

Control of Bovine Leptospirosis*

Lyle E. Hanson, D. V.M., Ph.D.
 Department of Pathology and Hygiene
 College of Veterinary Medicine
 University of Illinois, Urbana, Illinois

Introduction

Leptospirosis in the United States is an important cause of cattle losses. The disease may be severe or so mild it can be detected only with serologic tests. In recent years, studies indicate leptospirosis is widespread in the United States and is caused by a number of antigenically distinct serotypes which are often reservoirized in wildlife.

Leptospiral serotypes in cattle. — In the United States, five serotypes have been isolated from cattle (Table 1) (1,2,11,13,14). Studies in Illinois

TABLE 1
 Leptospires Isolated in the United States

Serotype	Hosts Isolated From	
	Domestic Animals	Wildlife
<i>L. icterohaemorrhagiae</i>	Swine, cattle	Norway rat, raccoon, skunk, opossum, muskrat, red fox, grey fox
<i>L. pomona</i>	Swine, cattle, sheep, horses, dog, cat	Skunk, wildcat, raccoon, opossum, woodchuck, red fox, deer, armadillo
<i>L. grippityphosa</i>	Swine, cattle	Raccoon, opossum, muskrat, fox squirrel, red fox, grey fox, mole, skunk
<i>L. hardjo</i>	Cattle	None
<i>L. canicola</i>	Swine, cattle, dog	Skunk, raccoon, armadillo

have resulted in isolations of *L. pomona*, *L. hardjo*, *L. grippityphosa* and *L. icterohaemorrhagiae* (2,3,7,13). *L. canicola*, although it has not been isolated from Illinois cattle, has been recovered from swine in Illinois and from cattle in the southeastern United States (12). Serologic tests also indicate antibodies for additional serotypes are present in many cattle.

A comprehensive study of one farm in northern Illinois over a five-year period resulted in isolations of *grippityphosa*, *icterohaemorrhagiae* and *hardjo* serotypes from the cattle and *icterohaemorrhagiae* serotype from the swine (13). *Grippityphosa*, *ballum* and *icterohaemorrhagiae* serotypes were isolated from wildlife on the same farm. A total of 264 isolations was made from 1161 wildlife. This

provides an indication of the extent of the distribution of pathogenic leptospire in domestic animals and wildlife on some farms.

Acute leptospirosis occurs after an incubation period of 10 to 20 days depending on the serotype and severity of exposure. The first signs are rise in body temperature, hemoglobinuria and agalactiae. In mild infections, the signs are often not recognized. In severe cases, depression, anemia, weakness, jaundice, convulsions and death may occur. Recent studies indicate the acute signs may be the result of the production of cytotoxins (16).

Fetal losses, resulting from abortion and stillbirths, occur two to four weeks after the onset of disease. The losses are usually observed in the last third of the gestation period but occasionally occur in the first third. Some infected cows give birth to live but weak calves. Some of these calves die during the first weeks of life and others live but have retarded growth.

Although the serotypes isolated from cattle vary in virulence, a wide degree of severity in pathogenicity may occur with infections due to the various serotypes. In the United States *pomona* and *grippityphosa* generally are the most pathogenic serotypes as acute signs are most often associated with these leptospire. *Hardjo*, apparently the most widely distributed serotype in many areas in the United States, is less often associated with serious fetal losses. However, *L. hardjo* is probably the most costly leptospiral serotype, as it has been associated with long-term herd infections on many farms. The herd histories indicate *L. hardjo* infections occur year after year, and occasional abortions result from the newly infected cattle. Recently, a *L. hardjo* infection in an Illinois herd resulted in acute signs of high fever, jaundice and depression. Although *L. icterohaemorrhagiae* and *L. canicola* infections in Illinois have been mild in Illinois cattle, reports from southern states and foreign countries indicate both of these serotypes are capable of causing severe infections.

