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Serological Prevalence of Tularemia in Cottontail Rabbits of Southern Illinois

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ABSTRACT: Sera of cottontail rabbits (*Sylvilagus floridanus*) collected in southern Illinois in 1983 and 1984 were screened for the presence of antibodies against *Francisella tularensis* by rapid slide agglutination and enzyme linked immunosorbent assay techniques; 6% of 118 and 16% of 119 samples were positive by these methods, respectively. Rabbits gained, lost and maintained titers over at least an 8 mo period. *Francisella tularensis tularensis* was isolated from one serologically negative, clinically healthy rabbit.

Key words: Tularemia, *Francisella tularensis*, cottontail rabbit, *Sylvilagus floridanus*, serological survey, prevalence.

Of the many infectious diseases known to affect cottontail rabbits (*Sylvilagus floridanus*), tularemia has the greatest potential to cause widespread mortality (Jacobson et al., 1978). Jellison et al. (1961) speculated that except for predation, tularemia may be the most frequent cause of death in cottontails. Few serological surveys for antibodies against *Francisella tularensis* have been conducted because early researchers (Yeatter and Thompson, 1943; Jellison et al., 1961) interpreted low prevalence of antibodies as an indication cottontails did not survive the disease.

Tularemia in humans has been reported frequently in the United States (Centers for Disease Control, 1988). In cottontails, the disease is presumed to be generally enzootic and occasionally epizootic (Jellison et al., 1961). Prior to studies of the epizootiology of tularemia in cottontails of southern Illinois (USA), we obtained baseline data on body condition, diseases and parasites (Lepitzki, 1986). Objectives of this study were to determine if there were serologic evidence of enzootic tularemia in cottontails and to determine concordance between rapid slide agglutination (RSA) and enzyme linked immunosorbent assay (ELISA) serological tests.

In 1983 and 1984, cottontail rabbits were captured near Southern Illinois University (Carbondale, Illinois, USA) and stocked in a 1.46 ha outdoor enclosure (Jackson County, 37°41′N, 89°15′W). The pen had a prior history of rabbit mortalities attributed, without confirmation, to tularemia (Yaich, 1981). Periodically, animals from the pen were collected for necropsy. Additional rabbits were collected from the road outside the pen, from Crab Orchard National Wildlife Refuge (Williamson County, Illinois, USA; 37°42′N, 89°3′W) and from Wayne Fitzgerrell State Park (Franklin and Jefferson Counties, Illinois, USA; 38°6′N, 88°57′W). The latter location experienced a major epizootic, presumably from tularemia, in summer 1980.

Live-trapped animals were transported to the laboratory, anesthetized with 40 mg/kg ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, New Jersey 07950, USA) and killed by exsanguination via cardiac puncture. Blood from animals that were shot was collected from bullet wounds and the heart. Samples were allowed to clot at room temperature and serum was separated by low speed centrifugation (3,000 rpm, 5 min), pipetted into sterile vials, and frozen (−20 C) until analysis. Commercial antigen and antibody kits (Difco Laboratories, Detroit, Michigan 48232, USA; Fisher Diagnostics, Orangeburg, New York 10962, USA) were used to detect antibodies against *F. tularensis* by RSA; the procedures of Van Kan et al. (1983) and Viljanen et al. (1983) were modified for ELISA. Titers of ≥1:80 were

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considered positive for RSA to prevent false positives due to cross-reactions with *Brucella* spp. (Eigelsbach, 1974). Even though the ELISA for *F. tularensis* did not cross-react with antibodies directed against *Brucella abortus*, titers below 1:80 were not considered positive because of non-specific reactivity.

Each rabbit was necropsied and tissues, fixed in 10% neutral buffered formalin, were sectioned using standard histological techniques and examined by light microscopy. Liver and spleen from 69 rabbits were stored frozen (−70 C) pending attempts to isolate *F. tularensis* using standard culture techniques for the organism (Eigelsbach, 1974).

