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TULAREMIA IN A MULE DEER

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Abstract: A case of tularemia was confirmed in a 51-year-old man who acquired the disease from a mule deer, *Odocoileus hemionus*. *Francisella tularensis* was isolated from bone marrow of the deer carcass.

INTRODUCTION

Tularemia is remarkable for its diverse modes of transmission and for the large number of animal species infected, including about 50 species of mammals in North America. Rabbits, hares, muskrats, voles and other small rodents are the usual hosts; larger species such as the fox, coyote, beaver, bobcat, badger, dog, cat, sheep, calf, goat and horse occasionally have been implicated. Although deer flies (*Chrysops discalis*) are a known source of transmission to man, there are surprisingly few reports of tularemia in deer, and nearly all of these are based on serologic evidence.[4,5,7,9,10,14,15] Deer flies readily feed on deer, but also feed on rabbits, apparently their usual source of infection with *Francisella tularensis*. There are a few published reports of apparent transmission of *F. tularensis* from deer to man[1,2,6,8,13] and in most of these no laboratory studies are described. We know of only one published report of isolation of *F. tularensis* from a deer (mule deer (*Odocoileus hemionus*) tissue sample, details not given[12]). Highly virulent *F. tularensis* have been isolated from the spleen of a mule deer from Colorado, 1971; and from the spleen and liver of a mule deer from Wyoming, 1973 (B. Thorpe, University of Northern Colorado personal communication 21 April 1975). Undoubtedly other cases have occurred, but were not reported in the literature or were in foreign literature not available to us. We report herein[5] a laboratory-proven case of tularemia acquired from a deer, and isolation of *F. tularensis* from bone marrow of the deer carcass. The case had interesting and unusual clinical features which will be reported separately in detail (Joel Ruskin, personal communication).

Case Report

A 51-year-old man was hospitalized in October, 1974, following a one-week history of fever, chills, malaise and diarrhea. Stool cultures were negative for pathogens, and various studies including sigmoidoscopy, barium enema, and rectal

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[5] See preliminary notification of case in California Morbidity, No. 6, February 14, 1975, California State Department of Health, Berkeley, California 94704, USA; and Morbidity and Mortality Weekly Report 24: 126, 131, April 5 1975; Center for Disease Control, Atlanta, Georgia, 30333, USA.

[6] Smith, V. L. Personal communication to W. L. Jellison on 12 March 1974 (courtesy of Dr. W. L. Jellison).

biopsy suggested a diagnosis of pseudomembranous ulcerative colitis. The diarrhea gradually resolved, but fever continued. A large, tender left axillary lymph node was noted, as was a scabbed, erythematous lesion on the left hand which the patient attributed to an injury from cutting wood. Culture of the lesion on the hand yielded *Staphylococcus aureus*. It was then learned that 3 days prior to symptoms, he had shot and field-dressed a mature male mule deer (*O. hemionus*) in southern Mono County, California. He handled no other animals and had no known fly- or tick-bites, but did drink from local streams. A diagnosis of tularemia was considered and his tularemia agglutinating antibody titer was found to be 1:320. Lymph node aspiration yielded a small amount of bloody fluid, which was negative by culture for *F. tularensis*. Several blood cultures were also negative, as was culture of the rectal biopsy specimen. No antibiotics had been given prior to obtaining these cultures. The patient was then treated with streptomycin for 5 days (becoming afebrile in 36 hrs), followed by oral doxycycline for 2 weeks. The hand lesion healed promptly; a second aspiration and culture of the axillary node was negative for *F. tularensis*. Diagnosis was then confirmed in the laboratory by serial tularemia agglutinating antibody titers: October 15—negative; October 22—1:320; October 23—1:640; November 8—1:5,120; January 10—1:640. Three sera also were tested in the Microbial Diseases Laboratory, California State Department of Health, using an *F. tularensis* agglutinating antigen prepared from the deer isolate: October 22—1:160; November 8—1:1,280; December 18—1:640. These sera had *Brucella* agglutination titers of 1:80.

The deer carcass had been cut and wrapped at a commercial meat locker. None of the other persons who handled the carcass became ill. The stored meat was incinerated after tests at the State Microbial Diseases Laboratory confirmed the presence of *F. tularensis*.

MATERIALS AND METHODS

Frozen deer steaks and roasts were submitted to the Microbial Diseases Laboratory on 10 January 1975. The meat was thawed and approximately 5 gm of marrow were removed from the bones. Smears of the bone marrow were heat-fixed and stained with Gram stain and with Wayson's stain.

Direct Fluorescent Antibody Test

Duplicate smears of bone marrow were made on each test slide and were lightly heat-fixed. To one smear was added an appropriate dilution of fluorescein isothiocyanate (FITC) conjugated *F. tularensis* antiserum[7] and to the other was added an appropriate dilution of FITC-conjugated normal rabbit serum as a negative control. Impression smears of *F. tularensis*-infected mouse spleen were used as positive controls. After addition of the conjugates and incubation for 30 min at room temperature, the slides were washed twice with pH 7.2 buffered saline and once with distilled water. The slides were counter-stained for 5 min with a 1:4,000 dilution of Evans blue stain, rinsed twice in carbonate-buffered saline (pH 9.0), once in distilled water, blotted and mounted with carbonate-buffered glycerol saline (1 part carbonate-buffered saline pH 9.0 and 9 parts glycerol).