The financial loss to the cattle owner results from fetal deaths, lost breeding periods, milk

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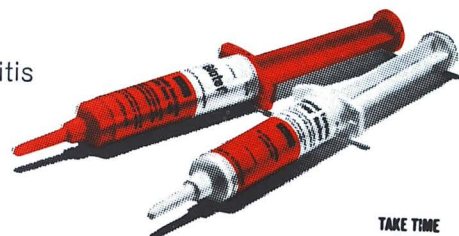
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reduction, retarded growth and occasionally deaths of adult cattle. The total cost is much larger than is usually apparent. A study of a recent outbreak in one Illinois dairy herd resulted in a milk reduction of 75% for a 10-day period (9). The death of four to five potential calves, agalactiae and other associated losses can cost an owner \$500 to \$1,000. An estimate of the cost of leptospirosis to Illinois cattle owners based upon reactor rates would indicate an annual loss of more than one million dollars.

Control measures. — Eradication of leptospirosis is not feasible because of the extensive reservoir in wildlife (Table 1). The wildlife hosts include deer, skunks, raccoons, opossums, house and field mice, rats, muskrats, bobcats, squirrels, woodchucks and foxes (12,13). An example of the incidence of wildlife infections is shown by a 22% isolation rate of leptospores from 1300 wildlife hosts collected during a period of five years on one Illinois farm (13). The most important wildlife reservoir hosts in the Midwest appear to be raccoons, skunks, opossums, rats and mice.

Control with antibiotics has been effective during the period of administration. Treatment of acute infections is indicated but must be used with the realization that residues will occur in the milk. Use of antibiotics as a prophylactic procedure has been effective but also will cause residue problems. Dihydrostreptomycin appears to be the most effective antibiotic, as effective concentrations result in both the kidneys and in the uterus.

Control of leptospirosis with bacterins provides the most effective procedure currently available. Bacterins stimulate the host to develop primarily neutralizing antibodies (IgG) detectable with hamster protection tests and only mildly stimulate the IgM antibody which can be measured with the microscopic agglutination test (6,8). Therefore, vaccination provides protection but does not interfere with field testing programs. Present information indicates the five serotypes isolated from U.S. cattle do not induce cross-immunity. Therefore, bacterins used in an area should contain the serotypes prevalent in that area. The Biologics Division of ARS has given approval for production of a polyvalent bacterin containing *pomona*, *grippotyphosa* and *hardjo* antigens for cattle and swine (15). Bacterins containing *canicola* and *icterohaemorrhagiae* antigens are currently available.

Materials and Methods

Cattle sera received from practicing veterinarians

in Illinois were tested for *pomona*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *canicola* serotype antibodies utilizing the microscopic agglutination (MA) test. The MA test was conducted according to the procedure previously described (10). Sera causing agglutination reactions of 50% or greater in dilutions of 1:100 or greater were regarded as positive.

Vaccination studies were conducted in a herd of approximately 900 purebred and grade Hereford cattle. The herd is located at the Dixon Springs Agricultural Center in southern Illinois. Serologic samples are collected each fall when the entire herd is assembled for grading and for pregnancy examinations. Serologic tests demonstrated that both *pomona* and *hardjo* serotypes were endemic in the herd since 1959. One-half of the herd was vaccinated twice a year from 1959 to 1964, and all the herd has been vaccinated once a year since 1964 with a commercial* *L. pomona* bacterin. Vaccination with an experimental** *L. hardjo* bacterin was started in 1967. One-half of the herd has been vaccinated each year (5).

The reactor rate in each yearly age group of cattle was determined and compared to determine the accumulative effect of yearly vaccination. This procedure was utilized to evaluate the response in animals vaccinated both following and prior to exposure in an endemic environment. The evaluation, utilizing herd reactor rates, contained serologic responses which occurred prior to vaccination and persisted for several years.

