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Isolation of *Leptospira grippotyphosa* from a Cow Following an Abortion. (29618)

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Serologic evidence of *Leptospira grippotyphosa* has been found in cattle in the United States over a number of years but without bacteriologic confirmation. Some cattle with positive sera have shown signs suggestive of *Leptospira pomona* infection, manifested by abortions, weak calves, and reduced milk flow, while other individuals have not exhibited any signs of disease(5,14).

Various serologic studies(4,11,13,14,17,19, 21,24) have indicated the presence of *L. grippotyphosa* antibodies in swine and wild animals as well as cattle. Deer sera obtained in Illinois between 1958 and 1960 had a reactor rate of 6% for *L. grippotyphosa* antibodies(8).

Aseptic meningitis in a man caused by *L. grippotyphosa* was reported in Illinois in 1953(2). The clinical diagnosis was confirmed by serologic studies. A year earlier Spain and Howard(23) reported a case of hepatitis in man caused by *L. grippotyphosa,* also serologically confirmed. A similar case was reported in 1954(12).

Several isolations of *L. grippotyphosa* have been made from wild animals in the United States(13,17,21). Clark *et al*(5) isolated *L. grippotyphosa* from voles on a Pennsylvania farm where several cattle developed antibodies following abortions.

Michin and Azinow(18) were the first to describe outbreaks of bovine leptospirosis due to *L. grippotyphosa* which occurred in the U.S.S.R. The disease varies from severe forms causing death of adult cattle to subclinical forms detectable only serologically(9).

Materials and methods. Abortions involving 2 cows of a dairy herd followed by development of *L. grippotyphosa* antibodies prompted collection of urine 7 days after the abortions. The animals at that time had agglutination-lysis titers of 1:100 and 1:1000. No signs other than abortion were observed in the cows.

Guinea pigs weighing 200 g and young weaned hamsters were intraperitoneally inoculated with ½ ml each of undiluted bovine urine. Gerbils(22) and white mice were used later for further determination of host susceptibility.

Rectal temperatures of the guinea pigs were recorded daily and their blood was taken, cultured either at the time of the first temperature rise or in the absence of a temperature rise at 7 days. Blood samples were obtained by heart puncture from the gerbils, white mice and hamsters 4 to 7 days after inoculation and cultured. Serum samples were obtained from all surviving animals approximately 3 weeks after inoculation for detection of antibodies. The agglutination-lysis test was used to detect *L. pomona, L. grippotyphosa, L. sejroe,* and *L. canicola* antibodies.

Portions of liver, spleen and kidney tissues, obtained from the experimental animals at necropsy, were ground in mortars and cultured. The media used in this study were Fletcher's semisolid and Stuart's liquid media enriched with 5, 10 and 15% rabbit serum and experimental media containing bovine albumin and oleic acid(7) obtained from the National Animal Disease Laboratory. Por-

tions of kidneys were also fixed in 10% formalin and stained with the Levaditi's silver technique.

Results. One hamster inoculated with urine from cow B699 died 7 days following inoculation and its kidneys were cultured in Stuart's medium. Leptospira organisms mixed with other bacteria were first detected in the Stuart's medium by darkfield examination 2 months later. The contaminating bacteria were eliminated through inoculation of hamsters, and the leptospires were reisolated in culture media from heart blood taken 4 to 7 days later.

One of 2 guinea pigs inoculated with the newly isolated leptospiral culture had a 2°C temperature rise 9 days after inoculation, which persisted for 8 days. Leptospires were isolated from the blood of the other guinea pig, which had no temperature rise, 7 days postinoculation. *L. grippotyphosa* antibodies were detected in the sera of both guinea pigs 21 days after inoculation.

Hamsters receiving the culture died 4 to 7 days after inoculation. Leptospires were isolated from the blood and demonstrated in kidney sections with Levaditi's stain.

