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# ***Leptospira interrogans* serovars *hardjo* and *pomona*: Causes of Infections in Dairy Cows and Humans in Florida**

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LEPTOSPIROSIS, although uncommon in human beings, occurs commonly in domestic animals throughout the world; it frequently affects cattle. In 1978, 108 cases of human leptospirosis were reported in the United States—water and various animal species were the most common sources of infection (1). In Florida, the disease was first diagnosed serologically in people in 1961 (2). A recent serologic survey of beef and dairy herds in

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Florida indicated a widespread infection of cattle with *Leptospira interrogans* serovar *hardjo* and to a lesser extent with *L. interrogans* serovar *pomona* (3). The results of other serologic surveys, particularly in the Southern United States, have suggested a widespread presence of *hardjo* infections in cattle (4,5).

The cardinal epidemiologic feature of leptospirosis is urinary excretion of leptospire from infected and carrier animals and transmission—either directly or via contaminated water—to susceptible hosts. Because of the generally sanitary urinary excretion habits of people, they are essentially dead end hosts for the organism.

We describe leptospiral infections among the cows and milking crews on three dairy farms in Florida. *Hardjo* and *pomona* serovars were implicated in these infections.

## **Study Subjects and Methods**

Between June 15 and August 28, 1978, 11 of 17 milkers on 3 dairy farms in Lafayette County, Fla., were seen by physicians. Some had been ill for up to 35 days before they sought medical help.

Blood specimens from 8 patients were cultured for leptospire on their initial visit, and serums from all

11 patients were examined for leptospiral antibodies. Also, urine specimens from two patients and cerebrospinal fluid (CSF) from one patient were cultured for leptospire. The leptospiral laboratory methods of Sulzer and Jones (6) were used; however, the culture medium was bovine albumin-polysorbate 80 (BA-P80) (7). The improved microscopic agglutination test (8) was used with 15 leptospiral serovars: *australis*, *autumnalis*, *ballum*, *canicola*, *grippotyphosa*, *icterohaemorrhagiae*, *pomona*, *pyrogenes*, *tarassovi* (representing non-hebdomadis serogroups) and *hardjo*, *hebdomadis*, *sejroe*, *szwajzak*, *wolffi*, and *georgia* (representing the hebdomadis serogroup).

Herd A consisted of about 1,000 Holstein dairy cows that were not vaccinated against leptospirosis. Herd B also contained about 1,000 Holsteins; these cows had been vaccinated 6–8 months before the outbreak with a leptospiral bacterin containing both *pomona* and *hardjo* organisms. Herd C consisted of about 100 Holsteins that had been vaccinated 5 months previously with a bacterin containing *pomona*, *hardjo*, and *grippotyphosa*. Leptospiral serology was performed on serums from 67 cows in herd A, 200 cows in herd B, and 28 cows in herd C; the preceding procedures and antigens were used. Also, urine was collected from 24 cows in herd A and cultured for leptospire in BA-P80 medium with a dilution technique and contaminant control method (6). The urine from 20 of these cows also was inoculated intraperitoneally into young hamsters; these hamsters were subsequently examined for evidence of leptospiral infection (6).

Leptospire isolated from the patients and cows were tentatively identified by cross-agglutination tests with antiserums to 11 serogroups, including 27 serovars of the hebdomadis serogroup. Hyperimmune antiserums were prepared by inoculation of rabbits with the isolates, and agglutinin-absorption tests were performed by use of the serovars to which the isolates reacted (6).

## Results

Leptospirosis was diagnosed in all the 11 patients examined. The patients' ages ranged from 6 to 24 years; the 6-year-old was the child of a milker and a visitor to the milking operation. Of the 11 patients, 10 were male, and all but the child were involved in the milking operations. Four cases occurred at dairy A, five at dairy B, and two at dairy C.

The symptoms reported by the patients included malaise (100 percent), fever (100 percent), nausea and vomiting (91 percent), myalgia and coryza (82 percent), chills (73 percent), adenopathy (64 percent), pharyngitis and anorexia (54 percent), stiff neck (45 percent), and photophobia, arthralgias, diarrhea, and

conjunctival suffusion (36 percent). Skin rashes were seen in 3 of the 11 patients. Four of five patients examined for CSF abnormalities had pleocytosis and elevated protein. Urinalyses for 6 of the 11 patients revealed proteinuria and pyuria in 3 of them, and 2 also exhibited hematuria.

