An Attempt to Determine the Incidence of Listeria Monocytogenes in the Brain of Mammals

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INTRODUCTION

In his excellent monograph (1) Seeliger records the isolation of *L. monocytogenes* from 32 animal and bird species throughout the world; in Canada, isolations have been made from 10 of these 32 species.

Little, if anything, is known of the pathogenicity and epidemiology of the disease; in one of the more recent papers (2) it has been suggested that the different pathological manifestations which result within and between species are mainly due to host susceptibility regardless of the portal of entry. Others (3) postulate that the encephalomyelitis which occurs follows invasion from the oral cavity. Although in any one particular outbreak it appears that all affected animals die, the possibility that a carrier state may exist cannot be ruled out with certainty. Due to the difficulty encountered in the isolation of this organism from certain tissues (4) and also to a lack of knowledge regarding its actual presence in brain tissue (5) a survey to estimate its occurrence in animals in Canada was felt to be of some value. Brain tissues from negative rabies cases and from a representative number of animals and birds submitted for routine necropsy were examined culturally. Reasons for selecting brain material for examination were; (a) its availability from the large number of negative rabies cases received; in many of which the Veterinarian or layman might have mistaken the nervous syndrome due to a Listeria encephalomyelitis with a similar syndrome seen in rabies; (b) the fact that in the majority of specimens presented for rabies examination, only the brain or head is submitted and; (c) contamination would be at a minimum in the brain tissue of animals which are dead upon arrival at the laboratory. That, in a survey of this type, examination of other organs as well as brain might have given more significant results, is admitted.

MATERIALS AND METHODS

1) *Specimens*

Whole brains of mammals and birds or the medullary portion thereof were collected at various laboratories of the Division[3]. As has been mentioned previously they were taken either from routine specimens submitted for necropsy or were brains from negative rabies specimens. At other than the Vancouver and in some cases the Hull Laboratories, the brains were kept in the frozen state and were submitted for bacteriological examination when a number had been collected.

2) *Bacteriological examination*

The medula and a portion of the cord, if present, were cut into small pieces and ground in a Tenbrock tissue grinder using tryptose phosphate broth (Difco) as a diluent. The resulting suspensions were dispensed into screwcap tubes for storage.

A loopful of this suspension was streaked onto plates of one or more of the following media; (a) beef heart in-

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fusion agar (6) with and without 5.0 percent bovine blood, (b) beef infusion agar containing 4.5 percent ovine blood and (c) either (a) or (b) which contained potassium tellurite in a final concentration of 0.025 percent. In work with the brain material mentioned in this report beef heart infusion agar and beef infusion agar containing 4.5 to 5.0 percent ovine or bovine blood were the media which gave best results. Plates were incubated under aerobic conditions at 37°C. for 24 to 48 hours.

The suspensions were stored at 4°C. If the initial attempt at isolation was negative, the suspensions were again cultured at the end of the 1st, 3rd, 6th and 12th week. Failure to isolate *L. monocytogenes* from a suspension after an incubation period of 12 weeks at 4°C. resulted in the specimen being considered negative.

Suspicious colonies appearing on the plates were transferred to at least two and sometimes three tryptose phosphate broths, one of which was incubated at 37°C., one at room temperature (20-25°C.) and the third, if seeded, at 4°C. Hanging drop preparations were prepared after an incubation period of not more than 18 hours for those cultures incubated at 37°C. and RT° and at about 24-30 hours for the culture at 4°C. Organisms which exhibited typical tumbling motility at RT° and 4°C. and were non-motile at 37°C. were sometimes further checked by seeding a motility test medium (eg. tryptose phosphate broth containing 0.2 percent agar and 8.0 percent gelatin) dispensed in small petri dishes and examining them at regular intervals for the swarming growth given by motile micro-organisms. The tryptose phosphate broth growths of the micro-organisms which gave the type of motility just mentioned were used to seed a selected group of carbohydrate and other biochemical media. These particular media were chosen (from experience) as representing a minimal number of differential tests giving constant results for known strains of *L. monocytogenes*. A list of these media and of the typical reactions given by some one hundred strains of *L. monocytogenes* checked therein are given in Table I.

