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Listeria monocytogenes in the Feces of White-Tailed Deer (*Odocoileus virginianus*)

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ABSTRACT

Five of 32 apparently healthy deer, *Odocoileus virginianus*, were shown to harbor *L. monocytogenes* in their alimentary tracts. Cultural, biochemical, serologic and virulence characterizations of the five fecal isolates of *L. monocytogenes* are presented. It is believed that these isolates are the first described from the alimentary tract of apparently normal deer. Two deer harbored serotype I strains of *L. monocytogenes*, the other three yielded serotype IV strains.

INTRODUCTION

Annually, during the month of December, approximately one third of the deer herd on the Edwin S. George Reserve, The University of Michigan, is removed by shooting. For the harvest of 1965, pertinent to this report, there was no deliberate selection of animals by age or by sex. Thirty-two deer were killed.

The animals were dressed usually within two hours of killing. Depending upon the type of management studies current among various Departments of the University of Michigan, the requisite specimens were collected in the dressing station. Although serology against selected bacterial species had been started in 1964, no bacterial isolation studies were initiated until the 1965 harvest. Due to the interest of several departments of the University in the ecology, epidemiology and interhost transmission modes of the psychroduric bacterial pathogenes, *Pasteurella pseudotuberculosis, Yersinia enterocolitica,* and *Listeria monocytogenes*, a preliminary survey of materials from the harvested deer was initiated in 1965.

MATERIALS AND METHODS

Tissues or Animal Products

At the dressing station, whole bloods were collected from jugular veins or thoracic cavities. Specimens of lung tissue were excised, and fecal pellets were expressed manually from a length of excised colon. These materials were held at approximately 4° C until taken to the laboratory.

Serum was removed from the blood clots in the laboratory. The lung tissues were homogenized in sterile phosphate-buffered isotonic saline (PBS) using sand in a mortar and stored at 4°C. The fecal pellets were transferred to screw-capped tubes containing trypticase soy broth[3] and 1 percent yeast extract (TSB/YE), 200 ug/ml Acti-dione[4], and 40 ug/ml potassium tellurite. The pellets were broken up with sterile glass rods,

[1] Supported by a Public Health Service Training Grant.
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[3] Baltimore Biological Laboratories, Inc., Baltimore, Maryland.
[4] Crystalline Cycloheximide, Upjohn Co., Kalamazoo, Michigan.

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then thoroughly mixed with a Vortex Jr. mixer[5]. The homogenized pellet suspensions were held at 4° C until processed to detect bacterial populations.

Isolation Media

L. monocytogenes was isolated in enriched media of 3.75 percent potassium thiocyanate (4) in TSB/YE at 22° C for 48 hours.

The selective solid medium used in this study was Bacto MacBride agar[6]. Tryptose agar plates were used concurrently. The Henry light and stereoscope assembly described by Gray (5) were used to visually differentiate *L. monocytogenes* from other Gram positive populations.

Cultural and Biochemical Characterization

The methods and materials required for cultural, morphologic and biochemical characterization of isolates were those described in *Diagnostic Procedures and Reagents* (6).

Serology

Preliminary serologic identification was accomplished by direct flourescent-labelled antibody methods (7). A representative fluor-labelled antiserum was provided for each serotype (I through IV).

Confirmatory serologic analysis was with individual antigenic preparations from the deer isolates, by the methods of Osebold *et al.* (8).

RESULTS

Attempts at direct isolation of *L. monocytogenes* with and without enrichment in potassium thiocyanate, and the use of either tryptose agar or MacBride selective medium, were negative when applied within one week of preparing the fecal suspension. However, after 30 and 45 days at 4°C, as suggested by Gray (9), positive results were obtained from 3 and 2 deer, respectively, using the enrichment-selective medium sequences.

Gram positive, catalase positive, motile, β-hemolytic, nonsporing rods were isolated after sequential exposure to potassium tellurite (40 ug/ml), to potassium thiocyanate (3.75 percent) and then phenylethanol (2.5 mg/ml). These were presumptive *L. monocytogenes* and required further biochemical characterization.

All isolates were unvarying in biochemical reactivity consistent with observations frequently made on Listeria: methyl red, acetylmethylcarbinol, glucose

TABLE 1. *Physical Characterization of Odocoileus virginianus and Their Fecal Listeria monocytogenes*

Deer No.	Age in Years	Sex	Weight (pounds) Live	Weight (pounds) Field Dressed	*Listeria monocytogenes* Serotypes
1226	1½	Male	161	126	serotype IV
1237	½	Female	76	59	serotype IV
1242	2½	Male	170	133.5	serotype I
1243	4½	Male	172	138.5	serotype IV
1244	1½	Male	140	107.5	serotype I

[5] Scientific Industries, Inc., Queens Village, Now York.

[6] Difco Laboratories, Detroit, Michigan.

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and maltose, and salicin positive. They did not utilize citrate and did not ferment sucrose, inulin, or xylose within 3 days at 35°C. Hydrogen sulfide was not produced, and litmus milk became slightly acidified with no clotting. Beta hemolysis occurred under the colonies on sheep blood agar, but never extended beyond the margin of the colony.

Mice were inoculated intraperitoneally with approximately 1 x 10^7 viable cells from an 18-hour, 23°C broth culture. Survivors were killed after the fifth post-inoculation day. Heart blood cultures and peritoneal swabs were taken from all dead or sacrificed animals and examined culturally and by direct fluor-labelled antibody methods. Only the isolate from deer M-1242, a serotype I, did not produce typical virulence or pathogenicity in experimental mice.

