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Characterization of *Erysipelothrix rhusiopathiae* Isolated from an Opossum (*Didelphis virginiana*) with Septicemia

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ABSTRACT: *Erysipelothrix rhusiopathiae* was isolated from a wild-caught opossum (*Didelphis virginiana*). The opossum was quarantined in isolation and removed from contact with other animals. After a 2-mo period it was found dead in its cage, and presented for postmortem examination. Pure cultures of *Erysipelothrix rhusiopathiae* (SLU isolate) were recovered from heart blood, liver, spleen and lungs. To compare pathogenicity, an experimental infection was attempted in CF1 mice with a single dose of 1.5×10^7 organisms of both an ATCC standard strain and SLU isolate of *E. rhusiopathiae*. Similar signs, lesions and results of culture were found for both strains. The findings suggest that opossums can be infected with *E. rhusiopathiae*.

Key words: *Erysipelothrix rhusiopathiae*, opossum, *Didelphis virginiana*, septicemia, identification, case history, experimental infection.

Erysipelothrix rhusiopathiae is primarily a pathogen of swine and produces the disease swine erysipelas. Infections in swine and other animals may be acute or chronic, and include septicemia, endocarditis, arthritis and cutaneous infections (Wood, 1984; Weaver, 1985). It is pathogenic for humans also (Steele, 1979). In humans, the most common form of infection is an inflammatory lesion of the skin which usually involves the hands and fingers, although septicemia and endocarditis have occurred (Steele, 1979). Acquisition of *E. rhusiopathiae* by humans is usually the result of contact with tissue of infected animals or contaminated animal products. This report describes the recovery of *E. rhusiopathiae* from an adult male, wild-caught opossum (*Didelphis virginiana*).

The adult male opossum was obtained from an undefined location within a five county study area of eastern Missouri, USA (primarily St. Charles and St. Louis Counties) under a wildlife collector's permit issued by the Missouri Department of Conservation (St. Louis, Missouri 63117, USA). On arrival at St. Louis University (St. Louis, Missouri 63104, USA), the opossum was placed in quarantine in an isolation cubicle in a stainless steel, 61- × 61- × 18.5-cm rack mounted cage and given free access to dry dog and cat food (Purina, St. Louis, Missouri 63166, USA) and water. The opossum was housed separately from other animal species. About 2 mo after arrival at the facility, the opossum was found dead. No previous clinical signs of illness had been observed. The opossum appeared to be in good condition with adequate body fat. The gross necropsy findings included splenomegaly, mottled liver and pale kidneys. Histologically, the spleen, liver and lungs were severely congested. Moderate focal degeneration of hepatocytes and few focal renal hemorrhages were noted also. Inflammation was minimal. Specimens were taken from heart blood, lungs, gallbladder, spleen and small and large intestines, and directly plated onto sheep blood agar and trypticase soy broth (TSB; Regional Media Laboratories, Lenexa, Kansas 66215, USA). Cultures were incubated aerobically and anaerobically at 37 C for 24 hr.

Erysipelothrix rhusiopathiae was isolated in moderate numbers (30 to 300 colony forming units) from the heart blood, liver, spleen and lungs. On sheep blood agar, the isolates were convex, circular, small and exhibited a greenish tinge which was identified as slight alpha hemolysis. The organism also was recovered in TSB from the liver, spleen and lungs. Growth in TSB was profuse. The organism grew equally well under anaerobic and aerobic conditions. Examination of gram stained preparations with a light microscope revealed small, gram positive rods with a

centrally located body in a majority of the cells; these bodies, presumed to be storage vacuoles, disappeared upon subculturing of the bacteria. The bacteria were plated onto sporulation media (Regional Media Laboratories) at 37 C for 7 days to induce spore formation. The bacteria failed to yield spores in 7 days. The organism was catalase negative; non-motile; did not reduce nitrate to nitrites or free nitrogen gas; was negative for mannitol, xylose, sucrose, and maltose fermentation and fermented glucose and lactose. It produced H_2S in the butt of a triple sugar iron (TSI; Regional Media Laboratories) medium tube, and made acid on both glucose and lactose in the TSI basal medium. Thus, this isolate had all the morphologic and biochemical characteristics of *E. rhusiopathiae* (Carter, 1984; Weaver, 1985).