TABLE I

Pertinent Biochemical Reactions and Other Biochemical Properties of L. *monocytogenes*. (Compiled from Literature and Study of some 100 Strains).

Hemolysis (Bovine or Ovine Blood)		—	α, β or Nil
Motility	37°C	—	Non Motile
(18 - 24 hrs.)	RT°	—	Motile
Glucose**		—	A^{1*}
Sucrose		—	A^{2-10}
Maltose		—	A^{1}
Salicin		—	A^{1}
Mannitol		—	O^{14}
Dulcitol		—	O^{14}
Trehalose		—	A^{1}
Esculin		—	A^{1}
Catalase		—	Positive[1]
Litmus Milk		—	Reduction at base[1], otherwise no change
Nitrate Reduction		—	Negative[2-4]
Indol Production		—	Negative [2-4]
H_2S Production		—	Negative[14]

*Indices denote number of days of aerobic incubation at 37°C at which these reactions are usually observed.

**All carbohydrates at 0.5 to 1.0 percent concentration in peptone water.

TABLE II.

Listeria Incidence in Relation to Rabies Status of Specimens Tested.

Specimen	Examined	Listeria Found	Frequency
Rabies Diagnosis Positive	1	1	1.0
Rabies Diagnosis Negative	257	0	—
Others	91	13	0.143
TOTAL	**349**	**14**	**0.040**

TABLE III

Listeria Isolated in Relation to Province or Area.

Location	Examined	Listeria Isolated	Frequency
Labrador	3	1	0.33
Nova Scotia	1	0	—
New Brunswick	1	0	—
Quebec	16	1	0.062
Ontario	99	5	0.05
Manitoba	13	0	—
Saskatchewan	37	0	—
Alberta	122	0	—
British Columbia	45	7	0.15
Northwest Territories	12	0	—
TOTALS	**349**	**14**	**0.040**

TABLE IV

Species Distribution

Species	Examined	Listeria Isolated	Frequency
Dog	105	1	0.009
Cow	46	0	—
Cat	31	0	—
Coyote	27	0	—
Fox	24	1	0.041
Chinchilla	21	8	0.38
Sheep	20	1	0.05
Pig	18	0	—
Mink	16	0	—
Wolf	7	0	—
Totals	315	11	0.034
Others[1] (4 or less specimens examined)	34	3	0.08
TOTALS	**349**	**14**	**0.04**

1Rabbit (4) — 1 positive, raccoon (4), lynx (4), bear (3), horse (3), chicken (2) — 2 positive, squirrel (2), goat (2), beaver (2), mouse (2), deer (1), weasel (1), flying squirrel (1), fish (1), porcupine (1), lemming (1).

RESULTS

Over a four year period 349 specimens were cultured for the presence of *L. monocytogenes*. All micro-organisms isolated which were suspected of being possible Listeria were subjected to the tests already described.

The specimens checked, species involved, origin and isolations are listed in Tables II, III and IV. Of the 349 specimens checked 258 were suspected rabies cases. In general, the medulla from these specimens were not cultured until they were shown by microscopic examination and in many cases by mouse inoculation, to be negative for rabies; however, one brain, from an Ontario fox was shown to be carrying both the rabies virus and *L. monocytogenes*.

Fourteen isolations of *L. monocytogenes* were made, from the brain tissues examined with the distribution among different animal species being as follows: dog — 1, fox — 1, sheep — 1, chicken — 2, chinchilla — 8, rabbit — 1.