Sera from 113 rabbits were tested by both RSA and ELISA; serological prevalence was 4% (5/113) and 15% (17/113), respectively. Rabbits positive by RSA were always positive by ELISA; however, the rapid slide was less sensitive and did not detect 12 sera which tested positive by ELISA. In four of five cases in which sera was positive by both tests, the ELISA titer was higher. Two of five and two of six additional rabbits tested by a single technique only also were positive resulting in an overall serological prevalence of 6% (7/118) by RSA and 16% (19/119) by ELISA. Serial blood samples of which at least one was positive were obtained from six rabbits: three lost titers (1:160 by RSA to negative by ELISA after 11 mo; 1:160 by ELISA to negative by ELISA after 8 mo; and 1:320 by ELISA to negative by ELISA after 4 mo), two gained titers (negative by RSA to 1:1,600 and 1:160 by ELISA, 4 and 6 mo later), and one maintained a 1:160 titer by ELISA over 8 mo and three blood samples.

Titers determined by ELISA were not ($P > 0.05$) influenced by season of collection (October to May versus June to September) ($Z = -0.7703$) or sex of rabbit ($Z = -1.0967$) (Wilcoxon rank-sum test; SAS Institute Inc., 1982) but were influenced by age (adult versus juvenile) ($Z = 3.591$, $P = 0.0003$) and location ($\chi^2 = 41.12$, $P = 0.0001$) (Kruskal-Wallis test, chi-square approximation; SAS Institute Inc., 1982). Adult rabbits had significantly higher ($P < 0.05$, Dunn's Multiple Comparison Procedure; Hollander and Wolfe, 1973) ELISA titers (mean rank = 69.13, $n = 46$) than juveniles (mean rank = 54.25, $n = 73$) and rabbits collected from Crab Orchard (mean rank = 103.69, $n = 8$) had significantly higher ($P < 0.05$) ELISA titers than rabbits from both the pen (mean rank = 55.38, $n = 97$) and the road (mean rank = 57.06, $n = 8$). A high mean rank score of rabbits from Crab Orchard probably reflects seven of eight rabbits collected from this site being positive by ELISA. Because all animals from Crab Orchard were adults, a significant overall age class effect was expected.

None of the animals had gross or microscopic lesions suggestive of tularemia. However, *F. tularensis* was cultured from one serologically negative animal and confirmed as *F. tularensis tularensis* or type A (Jellison et al., 1961) by the Centers for Disease Control (Atlanta, Georgia 30333, USA).

Comparisons of *F. tularensis* serological surveys are made difficult by the use of different serological tests of varying sensitivity and inconsistency in the setting of threshold titers determining significance of the tests. Prevalence of tularemia and titers of antibodies against *F. tularensis* in cottontails are usually low when determined by tube agglutination (McKeever et al., 1958; Burgdorfer et al., 1974; Jacobson et al., 1978) and fluorescent antibody tests (Andrews et al., 1980). The 16% serological prevalence detected by ELISA in our study was lower than the maximum 24% serological prevalence (during September to October) reported by Jacobson et al. (1978); however, these authors considered as positive titers that were as low as 1:5. In addition, they did not find any positive rabbits at any other time of the year; we found rabbits positive throughout the year. The highest titer of 1:2,560 by the ELISA also was well above the re-

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ported maximum titer (1:320) found in a cottontail using tube agglutination (McKeever et al., 1958).

Significance of the titers we detected is unknown. Jacobson et al. (1978) believed low titers (1:5 to >1:20) indicated prior exposure to tularemia. Demaree (1970 *in* Jacobson et al., 1978) reported titers of 1:20 in cottontails vaccinated against tularemia; these titers disappeared within 62 days. Our titers, both RSA and ELISA, tended to be higher and some persisted for much longer periods. We cannot speculate whether the high titers we found represent recent exposure or convalescence, or if rabbits with high titers would be immune to challenge.