Various dosages and preparations of penicillin were administered to all the patients, but the drug's efficacy in shortening the duration of the illness could not be evaluated satisfactorily. Every illness was self-limiting and without sequelae. None of the patients had jaundice or evidence of severe renal insufficiency.

**Dairy A patients.** Initial serum specimens for leptospiral serology indicated that the four patients from dairy A had been infected with leptospire. Serums of these patients agglutinated antigens of the hebdomadis serogroup, with highest titers to *hardjo* (1:6,400, 1:3,200, 1:3,200, and 1:200) and lesser cross-reacting titers or no titers to five other serovars of the hebdomadis serogroup. One patient also had lower titers to *pomona* and *tarassovi* (1:200 and 1:800). Followup serum specimens were obtained from two of the four patients. The first patient had an increase in *hardjo* titers from 1:6,400 to 1:12,800 in 12 days, and the other patient's *hardjo* titer remained at 1:3,200 after 7 days. Blood cultures from three patients, urine cultures from two patients, and a spinal fluid culture from one patient were negative for leptospire (table 1).

**Dairy A cows.** Leptospiral agglutination occurred at a serum dilution of 1:100 or higher with 58 (87 percent) of 67 serums from this dairy's cows. Against hebdomadis serogroup antigens, 52 (78 percent) of the serums agglutinated *hardjo*, ranging from 1:100 (13 serums) to 1:1,600 (6 serums); fewer serums cross-agglutinated with 4 other hebdomadis serovars. Serologic reactions to non-hebdomadis serogroups were absent or at very low levels; 9 serums reacted at 1:100 and 1 serum at 1:200 to *tarassovi* (table 2). Two isolations of leptospire were made, one from the kidney of a hamster inoculated with bovine urine and the other from bovine urine inoculated into BA-P80 medium. Preliminary cross-agglutination tests with leptospiral serogroup antiserums indicated that both isolates were of the hebdomadis serogroup. Cross-agglutination reactions with the hyperimmune antiserums of 27 hebdomadis serovars indicated that they were *hardjo* or closely related serovars (*wolffi*, *balcanica*, *trinidad*, *polonica*). Agglutinin-absorption studies, by use of the two isolates and their hyperimmune antiserums and the closely related serovars, identified the serovars as *hardjo*.

Table 1. Summary of leptospiral serologic results and cultures from dairy workers

Patient No. and antigen	Serologic results <sup>1</sup>			Blood cultures
	Titer	Followup titer	Number days later	
Dairy A				
Patient 1: <i>hardjo</i> .....	6,400	12,800	12	N.D.
Patient 2: <i>hardjo</i> .....	3,200	3,200	7	Negative
Patient 3: <i>hardjo</i> .....	3,200	N.D.	..	Negative
<i>tarassovi</i> .....	800	N.D.	..	
Patient 4: <i>hardjo</i> .....	200	N.D.	..	Negative
Dairy B				
Patient 1: <i>hardjo</i> .....	800	N.D.	..	N.D.
<i>pomona</i> .....	400	N.D.	..	
<i>icterohaemorrhagiae</i> .....	400	N.D.	..	
Patient 2: <i>hardjo</i> .....	200	200	15	Negative
<i>tarassovi</i> .....	Negative	800	15	
Patient 3: <i>hardjo</i> .....	3,200	N.D.	..	+ <i>pomona</i>
Patient 4: <i>pomona</i> .....	Negative	100	30	+ <i>pomona</i>
Patient 5: <i>autumnalis</i> .....	200	200	11	N.D.
<i>pomona</i> .....	Negative	400	..	
<i>tarassovi</i> .....	Negative	400	..	
Dairy C				
Patient 1: <i>hardjo</i> .....	12,800	6,400	27	Negative
Patient 2: <i>hardjo</i> .....	Negative	800	14	+ <i>hardjo</i>

<sup>1</sup> Reciprocal of serum dilution. NOTE: N.D. means not done.