DISCUSSION

Several of the specimens were of interest. The dog specimens from Labrador were decided following a heavy loss of sleigh dogs almost of an epizootic nature. t was supposed that the condition was canine distemper and in fact vaccine had been ordered and in some areas used. Laboratory investigation subsequently proved that there was a mixed infection of canine hepatitis and listeriosis, although probably not of a dual nature. Of the three carcasses submitted, two were diagnosed as hepatitis on histopathological examination while in the third, which was negative for hepatitis on section, *L. monocytogenes* was isolated from the medulla.

One of the chickens submitted was suspected of being infected with a nervous type of Newcastle Disease. On necropsy no visceral or thoracic lesions were noted. The brain was passaged through eggs and the embryo deaths were not typical of those usually caused by N.D.V. Cultural examination of the embryos and the original suspension revealed a pure culture of *L. monocytogenes*.

The isolation from the sheep was made from both the uterus which exhibited an acute metritis and from the brain. There were no other gross lesions evident.

The isolation from the mature rabbit which again did not exhibit gross lesions was made from the brain. This animal was from a laboratory rabbit colony and represented a single case.

As had already been mentioned *L. monocytogenes* and the virus of rabies were both demonstrated from the brain of the fox. This was the only rabies positive brain cultured.

It is of interest to note that *L. monocytogenes* was not isolated from any of the 257 negative rabies specimens examined even though a percentage of these were from animals exhibiting encephalitic symptoms. This was surprising as it was thought at the beginning of this survey that at least some Listeria would be found in this group.

SUMMARY

Over a four year period 349 specimens consisting of mammalian and avian brain tissues were cultured for the presence of *L. monocytogenes*. Two hundred and fifty-eight of these specimens were from cases of suspected rabies. *L. monocytogenes* was not isolated from any of the 257 negative rabies cases. It was isolated along with the rabies virus in an apparent dual infection from a fox brain. Fourteen isolations were made with a distribution among different animal species being as follows: dog — 1, fox — 1, sheep — 1, chicken — 2, chinchilla — 8, rabbit — 1.

RESUME

Pendant quatre ans on essaya d'isoler en cultures, *Monocytogènes* au moyen de 349 spécimens provenant de tissus célébraux de mammifères et d'oiseaux. Deux cent cinquante-huit de ceux-ci provenaient de cas soupçonnés de rage.

De 257 de ces cas négatifs pour la rage, *L. Monocytogènes* n'a pas pu être isolé. Cependant du cerveau d'un renard apparemment ateint d'infection double, il fut isolé ainsi que le virus rabique. Quatorze autres isolements furent réalisés ayant la distribution suivante pour les différentes espèces animales: Chien 1, renard 1, mouton 1, poulet 2, chinchilla 8, lapin 1.

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Abstract

Brucellosis in Livestock

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Brucellosis in livestock continues to be an important economic burden and public health threat in many sections of the United States.

Since 1934 a cooperative State-Federal program for the control and eradication of bovine brucellosis has been in operation. During that time the number of reactors disclosed through blood agglutination testing has declined from 11.5 to 1.8 percent of the animals checked. A similar reduction has occurred in the number of herds carrying the infection. For fiscal year 1935, infected animals were found in 36.2 percent of the blood-tested herds. As of June 30, 1957, this figure was 10.5 percent.

In October 1954 the bovine brucellosis eradication campaign was accelerated by additional Federal funds, and progress during the past 3 years exceeded that reported for any similar period since the program's inception. At present approximately half of all cattle in the United States are under supervision for the control and eradication of brucellosis.

The initial goal of the eradication campaign is to establish and maintain certified brucellosis-free areas. This designation signifies that the infection appears in no more than 1 percent of the animals and 5 percent of the herds. At the end of fiscal year 1957, 735 counties and 7 entire States were certified. Another 712 counties were actively working on programs leading to certification. This means that nearly 50 percent of all counties in the United States, Puerto Rico, and the Virgin Islands are either certified or are rapidly approaching that status. It is estimated that by June 30, 1958, a total of 16 States will be certified.

If the present level of field operations can be maintained, there is every reason to believe that the incidence of bovine brucellosis throughout the United States can be reduced to 1 percent or less by 1960.

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