A NEW TECHNIQUE FOR ISOLATING LISTERELLAE FROM THE BOVINE BRAIN

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It is relatively easy to isolate the causative agent from the brain in cases of ovine listerellosis. However, the direct isolation of the microorganism from the bovine brain has met with only partial success. This failure represents a challenge to microbiological technique. The investigation herein reported deals with a method whereby the percentage of direct isolations was increased.

Listerellosis in the sheep of Michigan has been reported in a previous publication (Gray *et al.*, 1946). That it is present in this state in cattle also has been suspected for some time. Previous to this year a number of bovine brains had been submitted to the Animal Pathology Diagnostic Laboratory for culture when listerellosis was suspected, but it had never been confirmed by isolation of *Listerella monocytogenes*.

All cultures had been prepared by grinding the medulla in a mortar with about 10 ml of tryptose broth, then agitating with glass beads in a shaking machine for about 20 minutes. A portion (0.3 ml) of the resulting suspension was plated on tryptose agar and incubated at 37 C for 24 hours. The remainder was stored in the refrigerator at 4 C.

Case 1. On February 26, 1947, a bovine brain was submitted for culture. A clinical diagnosis of listerellosis had been made. Cultures were negative after 24 hours' incubation at 37 C, but when the brain suspension which had been stored in the refrigerator for 3 months was again plated, there was a heavy growth of listerellae (figure 1).

Case 2. March 24, 1947, a feeder steer was submitted for necropsy. This was the third animal in this herd to die. All three had displayed symptoms typical of listerellosis. Cultures prepared from the brain showed six colonies of a gram-positive organism resembling listerellae. Five weeks later when this same suspension was plated on tryptose agar, the colonies were too numerous to count.

Case 3. April 5, 1947, a six-month-old Hereford was presented for necropsy. This animal had been circling for about a week and was killed. Brain cultures

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were badly contaminated and no listerellae were observed. After refrigeration for 2 months it was possible to observe colonies of *Listerella* among the contaminants by the use of a dissecting microscope, as described by Huddleson (1946). The colonies of *Listerella* are a bright green with a finely textured surface. This is so characteristic that listerellae can be identified even in cases with extreme contamination.

Case 4. April 15, 1947, a yearling from a farm adjacent to that of case 2 was brought in for observation. It showed symptoms of listerellosis and died within 4 days. Brain cultures were negative. Three weeks later it was possible to demonstrate numerous colonies of *Listerella* from the refrigerated brain suspension.

Case 5. May 8, 1947, the brain of a two-year-old Holstein was presented for culture. A few colonies of a gram-positive rod were isolated (figure 2). With the suspension from this brain, an attempt was made to measure quantitatively the apparent increase in numbers of colonies observed in the preceding cases.

TABLE 1

Case 5. Showing increase in number of colonies during a period of approximately one month

	DATE				
	5-10	5-20	5-28	6-5	6-12
Plate I	90*	270	1,600	C	I
Plate II	—	220	1,400	C	I

* Original plate. C—Contamination. I—Too numerous to count.

A 0.2-ml portion of the brain suspension was plated on tryptose agar, a bent glass rod being used to distribute the material evenly. The results of this test appear in table 1.

In the five cases presented there was a marked increase in the number of listerellae that could be demonstrated after the brain suspension had been allowed to stand in the refrigerator for a period of time. There is as yet no definite explanation for this phenomenon, but several possibilities exist. It was found that *Listerella* will grow quite readily at a temperature of 4 C. Inoculated (one 4-mm loop) tryptose agar slants containing about 0.5 ml nutrient broth at the base showed visible growth in the broth in 3 days, and in 7 days there was considerable growth on the slant when incubated at 4 C. There is also the possibility of further tissue disintegration, thus releasing the organisms from the cellular substance. Or the phenomenon may be explained by the presence of some unstable inhibitory substance in the brain tissue. Nutini and Lynch (1946*a*,*b*) have found a substance in an extract prepared from both the human and the bovine brain that is bactericidal to *Staphylococcus aureus*.

The evidence presented in this report strongly suggests the presence of a bacteriostatic factor for *Listerella monocytogenes* in the bovine brain. The presence of such substances in animal tissues may account for the specific im-

munity to infection inherent in certain tissues. Listerellosis as manifested in the ovine is extremely acute, generally, with a short fatal course. In the bovine the disease is less acute, and recoveries have been reported (Biester, 1941; Graham, 1943). Pounden (1947) has reported one outbreak of an extremely acute nature, but that may be considered as atypical. Bacteriologically, primary isolation of listerellae from the ovine seldom results in failure, but in the bovine primary isolations are made in less than a third of the suspects cul-

