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RODENT LEPTOSPIROSIS IN COLORADO

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Abstract: A study was conducted to evaluate leptospirosis in brown rats (*Rattus norvegicus*), muskrats (*Ondatra zibethicus*) and mice (*Mus musculus*) in southeastern Larimer County, Colorado. *Leptospira* serotype *icterohaemorrhagiae* was isolated from fourteen of 143 feral brown rats, an infection rate of 9.8%. Serological evidence of infection with this stereotype was found in 66.4% of the rats. Serological evidence of *L.* serotype *ballum* infection was present in three of 17 muskrats. Leptospires were seen in histological sections of kidney tissue from two of 61 feral mice. No isolations were made from cultures and serology was not done on mice.

INTRODUCTION

Leptospirosis is an infectious disease of the tropical and temperate parts of the world. The disease has been reported in domestic and wild animals and in man in Colorado. Diagnosis of Colorado cases has been primarily on serological evidence, demonstration of leptospires by dark field examination of tissue emulsions, or by histologic techniques. The organism has not been isolated or specifically identified in Colorado.

Roberts[5] examined 32 brown rats (*Rattus norvegicus*) trapped in eastern Larimer County, Colorado. Eight showed an antibody titer of at least 1:1,000 for *Leptospira ichterohaemorrhagiae*. Live antigens of the serotypes *L. pomona, L. canicola, L. hardjo, L. grippotyphosa* and *L. icterohaemorrhagiae* were used in the microagglutination test. No attempt was made to isolate the organism.

There has been 165 positive leptospirosis cases in domestic animals at the Colorado State University Diagnostic Laboratory between 1968 and 1975. Tests and subsequent diagnosis were based on recommendation of the attending veterinarian. Diagnosis was based on either actual visualization of the organism in tissue fluids or homogenates or by slide agglutination test using killed stained antigens. Bacterial isolation was not attempted. Evidence of leptospirosis in the above animals led to the present study which was an attempt to isolate and identify the causative organism from certain feral and wild rodents in eastern Larimer County, Colorado.

MATERIALS AND METHODS

All animals used in this study were taken with live traps. They were anesthetized with ether and the ventral surface of each animal painted with Wescodine.[1] Blood was aseptically taken by cardiac puncture. Two drops of blood were used to inoculate a tube of bovine serum albumin (BSA) semi-solid medium.[1] The remainder was placed into a sterile blood collecting tube and left to coagulate. The serum was removed and stored at —4 C for later determination of antibody titer.

Both kidneys were removed; one was cut longitudinally and fixed in 10% neutral formalin, sectioned and stained later by the silver impregnation method.[2] The second kidney was placed in a 10 ml syringe without a needle. The tissue was expressed from the syringe into 10 ml of BSA liquid medium, mixed on a vortex mixer for one min and allowed to stand for one hr at room temperature. Each tissue homogenate was serially diluted to 10^{-5} with additional liquid medium to dilute any possible contaminant. The tubes

[1] West Chemical Products, Inc., 11075 East 47th Avenue, Denver, Colorado 80202, USA.

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were allowed to stand for 10 min, then 1 ml from the top layer of each dilution was transferred to a tube of BSA semi-solid medium.

Both the semi-solid and the liquid media were incubated at 30 C. The tubes were examined the first 2 days of incubation for bacterial contamination. Those showing contamination were discarded. Contamination usually occurred in the no-dilution or 10^{-1} dilution tubes. Remaining tubes were examined weekly for one month using a dark field microscope. Isolates were identified by use of specific antisera.

Blood serum collected from each animal was subjected to a microagglutination test to detect the presence of antibodies.[6] Pooled live antigen was prepared by mixing equal volumes of 3-week-old cultures of *L.* serotype *icterohaemorrhagiae, grippotyphosa,* and *ballum.* Tubes were placed in a 30 C incubator for one hr. Results were determined microscopically by observing one drop of mixture from each tube using a dark field microscope. The same test was repeated on those sera showing positive reaction with the pooled antigens but using the above individual serotypes as live antigens. The titer was considered to be the highest dilution showing 50% agglutination when compared with the control mixture of saline and antigen.

