample was taken from the Arne Nelson site, since all pit-silage had been fed to the cattle by the time late winter samples were taken. Two sampling sites (DN, AN) were made inaccessable

for the early winter sampling due to large snow drifts left from a January blizzard. Generally, the incidence of Listeria isolates from pit-silage was highest in the set of winter samples.

TABLE 14

Isolation of Listeria from silage samples collected in the Fall (F), Early Winter (W1), Late Winter (W2), and Spring (S).

Sample Type	WM	BS	AN	DN	DSU	Time
Pit-silage	+	-	+	-	ND*	F
	+	+	ND	ND	ND	W1
	+	+	ND	+	ND	W2
	+	-	ND	ND	ND	S
Silo-silage	ND	ND	-	ND	-	F
	ND	ND	-	+	-,-	W1
	ND	ND	+	+	+	W2
	+	ND	+	+	-	S

* Samples not collected

Silo-silage was not expected to yield Listeria isolates, since the silo-silage samples collected were of good quality silage, low in pH, as compared to the spoiled pit-silage samples. However, the methods of isolation used uncovered seven Listeria isolates in the 14 samples collected. This demonstrates that silo-silage of good quality is not necessarily free from Listeria organisms. No samples were taken at the Willis Meyer site until in spring, after the feeding of pit-silage was discontinued. Both pit and silo-silage were of good quality. The Bill Sheffeld site had not silo-silage for sampling. The incidence for isolating Listeria from silo-silage seems to be highest for the late winter and spring samples.

Silage appears to be a very important vehicle in the transmission of Listeria. Once ingested by the animal, the Listeria is either passed through the animal or, if of sufficient pathogenicity and concentration, may cause infection in the susceptible host.

Alfalfa samples (see Table 15) were collected from all five sites (WM, BS, AN, DN, DSU). Listeria was isolated from both types of alfalfa; however, no isolates were obtained from samples collected in either the fall or spring. The recovery of Listeria from these samples (baled and chopped alfalfal) required 30-60 days of incubation at 5° C. The Dale Nelson site recorded the highest incidence (2/4) of isolates of Listeria from alfalfa, while the Dairy Science Unit recorded the lowest (0/4). At both of these sites, the alfalfa was stored under a large roof to protect it from spoilage.

TABLE 15

Isolation of Listeria from alfalfa samples collected in the Fall (F), Early Winter (W1), Late Winter (W2),and Spring (S).

Sample Type	WM	BS	AN	DN	DSU	Time
Chopped alfalfa	-	ND**	-*			F
	+	-	-*			W1
	-	+	+*	ND	ND	W2
	-	ND	ND			S
Baled alfalfa				-	-	F
				+	-	W1
	ND	ND	ND	+	-	W2
				-	-	S

* Alfalfa silo silage
** Samples not collected

The isolation of Listeria from the alfalfa samples was quite surprising considering the environment provided by the sample material. Samples were very dry, loosely packed, and in some cases (chopped alfalfa) quite moldy on the outer surface. This type of environment suggests that Listeria is able to survive under highly adverse conditions. The numbers of Listeria present in the samples is low, this is seen in the length of incubation time required for its isolation.

Alfalfa that had been chopped and placed into the silo (AN) provided a more moist environment, and in the late winter alfalfa-silage sample (taken from the Arne Nelson site), Listeria appeared to exist at much higher levels than in either baled or chopped alfalfa.

All of the Listeria isolations were obtained from the early and late winter alfalfa samples. Isolation of Listeria during the winter period appears to indicate a shift to a more favorable environment for the maintenance of Listeria.

Vegetation is clearly of major importance in the maintenance and transmission of Listeria monocytogenes. All types of vegetation including silage, alfalfa, and decaying corn stalks allowed for the maintenance of Listeria organisms.

Listeria was quite prevalent in the water samples (Table 16). This occurrence, especially in automatic watering systems, appeared to be directly related to contamination with silage. However, Listeria isolates found in creek water are less likely to be a direct result of silage contamination, since these isolates were obtained before the feeding of silage had begun. The early winter sample of creek water was taken through a hole chopped in the ice where the cows were allowed

to drink. The water was flowing rapidly during all sampling periods.

TABLE 16

Isolation of Listeria from water samples collected in the Fall (F), Early Winter (W1), Late Winter (W2),and Spring (S).