Table 1 provides a limited description of the deer which were proven to carry *L. monocytogenes* in feces, and the serotype of the isolated strains. Thus 15.7 percent (5 of 32) of the deer carried *L. monocytogenes* in their alimentary tracts at the time of harvest.

DISCUSSION

Isolations of *Listeria monocytogenes* from diseased deer have been rare (1, 2, 3). To the best of our knowledge, isolations of *L. monocytogenes* from "normal" wild deer have not been reported previously. We do not know the significance of this finding, but research on the occurrence of *L. monocytogenes* in the George Reserve deer and in other parts of the Reserve environment, is continuing. Hopefully, it will lead to new understanding of the epizootiology of this organism. For present purposes, it is felt that a discussion of the techniques used for isolation of *L. monocytogenes* should be presented.

Unlike the detection of etiologic agents in overt disease states, successful isolation of agents in subclinical or carrier states depends largely upon the efficiency of enrichment and selective systems. For this reason, unusual and perhaps unrealistic emphasis was given the potassium thiocyanate enrichment method. It had been shown that, using known virulent strains of *L. monocytogenes,* tolerance by, and proliferation of, these bacteria could be anticipated with an input of 1 to 10 viable cells in a substrate (TSB/YE) which contained 40 ug/ml potassium tellurite. This established the rationale for the holding medium for the fecal suspensions.

Controlled growth studies, using known virulent strains of *L. monocytogenes,* consistently showed that a calculated input of 1 viable cell would proliferate, independently of other Gram positive bacteria, in TSB/YE with 3.75 percent potassium thiocyanate. This proved that one could reasonably expect survival and proliferation of *L. monocytogenes* at 35°C, within 48 hours, in a suitable substrate containing 3.75 percent potassium thiocyanate.

Both the holding medium (with potassium tellurite) and the primary enrichment medium (with potassium thiocyanate) contributed largely to the obliteration of Gram negative organisms. However, neither materially depressed unwanted Gram positive bacteria, i.e., streptococci, diphtheriods, and unclassified aerobic, non-sporing rods.

The major selectivity of MacBride agar is referrable to phenylethanol, which generally inhibits Gram negative bacteria, and also Gram positive sporing bacteria. There is nothing particularly diagnostic about the surface colonies of *L. monocytogenes* upon this meduim. It is true that the Henry light and stereoscopic assembly helps to avoid the isolation of streptococci, but it generally cannot be of much assistance in discriminating between some of the aerobic, non-sporing Gram positive rods.

It is recognized that maintenance of listeria materials at 4°C with potassium

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tellurite, enrichment with potassium thiocyanate, and selective plating with phenylethanolic media are of great assistance for the isolation of *L. monocytogenes*. Nevertheless, we believe that a distinctive, discriminating, selective medium still is required in this manipulative sequence, especially for so-called "normal flora" studies.

LITERATURE CITED

1. THAMM, H. 1957. Listeriose unter Rehwild. Zentrl. Bakteriol., I. Orig. 167: 417-418.
2. HORTER, R. and F. HUNSTEGER. 1960. Kasuisticher Beitrag zur Listeriose. Deut. Tierarztl. Wochschr. 67: 11-14.
3. NILSSON, A. and K. A. KARLSSON. 1959. *Listeria monocytogenes* isolations from animals in Sweden during 1948 to 1957. Nord. Vet. Med. 11: 305-315.
4. LEHNERT, C. 1964. Bakteriologische, serologische and tierexperimentelle Untersuchungen zur Pathogenese, Epizootologie and Prophylaxe der Listeriose. Arch. f. Expt'l. Veterinarmed. 18: (5) 981-1027, (6): 1247-1302.
5. GRAY, M. L. 1957. A rapid method for the detection of colonies of *Listeria monocytogenes*. Zentrl. Bakteriol. Parasitenk., Abt. I. Orig. 169: 373-377.
6. Am. Public Health Assoc. 1963. *Diagnostic Procedures and Reagents*. 4th Edit. Am Public Health Assoc., Inc., New York, N. Y.
7. MARSHALL, J. D., JR., W. C. EVELAND, and W. C. SMITH. 1958. Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technic with a modification of its application. Proc. Soc. Exptl. Biol. Med. 90: 898-900.
8. OSEBOLD, J. W., O. AALUND, and C. E. CHRISP. 1965. Chemical and immunological composition of surface structures of *Listeria monocytogenes*. J. Bacteriol. 89: 84-88.
9. GRAY, M. L., H. J. STAFSETH, F. THORP, JR., L. B. SCHOLL, and W .F. RILEY, JR. 1948. A new technique for isolating Listerellae from the bovine brain. J. Bacteriol. 55: 471-476.

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This 4-day program is conducted by Dr. R. Stuart Mackay, Senior Scientist on the Galapagos International Scientific Project and now at Boston University. It is designed to provide a comprehensive introduction to the field for those engaged in research in the biological and health sciences.

Prerequisite for taking the Bio-Medical Telemetry course is a B. Sc. degree. Tuition is $125. Ten honoraria are offered to academically-based scientists by the co-sponsoring American Institute of Biological Sciences, (BIAC, 3900 Wisconsin Avenue, N.W., Washington, D. C. 20016). Tuition information may be obtained from the Office of Conference Development, Boston University, Boston, Massachusetts 02215.

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