Results

The reactor rates in 1970 indicated *L. hardjo* was the most prevalent serotype in Illinois. Sera from 13,580 cattle were tested for a *L. pomona* reactor rate of 1.78%. This is the lowest *L. pomona* reactor rate in 16 years. A total of 5,420 cattle sera were tested with four additional antigens. The reactor rate was 11.7% for *L. hardjo*, 4.2% for *L. grippotyphosa* and less than 1% for *L. icterohaemorrhagiae* and *L. canicola* (Table 2).

TABLE 2
Reactor Rates of Illinois Cattle Sera in 1970

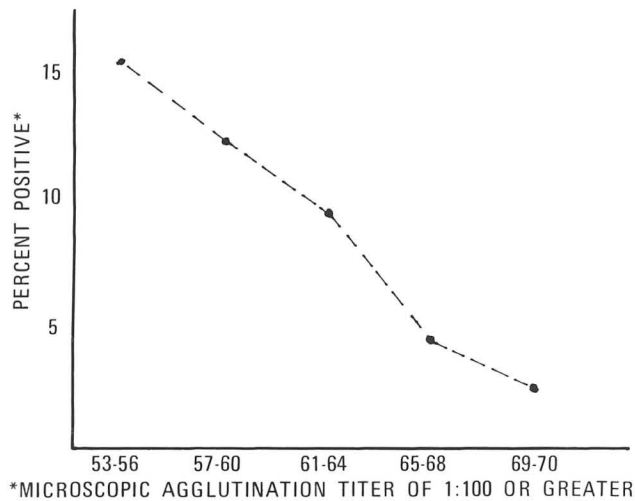
Serotype	1960	1970
<i>L. pomona</i>	9.0%	1.8%
<i>L. hardjo</i>	11.2%	11.7%
<i>L. grippotyphosa</i>	3.4%	4.2%
<i>L. icterohaemorrhagiae</i>	< 1 %	< 1 %
<i>L. canicola</i>	2.4%	< 1 %

*Fort Dodge Laboratories, Inc., Fort Dodge, Iowa

**Affiliated Laboratories Corporation, White Hall, Illinois

The constant decrease in *L. pomona* reactor rates, as shown in Figure 1, suggests an effect of vaccination. A similar pattern of a decreased *L. pomona* reactor rate has been observed in swine.

FIGURE 1
REACTOR RATES OF *L. POMONA* IN ILLINOIS CATTLE



Leptospirosis vaccination studies conducted at the Dixon Springs Agricultural Center have resulted in a significant decrease in reactor rates to *L. pomona* and *L. hardjo* serotypes. A record of the MA positive sera demonstrated a progressive reduction in reactor rates in each age group following *L. pomona* vaccination initiated in 1960. A negative response originated in the calves in 1962 and in calves and in the yearlings in 1963. The resistant population, as indicated by negative MA tests, increased progressively each year until 1969 and 1970 when no reactors were demonstrated.

A similar pattern occurred following the initial vaccination with *L. hardjo* bacterin of 50% of the herd in 1965. The second year (1967) after institution of vaccination only a few reactors were present in the calves and one-year-old animals. One year later (1968) only a few animals under three years of age were positive. In 1969 only a few reactors were present in cattle under four years of age. In 1970 all but one animal under five years of age had sera with no detectable *L. hardjo* antibodies.

Discussion

Clinical leptospirosis in United States cattle is caused by five serotypes. Four of the five serotypes have been isolated from Illinois cattle. Reactor rates from Illinois indicate that *L. hardjo* has been the most common serotype for several years. During the past 10 years, the *L. hardjo* rate has remained constant (8-10%) while the *L. pomona*

rate has significantly decreased (from 16 to 1.8%).