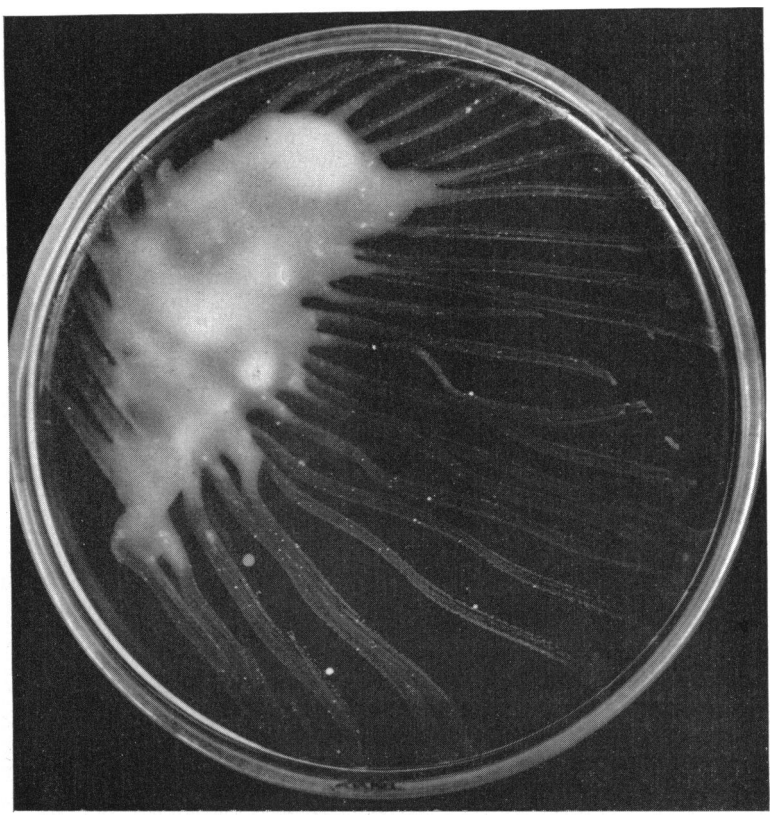

FIG. 1. SHOWING HEAVY GROWTH OF LISTERELLA AFTER A BOVINE BRAIN SUSPENSION HAD BEEN STORED IN THE REFRIGERATOR FOR THREE MONTHS

tured even though listerellosis is confirmed by histopathologic sections (compare figures 2 and 3). These observations are similar to those reported by Gifford and Jungherr (1947). Cultures recently isolated from the ovine will produce a conjunctivitis in rabbits in about 18 hours, whereas those recently isolated from the bovine require 72 hours or more to produce a conjunctivitis. In general, cultures of a bovine origin show a latent pathogenicity as compared to the ovine strains.

Biochemical tests. All media and procedures were in accordance with those described in *The Manual of Methods for the Pure Culture Study of Bacteria.*

Indole was not produced; nitrates were not reduced; litmus milk was reduced at first and after about 10 days showed a slight acid reaction; H_2S was produced in 24 hours; a slight zone of beta hemolysis appeared on blood agar; and starch was not hydrolyzed. The results of the fermentation reactions are shown in table 2.

Animal inoculation. Two drops of a 24-hour agar slant growth of the culture from case 2, suspended in 5 ml broth and instilled in the conjunctival sac of an adult rabbit, produced only a slight reddening of the lids in 5 days of observation This same animal was then given 0.5 ml intravenously of a similar suspension

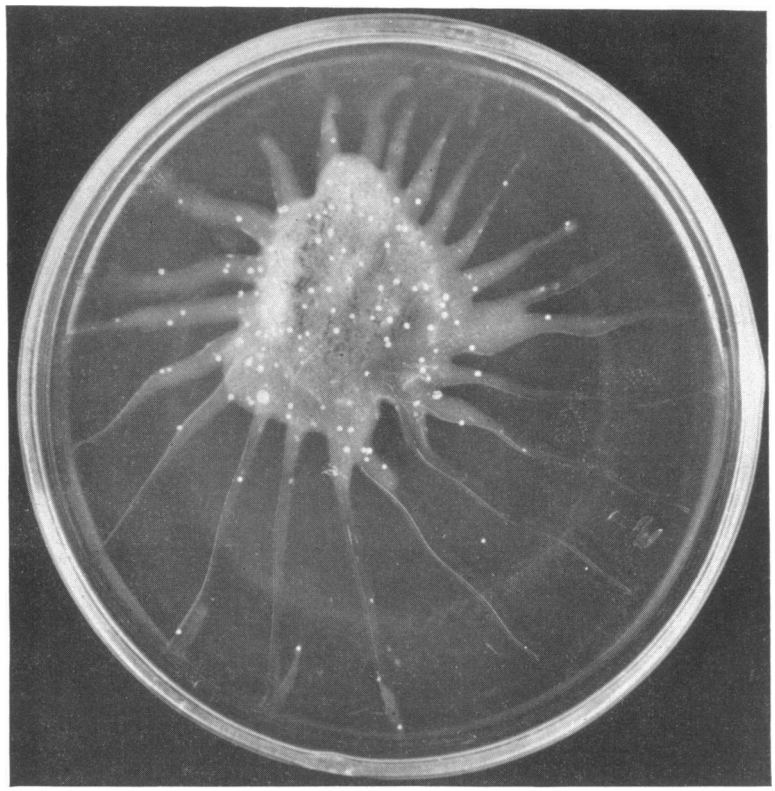

FIG. 2. PRIMARY CULTURE OF BOVINE BRAIN. CASE 5

The animal died in 48 hours. No gross lesions other than slight degeneration in the liver were observed. Listerellae were isolated from the heart blood and liver.