Culture and Animal Inoculation Procedures

A portion of the specimen was diluted with saline to make a slurry for use in culture procedures and animal inoculations. The remaining portion of the specimen was refrozen at —70 C. The slurry was inoculated onto blood glucose cystine agar plates[11] and incubated for 48 hr at

[7] FITC conjugates were prepared in this laboratory by the method of Cherry *et al.*[3] using rabbit antisera to *F. tularensis* strain B38 (ATCC #6223), and also to the *F. tularensis* strain isolated from the deer bone marrow. An FITC conjugate prepared from a pool of normal rabbit sera and one prepared from the pre-immunization serum of the rabbit were used as negative controls.

35 C. Two mice were inoculated subcutaneously with approximately 0.3 ml each of the supernatant fluid from the slurry. A guinea pig was inoculated by rubbing the slurry over abraded skin surface (percutaneous method).

Biochemical Tests

Carbohydrate fermentation tests were performed by inoculating the test organism onto slants of cystine-heart infusion agar (without blood) each containing one percent concentrations of one of the following: glucose, glycerol, levulose, maltose, or sucrose and Andrades' indicator. The slants were incubated aerobically at 35 C and observed for evidence of acid production over a period of 3 weeks.

Serologic Tests for Identification of Organisms

The antiserum for use in agglutination tests was prepared in rabbits by inoculation of *F. tularensis* strain B38 (ATCC #6223). The titer of the antiserum to the homologous strain was 1:1280. The organisms to be tested were washed from 48-72 hr growth on blood glucose cystine agar using 0.5% formalinized saline. The resulting suspension was adjusted to a No. 2 McFarland standard and used as antigen in the agglutination test. The test was performed in tubes containing 0.5 ml of serial dilutions of the serum; 0.5 ml of the antigen was added to each tube. The tests were incubated in a water bath at 37 C for 30 min and were then centrifuged for 7 min at 2000 rpm. The highest dilution giving complete agglutination was considered to be the titer.

RESULTS

The direct smears of the bone marrow stained with Gram or Wayson's stain indicated the presence of many types of bacteria. The presence of small coccobacilli were suspected, but the amount of detritus and white blood cells present in the smears made interpretation impossible. The same problem was present in interpretation of the direct immunofluorescent tests. Small numbers of fluorescing objects of a size compatible with that of *F. tularensis* organisms were observed in the direct smears of the bone marrow stained with both *F. tularensis* conjugates. These tests were considered equivocal, however, because occasional fluorescing specks occurred in the duplicate bone marrow smears stained with normal rabbit serum conjugates.

Cultures of the bone marrow directly on blood glucose cystine agar were overgrown with organisms other than *F. tularensis* and were therefore unsatisfactory.

The two mice inoculated with the bone marrow slurry were ill on the fourth day post-inoculation. One was killed and the liver and spleen were removed. Impression smears of these organs stained with Gram stain showed numerous Gram negative small cocci and coccobacilli; similar smears stained with Wayson's stain showed presence of small, poorly staining coccoid organisms. The fluorescent antibody tests on smears of the mouse liver and spleen showed numerous fluorescing organisms when either of the *F. tularensis* conjugates was used; duplicate smears stained with the normal rabbit serum conjugate were negative. Inoculation of blood glucose cystine agar with material from the liver and spleen produced growth which was positive by the *F. tularensis* fluorescent antibody tests, was agglutinated by *F. tularensis* antiserum to titer of the serum, and gave biochemical reactions compatible with *F. tularensis*; acid formation at 4 days in cystine-heart agar containing glucose or glycerol, and growth without acid formation in cystine-heart agar containing maltose, sucrose or levulose.

The guinea pig inoculated cutaneously did not show clinical signs 3 weeks later, when it was killed. Impression smears of the spleen and liver were negative by Gram and Wayson's stains and by fluorescent antibody tests.

A portion of the bone marrow specimen, which had been refrozen was thawed, and another guinea pig was inoculated subcutaneously. It was killed 2 weeks later, and impression smears of the spleen and liver also were negative by Gram and Wayson's stains and by fluorescent antibody tests.

DISCUSSION

The clinical presentation, epidemiologic history, and isolation of *F. tularensis* from the deer carcass indicate that the patient was infected by percutaneous inoculation of the hand. As far as we can determine, this is the first reported human case of tularemia acquired from a culture-proven infected deer. Although evidently a rare event, this case should serve as a warning for hunters to use reasonable precautions in butchering deer as well as rabbits, the commonly-known hosts. The deer could have been infected by fly- or tick-bite, or possibly by ingestion of contaminated water. Further study of deer as experimental hosts for *F. tularensis* infection is indicated, since little evidently has been done on this subject. Infected deer might be a source for infection of *Chrysops* or tick species, and thus help to disseminate *F. tularensis* in nature.

This case report emphasizes the need to use all available laboratory methods in studying unusual cases. *F. tularensis* could not be isolated from the patient by routine culture procedures, but the diagnosis was confirmed serologically. Apparently relatively few *F. tularensis* organisms were present in the bone marrow when it was tested, perhaps partly due to loss during storage in the freezer, or to overgrowth by other contaminating bacteria. The value of mouse inoculation as an "enrichment" technique to overcome some of these problems was evident.

We encourage others to report proven or probable cases of tularemia in deer and other unusual hosts, and thus add to our knowledge of this ecologically complex, important zoonotic disease.

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