As extensive reservoirs of leptospiral infections exist in the wildlife in the United States, control of bovine leptospirosis is dependent primarily upon vaccination. Recent studies indicate leptospiral bacterins can induce effective resistance in cattle and swine. Studies reported in this paper indicate both commercial *L. pomona* and an experimental *L. hardjo* bacterins were highly effective in reducing the reactor rate in a large herd endemically infected with both serotypes. The findings demonstrate that field studies can provide an indication of the efficacy of a leptospiral bacterin through the evaluation of reactor rates for various age groups.

Conclusions

Five pathogenic serotypes have been isolated from United States cattle. All five serotypes have been demonstrated to be capable of causing disease. Four of the five serotypes have been isolated from Illinois cattle.

Leptospirosis infections cause serious economic losses in the cattle industry due to fetal deaths, milk loss, slow weight gains and deaths of calves and cows.

Wildlife of the United States are frequently infected with leptospires and play a major role in the dissemination of several serotypes. In Illinois, leptospires have been isolated from skunks, raccoons, opossums, squirrels, muskrats, Norway rats, and mice.

Transmission may occur by skin contact with urine or infested surface waters, coitus and through the food chain.

Control of leptospirosis is most effective through annual vaccination with serotypes prevalent in the area. Vaccination provides a relatively inexpensive method of control without interfering with currently used diagnostic tests.

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animals had only 12% to 28% of the normal level of Factor VII (Table 3).

Table 3
Clotting Factor Assays on Ten Normal Cattle*

	Factor II	Factor VII	Factor X
Cow 1	36%	18%	50%
Cow 2	32	12	40
Cow 3	35	24	60
Cow 4	44	20	70
Cow 5	41	20	76
Cow 6	35	16	44
Cow 7	35	22	68
Cow 8	32	14	60
Cow 9	58	28	60
Cow 10	42	21	59

*Percentage values were based on percent of normal human values found in plasma.

Because the bleeding syndrome appeared to be Vitamin K related, the original group of free-bleeders were fed 50 mgm. Menadione per day for three weeks. Factor VII assays were then determined and were found to have risen to a range of 37-48% of normal (Table 4).

Table 4
Factor VII Levels—Percent of Normal

Animal No.	Pretreatment	Post-Treatment
1	15	37
2	14	48
3	15	45
4	14	39
5	14	40
6	12	41
7	9	39

Treatment - 50 mgm. head/day/30 days

Silage samples were submitted to the laboratory of one of the authors (R.R.D.) for mycological and mycotoxin examination. *Fusarium trincinctum* was isolated with relative ease. Acetone extracts of the *Fusarium* species were assayed for antibiotic activity using the method of Burmeister and Hesseltine (1). A T₂-like mycotoxin was identified using this biological assay.

Discussion

The tendency for corn silage-fed animals to bleed freely has been a clinical observation by many veterinarians for many years. The exact nature of the syndrome and the pathogenesis have not been determined. Recently Osweiler and his group (2) have studied a porcine hemorrhagic disease. Their findings indicate the experimental disease is characterized by prolonged prothrombin time with Factor X deficiency and an associated Factor VII deficiency. These data suggest a Vitamin K deficiency. From the data presented here it would appear that this bovine hemorrhagic syndrome is at least related to a vitamin K

deficiency, whether it be a simple deficiency, an antagonist, or interference with the liver's synthesis of Factor VII is unknown. With the unidentified mycotoxin, we were able to show growth inhibition to *E. coli* and numerous *Sarcina* species. Gustafsson (3) has shown that substitution of *E. coli* or *Sarcina*-like microorganisms completely reverses the vitamin K deficiency symptoms produced in germ-free rats. It is possible that these silage-fed animals become depleted in the vitamin K dependent plasma clotting factors due to the presence of this unidentified mycotoxin. If this is the case, it is not clear why Factor VII appears to be the most severely affected of the three vitamin K dependent factors.

Summary

A surgically induced hemorrhagic syndrome of feedlot cattle is described. The syndrome appears to be related to a deficiency of one of the vitamin K dependent factors, especially Factor VII. Oral medication with Menadione appeared to be effective as a means of prevention.

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