The biochemical characteristics of our isolate (SLU) were compared to the American Type Culture Collection (ATCC; Rockville, Maryland 20852, USA) type strain (19414). Our isolate had nearly identical morphologic characteristics and biochemical reactions when compared to the ATCC type strain; however, small differences were noted. For example, when equal concentrations of organisms of ATCC strain and SLU isolate were inoculated into individual TSI slants, slightly greater H_2S production was noted with the SLU isolate. The Missouri State Department of Public Health Bacteriology Laboratory (Jefferson City, Missouri 65102, USA) and the USDA National Animal Disease Center (Ames, Iowa 50010, USA) confirmed our isolate as *E. rhusiopathiae*.

Serotyping the organism was attempted at the National Animal Disease Center. The isolate did not belong to any of the 22 published serotypes, but it did possess a serotype antigen identical to an isolate received from Copenhagen, Denmark. The isolate had been recovered from pig manure (Richard Wood, pers. comm.).

Antibiotic susceptibility was identical for both the SLU isolate and ATCC type strain. To augment our identification and to assess and compare pathogenicity in a classic model, we inoculated CF1 mice (SASCO, Inc., Omaha, Nebraska 63101, USA) intraperitoneally with both ATCC and SLU strains. The bacteria were grown to approximately 1.5×10^8 organism/ml. Three mice in one group were inoculated with 3 to 6×10^7 ATCC organisms and a second group of three mice was similarly inoculated with the SLU isolate. An additional two mice received similar volumes of normal saline as controls. All mice inoculated with *E. rhusiopathiae* exhibited signs of illness within 24 hr postinoculation, including labored breathing, lethargy and pasty feces. The mice were killed with carbon dioxide gas for postmortem examination. Liver necrosis was the primary pathologic finding. *Erysipelothrix rhusiopathiae* was recovered from the heart blood and liver of all SLU infected mice and from two of the ATCC inoculated mice. The control animals remained healthy, showed no gross lesions and were negative with culture for pathogenic bacteria.

The experiment was repeated in an additional eight mice using subcutaneous inoculation and a single concentration of ATCC or SLU strain of 1.5×10^7 organisms. Clinical signs and results of culture were similar to the first study. However, signs of disease did not appear until 72 hr postinoculation. Both strains inoculated either subcutaneously or intraperitoneally produced similar signs, lesions and cultures in mice.

Our studies show that the SLU organism, originally isolated from a wild caught opossum, was morphologically and biochemically similar to the ATCC type strain of *E. rhusiopathiae*. It was equally pathogenic to mice by both subcutaneous and intraperitoneal inoculation, but it did not cross-react with any known serotype. In our opossum the lack of classical lesions of erysipelas such as arthritis, endocarditis and cutaneous necrosis is not surprising. A review of the literature by Wood and Shuman (1981) indicated that no specific signs or lesions are associated with *E. rhusio-*

pathiae in many wild mammals. They concluded that signs and lesions are representative of a septicemia; a pathognomonic lesion was not observed. It is not certain how this disease is transmitted in nature. However the organism has been recovered from soil, water, dead water rats, fecal droppings and insects, indicating that *E. rhusiopathiae* is common in the environment. Ingestion of the organism or wound contamination are the most likely modes of transmission (Wood and Shuman, 1981). Because of omnivorous feeding habits, the chance of the opossum consuming food contaminated with *E. rhusiopathiae* is perhaps greater than it would be for many other wild animals. In addition, the opossum's wide range of habitat (both urban and rural) raises the question of their role as potential reservoirs of the disease for both livestock and humans. We have initiated studies of the pathogenesis of *E. rhusiopathiae* infection to gain a better understanding of the disease and its epidemiological implications in opossums.

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