Kidney tissue was stained by the silver impregnation method of Lavaditi[2] as modified by Parnas.[3] Sections were mounted and examined for Leptospires.

RESULTS AND DISCUSSION

A total of 221 animals were used for the study (Table 1). The animals were collected at landfill areas, around feedlots, near residential areas and in the riparian border of a river. Fourteen brown rats yielded positive culture for *L.* serotype *icterohaemorrhagiae.* Blood sera taken from 95 brown rats agglutinated *L. icterohaemorrhagiae.* Titers ranged from 1:10 to 1:10,000. Leptospira could not be cultured from 17 muskrats (*Ondatra zebithicus*) or from 61 mice (*Mus musculus*) even though agglutinins for *L.* serotype *ballum* were present in three muskrats and leptospires were seen in the kidneys of two mice.

Finding serotype *icterohaemorrhagiae* in rats was to be expected since rats are a major carrier of this serotype. The presence of this serotype in Colorado is now proven. Finding agglutinins to *ballum* in muskrats was not expected. Positive confirmation of infection by *ballum* in Colorado muskrats awaits isolation of the organism. This serotype has been isolated from muskrats elsewhere in the

TABLE 1. Frequency distribution of cultural, histological and serological reactions of three rodent species from Larimer County, Colorado.

Species	Number Examined	Culture Positive	Histology Positive	Serology Positive	Percent Positive (all procedures)
Brown Rat (*Rattus norvegicus*)	143	14[a]	15	95[b]	67.8
Muskrat (*Ondatra zibethicus*)	17	0	0	3[c]	5.7
Mouse (*Mus musculus*)	61	0	2	—[d]	3.3

[a] all isolates were *L. icterohaemorrhagiae.*
[b] all positive for *L. icterohaemorrhagiae.*
[c] all positive for *L. ballum* (titers ranged from 1:100 to 1:10,000).
[d] no blood sera taken.

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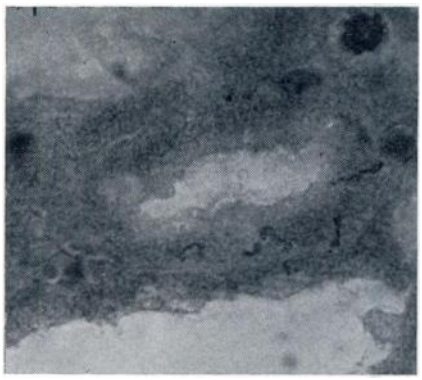

FIGURE 1. Leptospiral organisms between epithelial cells of a kidney tubule of a brown rat and stained by the silver impregnation method (3,700 X).

United States.[4] The presence of leptospires in the kidneys of mice indicates that this animal is a carrier in Colorado. Silver staining of kidney tissue was found to be a useful adjunct to serology and culture in the detection of leptospirosis (Figure 1). However, the method did not assist in identificaion of serotype. The two mice which were histologically positive were culturally negative. The variable course of leptospiral infections in animals and difficulty in culturing the organisms and interpreting serology point to the need for multiple diagnostic techniques for this disease.

As far as these authors can ascertain, the evidence presented here is the first authentic isolation of leptospiral organisms in Colorado. Improvement of diagnostic and reporting practices will reveal the significance of the disease in man, domestic animals and wild ranging animals. The evidence of high prevalence in feral rats (67.8% by all procedures) constitute a reservoir of *Leptospira* which could find its way into other animal populations of eastern Colorado. The present research shows the need for further investigation of other species of animals such as skunks and raccoons which inhabit this part of Colorado and which are known to act as *Leptospira* carriers elsewhere. The research also indicates that, logically, serotype *ballum* should be sought where mice, muskrats and brown rats are present in the same geographical area.

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