Inoculated white mice did not develop signs of infection. Leptospires were isolated from the kidneys of a mouse and observed in stained sections 28 days following inoculation. *L. grippotyphosa* antibodies were detected in their sera.

Gerbils developed signs 5 to 8 days following inoculation with the culture. Necropsies were performed when animals became moribund 1 to 2 days after onset of signs which included trembling and prostration. Leptospires were consistently isolated in culture media from kidney suspension and demonstrated in silver stained kidney sections.

During recoveries of *L. grippotyphosa* from small animals it became apparent that little if any growth was occurring in conventional serum enriched media. With serial transfer, organisms gradually diminished in numbers and it appeared that the organisms could only be maintained by *in vivo* animal passage. However, a number of recently developed semisolid and liquid bovine albumin, sodium oleate and Tween-80 media proved adequate for *in vitro* culture(7).

TABLE I. *Leptospira grippotyphosa* Antibody Reactor Rate of Illinois Cattle and Swine Sera.*

Year	No. cattle sera tested	% Positive	No. swine sera tested	% Positive
1959	473	2.95	114	0
1960	782	4.98	251	3.58
1961	926	4.80	240	3.20
1962	1431	2.58	707	1.41
1963	914	4.15	845	1.06

* Sera obtained from animals with signs suggestive of *L. pomona* infections.

The extent of the disease in Illinois is suggested by the results of testing swine and cattle sera from animals showing signs suggestive of *L. pomona* infection, but not reacting to *L. pomona* from 1959 through 1963 (Table I).

Discussion. Gerbils and hamsters were found to be the most susceptible laboratory animals studied for the *L. grippotyphosa* isolate. Inoculation of either species with cultures or tissue suspensions repeatedly resulted in clinical signs occurring 5 to 8 days after inoculation followed by death 1 to 2 days later. Guinea pigs and white mice showed little evidence of disease but developed antibodies and leptospiruria which persisted as long as 21 days. The relatively low susceptibility of guinea pigs for the new isolate suggests one reason why previous isolation attempts have been unsuccessful.

The difficulties encountered in propagating the newly isolated organism in routinely used media suggest another cause for previous isolation failures. Inhibitory substances in the serum enriched media or absence of critical nutrients or both may be responsible for lack of growth. Noguchi(20) as long ago as 1918 suggested that 10% serum was optimal for growth. Fukushima(10) observed growth inhibition at serum concentrations of 15, 20 and 30%. Similar observations were made by Chang(3). Helprin and Hiatt(15) found that serum concentrations greater than 6% were inhibitory to respiration of *Leptospira icterohaemorrhagiae*. Kraminskaia(16) using recommended media in Leptospira growth studies found periods when almost complete cessation of multiplication occurred. Elling-

hausen(6) observed that the growth response of *L. pomona* to increasing concentrations of rabbit serum was linear from 0 to 8% but the linearity sharply disappeared at 10, 12 and 14%. Alexander *et al*(1) describing attempts to cultivate a new fastidious pathogenic leptospire, *Leptospira naam,* subserotype *dakotii,* reviewed the difficulties encountered in obtaining adequate growth in liquid media. With *L. naam,* concentrations of 10 to 30 $\times$ 10^6 organisms/ml were maximal and could only be achieved in conventional liquid media after a 14-day incubation period.

In view of the success of this study with new semisolid and liquid bovine albumin Tween-80 media(7), it is suggested that the medium be utilized for isolation of leptospiral organisms.

Summary. L. grippotyphosa was isolated from the urine of a cow 7 days after abortion. The isolant grew poorly in Stuart's liquid medium and Fletcher's semisolid medium. Experimental semisolid and liquid media containing bovine albumin fraction V and Tween-80 proved valuable as isolation and growth media. Gerbils and hamsters were more susceptible than guinea pigs and white mice to the newly isolated organism.

Serologic evidence indicates *L. grippotyphosa* is widely distributed in Illinois cattle and swine.

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