Two drops of a similar suspension of culture from case 4 were instilled into a rabbit's eye. After 96 hours the eye was slightly congested. This condition persisted for 8 days. At this time (the eighth day) listerellae could not be isolated from the eye. On the ninth day the eye became more congested, and on the tenth day a severe conjunctivitis and keritits were present. This persisted for 3 days. Swabs taken from the eye during this time when plated on tryptose agar showed a heavy growth of listerellae. When the conjunctivitis cleared, the

animal appeared very ill. It was depressed, did not eat, and drank large quantities of water. No temperature was taken, but the ears were very hot to the

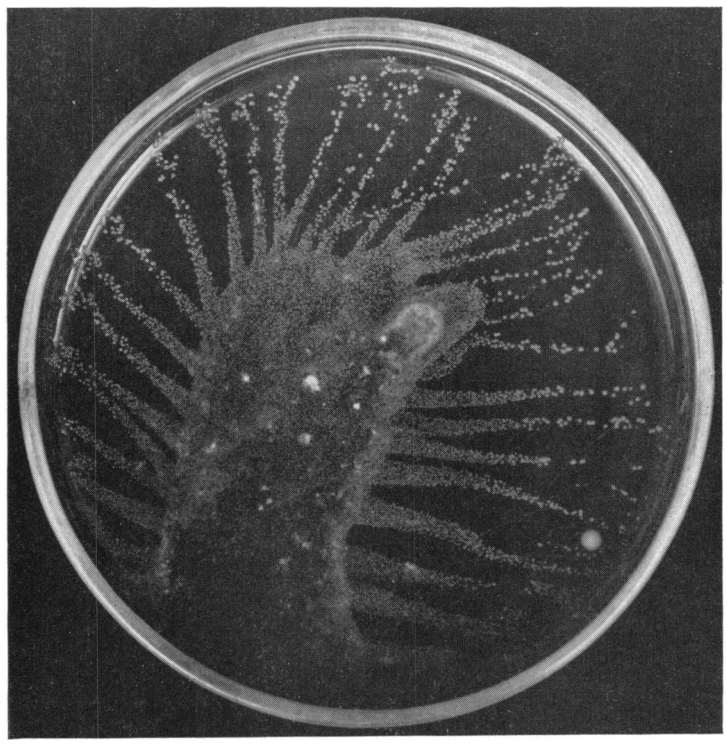

FIG. 3. TYPICAL PRIMARY CULTURE OF OVINE BRAIN
This animal was from the same farm as case 3 of this report

TABLE 2

Fermentation reactions

CULTURE	GLUCOSE	GALACTOSE	FRUCTOSE	ARABINOSE	XYLOSE	LACTOSE	MALTOSE	SUCROSE	TREHALOSE	RAFFINOSE	DEXTRIN	INULIN	INOSITOL	DULCITOL	MANNITOL	SORBITOL	GLYCEROL	SALICIN
1	+	−	+	−	−	∓	+	+	+	−	+	−	−	−	−	−	±	+
2	+	−	+	−	−	∓	+	±	+	−	+	−	−	−	−	−	±	±
3	+	−	+	−	−	±	+	+	+	−	+	−	−	−	−	−	±	±
4	+	−	+	−	−	±	+	∓	+	−	−	−	−	−	−	−	±	+
5	+	−	+	−	−	−	+	±	+	−	+	−	−	−	−	−	∓	+

+—acid; ±—marked acid; ∓—slight acid; −—no acid.
No gas was produced in any of the media.

touch. It showed all the symptoms of listerellosis. They persisted for 48 hours, at the end of which time the animal was comatose, and was killed for necropsy. There were no gross lesions other than a slight degeneration of the

liver. The heart blood, liver, and medulla were cultured on tryptose agar. Listerellae were isolated from the medulla only. Paraffin sections of the medulla showed the typical paravascular cuffing found in listerellosis.

A similar suspension of a culture isolated from a sheep, which was brought in from the same farm as case 3 of this report, produced a marked conjunctivitis and kerititis in the eye of an adult rabbit in 24 hours. This condition persisted for 10 days. Listerellae were isolated from the eye for 12 days following instillation.

SUMMARY

Five cases of listerellosis in the bovine have been reported and confirmed by laboratory study. In three of the five cases isolations were made after the brain suspension had been refrigerated for from 5 weeks to 3 months. A preliminary report is made relative to some substance in the bovine brain which may interfere with the primary isolation of listerellae, but which may be destroyed by refrigeration. A method of readily identifying colonies of *Listerella* is also described.

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