Sample Type	WM	BS	AN	DN	DSU	Time
Automatic watering Systems	-		+	-	-	F
	+		+	+	-	W1
	+	ND	+	+	+	W2
	+		+	+	-,-	S
Creek water		+				F
		+				W1
	ND*	-	ND	ND	ND	W2
		+				

* Samples not collected

The water at the DSU site was found to be relatively free of Listeria. This water was provided by a Brookings city water line and did not possess large numbers of organisms. The chlorine residual in the water was possibly responsible for the reduced bacterial growth. Listeria was isolated from the silage and the water during the same period, indicating a possible direct relationship between the two isolations.

Another similar result was evident at the Willis Meyer site, no Listeria was found in the fall water sample. Though silage contained Listeria in the fall, it was not being fed to the cows, thus, now silage contamination of the water had taken place. Once silage was fed, however, the water became contaminated with Listeria. Silage may

also have been responsible for the Listeria found in water samples at the AN and DN sites; silage containing large numbers of Listeria was fed near an automatic watering system. Again, the cows had a direct access to both the silage and the water.

The Listeria was present in the automatic watering systems in sufficient numbers to allow its early detection. This type of system may serve as an excellent source for the transmission of Listeria. Further study should investigate the role that automatic watering systems may play in the maintenance and transmission of disease causing organisms.

TABLE 17

Comparison of the number of positive samples from the environment independent of site location.

Time	Soil	Feces	Silage	Alfalfa	Water	Total
Fall	5/8	2/4	2/6	0/5	2/5	11/28
Early Winter	8/9	3/5	3/6	2/5	4/5	20/30
Late Winter	6/10	5/5	6/6	3/5	4/5	24/31
Spring	7/11	3/5	4/6	0/3	4/6	18/31
Total	26/38 (68.4%)	13/19 (68.4%)	15/24 (62.6%)	5/18 (27.8%)	14/21 (66.7%)	73/120

The results in Table 17 indicate that soil and feces are the

sample types in which Listeria was most frequently isolated. Water and silage also showed a high incidence of Listeria isolations. This similar high incidence of Listeria in these samples suggests a possible interrelationship of reservoirs and vehicles of transmission.

The silage is capable of supporting and maintaining the growth of the Listeria since in such an environment conditions are optimal for the growth of Listeria (pH range, temperature range). Silage presents a favorable environment for these organisms which are either supplied by soil contamination when cutting the vegetation for silage or are present in the vegetation when it is ensiled. The isolation of Listeria from alfalfa samples may support the idea that the Listeria organisms are present in the vegetation, since if just soil contamination was involved, 60 days would not have been required for the isolation of Listeria from the alfalfa.

The consumption of silage and water appears to be an excellent source for transmitting Listeria in high numbers to animals. Only susceptible animals will succumb to this pathogen, while others serve as reservoirs and excrete viable organisms in the soil. A reservoir for Listeria could be established in the soil under appropriate conditions or the soil could serve as a means of transmitting the organism to vegetation, silage and water.

The biochemical results of the 76 isolates suggest the presence of several strains of Listeria in the environment. Further work is necessary to establish the incidence of these strains in the environment and their pathogenicity.

The South Dakota Animal Disease Research and Diagnostic Laboratory has recorded a significant level of bovine (106 cases) and ovine (48 cases) listeriosis. Figure 7 indicates the prevalence of animal listeriosis cases and their geographical distribution in South Dakota. The East River area possesses a higher level of listeriosis cases than the West River area. This occurrence may be due to the more common practice in East River of feeding silage, or due to the soil type, yearly rainfall levels or proximity of the Brookings Diagnostic Laboratory. The higher incidence in cattle and sheep possibly reflects a greater exposure and susceptibility to Listeria. The current results indicate the prevalence of Listeria in the environment in close association with cattle and sheep. The highest incidence of isolation occurred between January and March,which correlated directly with the peak listeriosis periods. The vehicle responsible for this high exposure could directly involve the feeding of silage and the drinking of water containing sufficient numbers of Listeria organism.