We interpret the 16% prevalence of rabbits possessing antibodies as evidence of enzootic tularemia in rabbit populations from three discrete areas of southern Illinois. Also, the data suggest tularemia is not always fatal. This assumption is supported by the isolation of *F. tularensis* type A tularemia from a normal rabbit, albeit we may have killed the animal prior to onset of disease.

Ralph Harnishfeger collected blood and did some of the early necropsies. Brenda Bunn assisted with most of the remaining rabbit collections and necropsies. Meir Lev (Department of Microbiology, Southern Illinois University, Carbondale, Illinois 62901, USA) cultured frozen livers and spleens for tularemia. Technical aspects of ELISA were performed by Kay Elliot (Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield, Illinois 62708, USA). This project was a Federal Aid Study of the Illinois Statewide Wildlife Surveys and Investigations W-49-R(SI)-30 through 32, with the Illinois Department of Conservation and the Cooperative Wildlife Research Laboratory, Southern Illinois University at Carbondale, cooperating.

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BOOK REVIEW . . .

La Brucelosis de los animales en América y su relación con la infección humana, Casimiro García-Carillo. Office International des Epizooties, 12, rue de Prony, 75017 Paris, France. 1987. 303 pp.

The book is a compilation of factual data on brucellosis in the Americas. The largest amount of material presented includes information on the occurrence of this infection in domestic animals, wild mammals, and its impact on man in this continent. Additional information on the economical aspect in terms of animal health losses and medical expenses incurred during treatment of infected people on a country to country basis also is presented.

When it comes to control of brucellosis, it is encouraging to know that control programs are underway in most countries. Canada is one of the few countries which enjoys a brucellosis-free status. It is projected that in the near future, as a consequence of ongoing control programs, United States, Cuba, and Jamaica will be free of brucellosis as well. The efficacy of the control of brucellosis in Latin America, however, is continuously hampered because of the prevailing financial difficulties and the inherent political instability.

The positive role of the Panamerican Center for Zoonoses in Argentina in the control of brucellosis, particularly in Latin America, is emphasized. This Center is responsible for the production, standardization, and supply of brucella antigens to Latin American countries. It is also the reference center for the identification and characterization of new brucella isolates.

Due to the nature and importance of the topic under discussion, and since the common target is the elimination of brucellosis from the New World, it would have been desirable to include a section on control of brucellosis in Latin America. I am referring to the need for an outline of the basic technical principles for a successful control program taking into consideration the unique existing situations among Latin American countries. Here again, I see a unique opportunity for a direct participation of the Panamerican Center for Zoonoses. Admittedly, control programs of this nature cannot work without both realistic financial and technical support.

The second part of the book deals with a general overview of brucellosis in the Americas. It summarizes the occurrence of brucellosis by animal species, country or region, and whenever possible, the identification of the brucella species involved is documented. Taking as an example, the estimated annual losses from bovine brucellosis for several Latin American countries are presented. Although this information is not current, one can appreciate the negative implications of these losses on the already battered economies of those countries. Regarding the epidemiology of the disease, a direct correlation is seen between the occurrence of brucellosis in livestock in the Americas and the rate of this infection in man.

The illustrations used in the form of maps or tables are appropriate, and are easy to follow and understand. Except for a few typographical errors, the same applies to the illustrations in the first part of the book.

In summary, the author should be complimented for taking the initiative in writing this book. The search for information must have been a difficult task in itself if we consider that in several instances he had to resort to government files. Reading the book, I found a wealth of information of interest to the veterinary, medical, and related biomedical sciences. For example, I found it very interesting to know that the distribution of *Brucella suis* in the swine population of the Americas is nearly as wide as that of *Brucella abortus* in cattle. With all the implications that this pathogen has for human health, it also is interesting and important to know that certain *Brucella suis* isolates from Argentina and Colombia have unique and different characteristics from the brucella biotypes so far recognized.

I recommend the book to all of those presently involved with the control of brucellosis in the Americas.

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