Experimental Studies on Bovine Q Fever

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In studies of early outbreaks of Q fever in Australia and Europe, various sources of infection were suggested, but the epidemiology remained somewhat obscure. Australian cases were associated with contact with cattle; however, natural infection in cattle was not demonstrated with certainty (1-3). In the United States, an outbreak occurred in 1946 among slaughterhouse employees in Amarillo, Tex. A single shipment of cattle was presumed to be the source of infection in this outbreak, but again, the naturally infected source could not be identified. In the spring of 1947, observations by Young (4) provided evidence of an endemic focus of infection in southern California. He observed cases of Q fever, diagnosed clinically and later confirmed serologically, following arid seasons and dust storms. A brief field study by Shepard and Huebner (5) disclosed additional cases, and furthermore, serologic evidence of infection in dairy cattle was found.

Through concerted efforts of the Public Health Service, the California State Departments of Health and Agriculture, and the Los Angeles City and County Health Departments, an extensive study of Q fever in the Los Angeles area was undertaken. One of the first findings of this group was the isolation of *Coxiella burnetii* from the milk of a high percentage of dairy cattle in the area (6). Thus, for the first time, the causative agent of Q fever was isolated from cattle with which human cases had had either direct or indirect association.

After the discovery of natural infections among dairy cattle, it became of prime importance to obtain a complete knowledge of the nature of Q fever infection in this species. Infected dairy cattle probably had been examined on many occasions by practicing veterinarians, yet they were unaware that a new disease was present. The carcasses of infected cattle had been processed through local packing plants, yet veterinary inspectors had observed no unusual pathology during their post-mortem examinations. Thus, a basic knowledge of the symptomatology and pathology of this disease was desirable. As a prerequisite to eventual control, the pathogenesis and epizootiology of the disease must be well understood. Some of the aspects of this disease possibly could be investigated in the endemic area; however, because of the adverse effect on operational economy, most dairymen were reluctant to cooperate on well controlled studies. Furthermore, observations on the experimental disease in cattle would provide useful leads in such field investigations as were possible to conduct. Hence, controlled experimental studies in the laboratory were necessary to obtain a thorough knowledge of bovine Q fever.

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EXPERIMENTAL STUDIES IN CATTLE

Experimental studies of Q fever in cattle were initiated by Parker and associates (7-9) in mid-1947 at the Rocky Mountain Laboratory, Hamilton, Mont. The first attempt to infect calves involved four heifers inoculated intravenously, intranasally, intravaginally, and by feeding contaminated bran, respectively. Large doses were employed, yet all four calves were refractive to infection.

After *C. burnetii* was recovered from milk of naturally infected cows in California, an attempt was made to infect two lactating cows by inoculation via the teat canal or by injecting *C. burnetii* into the mammary gland substance. Inoculated quarters of these cows continued to shed rickettsiae for extended periods, varying from 17 days to 200 days after inoculation.

A nonpregnant lactating cow became infected after introduction of a mixture of a yolk-sac culture of *C. burnetii* and semen (artificial insemination) into the cervical canal. The milk of a single quarter became infectious and remained so for more than 400 days. Since the urine of this cow was infectious for a brief period after inoculation, it was postulated, at that time, that infection may have occurred through contamination of the teat orifice with infective urine. These were the first successful attempts to infect lactating cows.

The symptomatology of the experimental disease in cattle has varied considerably, depending upon the dosage and strain of *C. burnetii*, and upon the route of inoculation. Cows inoculated with the Nine Mile strain of *C. burnetii* via the teat canal

manifested no clinical symptoms, whereas cows inoculated by the same route with a comparable dosage of a California strain developed clinical symptoms of brief duration, characterized chiefly by mastitis, fever, depression, and anorexia. Cows and calves inoculated intradermally with either the Nine Mile strain or a California strain also developed clinical illness. Lactating cows which became infected after genital exposure or through inhalation of *C. burnetii* manifested no clinical illness. In all probability, the natural disease in cattle is not associated with any marked symptomatology.

The pathology of the disease was studied in four normal lactating cows inoculated with a California strain of *C. burnetii* via the teat canal, and sacrificed at varying intervals after inoculation. Gross lesions at autopsy were limited chiefly to the mammary gland and regional lymph nodes. Inoculated quarters of the cow sacrificed during the acute phase of infection were very edematous with heavy serous accumulations in the subcutaneous tissue of the udder. A marked serous lymphadenitis was noted in the regional lymph nodes receiving lymph drainage from the inoculated quarters. In cows sacrificed during the chronic phase, only a serous lymphadenitis of lymph nodes receiving lymph drainage from infected quarters was observed. Histopathologically, a subacute interstitial mastitis was the most consistent finding.

In early studies, certain experimental data suggested that Q fever in cattle was a localized infection of the mammary gland, the rickettsiae gaining entrance via the teat canal. Thus, the milking process could be an important factor in its spread. To test this thesis, five cows with normal mammary glands were repeatedly exposed by dipping their teats in infectious milk both before and after milking (hand milking). During and subsequent to the 9-month period of exposure, frequent tests of milk for infectiousness revealed essentially no evidence of infection. Furthermore, there was no serologic evidence that any of the five cows had become infected. These five cows were then exposed in similar fashion to milk containing both *C. burnetii* and *Streptococcus agalactiae* for a period of 4½ months. This was done to ascertain whether concurrent bacterial mastitis predisposed cattle to Q fever infection, and also to determine whether these cows would contract streptococcal mastitis, a disease whose portal of entry is the teat canal. Three animals acquired streptococcal mastitis, whereas none contracted Q fever. On the basis of this experiment, it appears that the process of hand milking is not an important factor in the cow-to-cow spread of Q fever.

In view of the negative results obtained in the preceding experiment, other probable routes of infection were explored. Two lactating cows were exposed by supraconjunctival instillation of 0.1 ml. of a 10^{-4} dilution of a yolk-sac culture of *C. burnetii* (California strain). After such exposure and an additional one given 61 days later, essentially no evidence of infection was demonstrated in either cow.

The spinose ear tick, *Otobius megnini* Duge, is indigenous to southwestern United States and therefore includes both endemic areas in Texas and California. Jellison, *et al.* (10) reported the isolation of Q fever rickettsiae from ticks of this species collected from cattle on infected dairies in southern California. Although the role of this tick in the epizootiology of Q fever has not been thoroughly investigated, laboratory attempts to transmit Q fever to cattle with this tick have been essentially negative. Of four cows which were hosts to the infected nymphs or larvae which were progeny of infected female ticks, only one developed specific complement-fixing antibodies; however, *C. burnetii* was not recovered from either milk or blood of this cow.

The dermis of cattle readily supports the growth of *C. burnetii*. This was first demonstrated in two bull calves inoculated intradermally with 1 ml. of a 10 percent yolk-sac culture. These calves experienced a febrile reaction and a rickettsemia of brief duration, and local skin lesions were produced at the site of inoculation. Similar results were obtained in a lactating pregnant cow exposed by the same route. Other observations on this cow included the following: (a) abortion occurred the 7th day after inoculation and *C. burnetii* were recovered from the placenta, (b) the milk of all quarters contained Q fever rickettsiae for about 2 weeks, and (c) the urine, particularly during the 3d week after abortion, was infectious. The presence of *C. burnetii* in the urine could represent actual elimination of the organism in the urine or contamination with postparturient uterine discharges.

Since the air-borne spread of Q fever rickettsiae was suggested in previous outbreaks of Q fever, it was desirable to determine whether cattle could be infected by inhalation of *C. burnetii*. In the first attempt, a pregnant lactating cow was exposed