Susceptibility on the other hand may be due to climatic factors. When cases were tabulated monthly, a distinct cyclic pattern is noted (Figure 8). The seasonal occurrence was in the early winter (December) and late spring (May), with most cases occurring in February and March. The seasonal occurrence begins just as the lowest maximum and minimum temperature of the year have occurred. Such a high occurrence,after the coldest period of each year,would suggest a possible combination of events (increased exposure and susceptibility). This climatic effect could possibly result in lowering the animal's resistance to a point

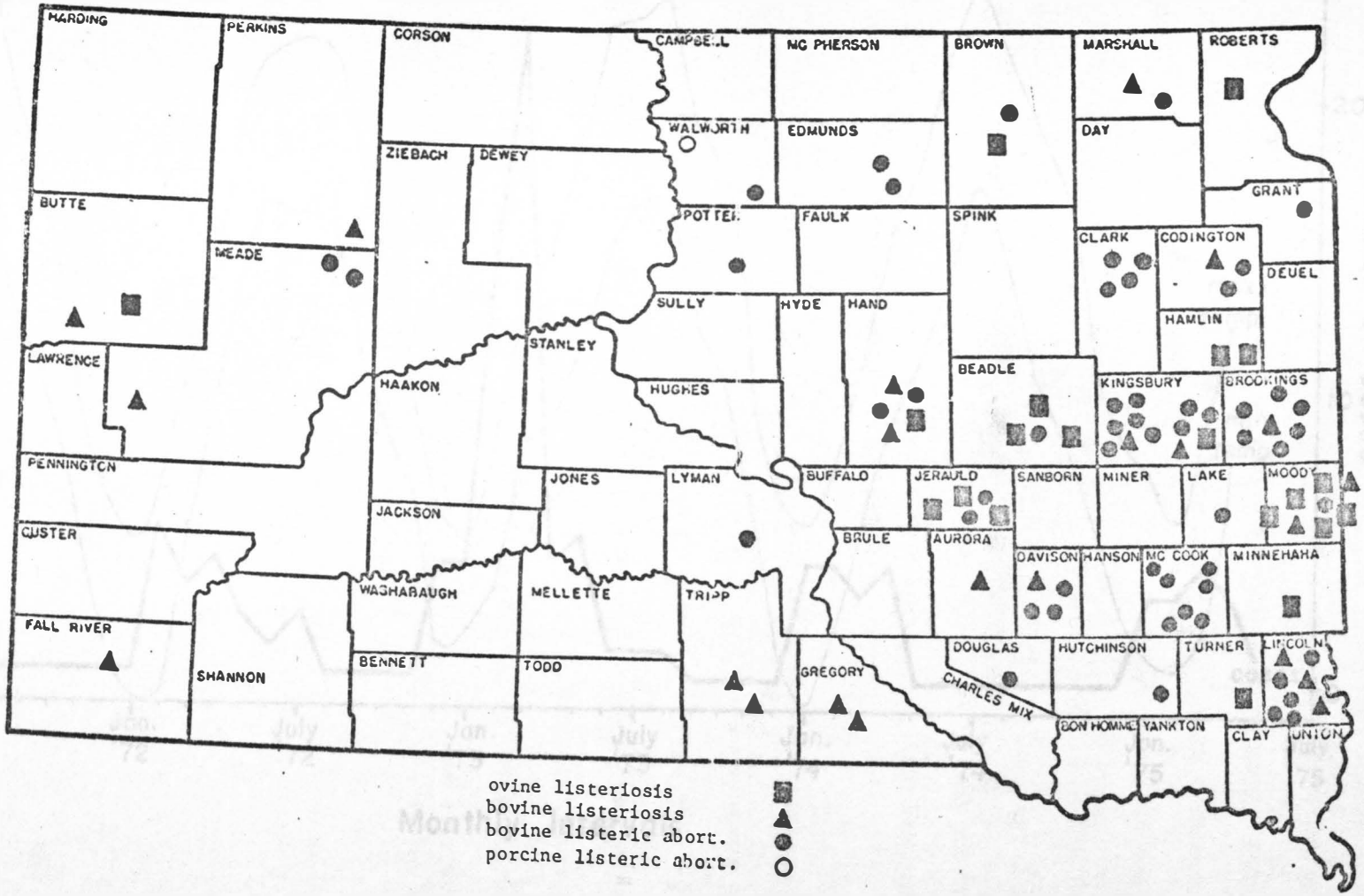

Figure 7. Geographical distribution of animal listeriosis cases in South Dakota.

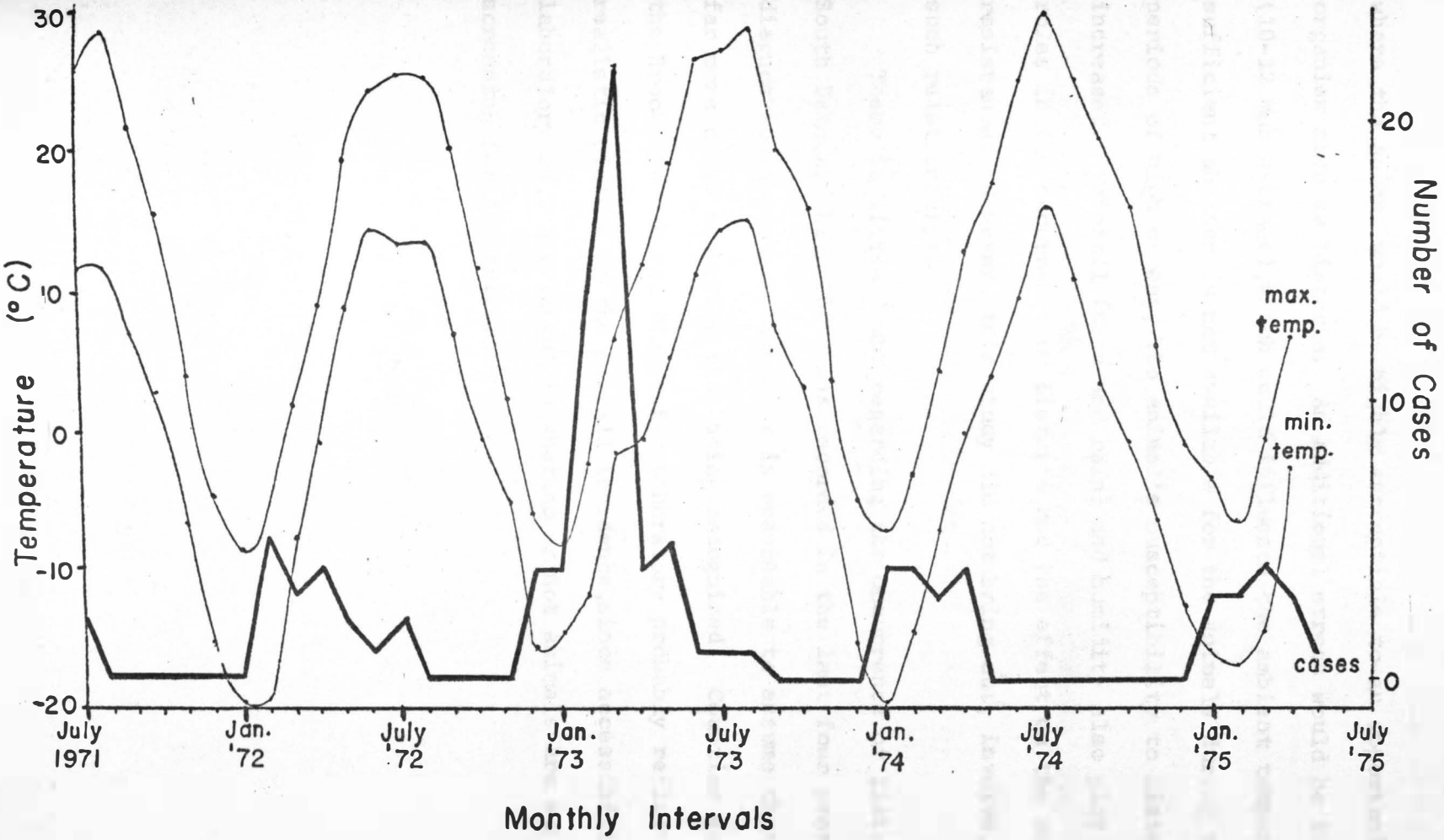

Figure 8. Seasonal distribution of reported listeriosis cases in South Dakota in relationship to average maximum and minimum temperature by year and monthly periods.

where the animal would be highly susceptible to an opportunistic organism such as Listeria. An additional stress would be the wind (10-12 mph average),which would influence the ambient temperature. If sufficient shelter is not available for the animals during these periods of high stress, the animal's susceptibility to Listeria may be increased. Rainfall (snow and rain) and humidity also play possible roles in the occurrence of Listeria and the effect on the animal's resistance. However, this study did not bring out, involve, or study such relationships.

There is little doubt regarding the occurrence of listeriosis in South Dakota. With 155 cases recorded in the last four years at one diagnostic laboratory alone, it is reasonable to assume that there are far more cases occurring than being recognized. Counties surrounding the Brookings Animal Diagnostic Laboratory probably reflect a more realistic picture of the overall incidence,since accessibility to the laboratory is a big factor in whether or not animals are subject to screening for listeriosis.

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