**Dairy B patients.** One patient had serologic titers to *hardjo* (1:800), *pomona* (1:400), and *icterohaemorrhagiae* (1:400) on initial examination. A followup serum specimen was not available. A second patient had a titer of 1:200 to *hardjo* initially as well as 15 days later; during this interval, the serologic response with *tarassovi* converted from negative to 1:800. Leptospire were not isolated from cultures of the blood specimen taken from the second patient on initial examination. A third patient had a serum titer to *hardjo* (1:3,200) and to *wolffi* (1:3,200); a followup specimen was not available. Serovar *pomona* was isolated from this patient's initial blood specimen. A fourth patient had no leptospiral antibodies on first examination, but he had a titer to *pomona* (1:100) 30 days later. Serovar *pomona* was isolated from the blood specimen of this patient during a time when he was febrile. The fifth

patient had a titer to *autumnalis* (1:200) when first seen, and 11 days later he had titers of 1:400 to *pomona* and 1:200 to *autumnalis*—two serovars that cross-react. During these 11 days, the serologic response with *tarassovi* converted from negative to 1:400 (table 1).

**Dairy B cows.** Leptospiral antibodies were observed in serum dilutions of 1:100 or more in 180 (90 percent) of 200 serums tested from this dairy's cows. The most prevalent serologic reactions were to *hardjo* (64.5 percent), titers ranging from 1:100 (50 serums) to 1:3,200 (2 serums), with many cross-reactions to the other hebdomadis serovars. Serologic reactions to non-hebdomadis serogroups were most prevalent to *tarassovi* (42 percent) and *pomona* (33 percent), as shown in table 2; the *tarassovi* titers ranged from 1:100 (36

**Table 2. Summary of leptospiral serologic (microagglutination test) results in cows<sup>1</sup>**

Number of cows and antigens eliciting highest responses	Percent positive
<b>Dairy A, 67 cows:</b>	
<i>hardjo</i> .....	78
<i>tarassovi</i> .....	9
<b>Dairy B, 200 cows:</b>	
<i>hardjo</i> .....	64
<i>tarassovi</i> .....	42
<i>pomona</i> .....	33
<b>Dairy C, 28 cows:</b>	
<i>pomona</i> .....	36
<i>hardjo</i> .....	21
<i>grippotyphosa</i> .....	21

<sup>1</sup> Minor reactions to other antigens not shown.

serums) to 1:800 (10 serums), and the *pomona* titers ranged from 1:100 (24 serums) to 1:3,200 (11 serums).

**Dairy C patients.** Of the two milkers at dairy C, one had a serologic titer of 1:12,800 to *hardjo* and lesser cross-reacting titers to two other hebdomadis serovars when first seen. The blood specimen cultured at this time was negative for leptospire. After 27 days, the serum titer had dropped to 1:6,400. The leptospiral serologic test results for the second patient were negative initially, and *hardjo* was isolated from the blood specimen cultured at this time. The serum titer reached 1:800 to *hardjo* 14 days later (table 1).

**Dairy C cows.** Leptospiral antibodies were found in serum dilutions of 1:100 or higher in 24 (86 percent) of the 28 serums tested. Six serums (21 percent) agglutinated *hardjo* antigen at serum dilutions of 1:100 (4 serums), 1:400 (2 serums), and 1:1,600 (1 serum). Eleven serums (36 percent) agglutinated *pomona* antigen at serum dilutions of 1:100 (3 serums), 1:200 (6 serums), 1:400 (1 serum), and 1:800 (1 serum). Six serums (21 percent) agglutinated *grippotyphosa* antigens at serum dilutions of 1:100 (4 serums), 1:200 (1 serum), and 1:800 (1 serum), as shown in table 2. Many cross-reactions occurred with the hebdomadis antigens *sejroe* and *wolffi* and the serums.

## Discussion

The clinical manifestations in the affected dairy workers were similar, but not identical, to those mentioned in other reports of anicteric leptospirosis in human beings (9-12). In the outbreak reported from Israel (11), *hardjo* was isolated from dairy workers who did not exhibit jaundice, conjunctivitis, or neurologic, cardiac, or pulmonary signs. In the outbreak reported

here, conjunctival symptoms and central nervous system abnormalities were observed. The clinical features of an outbreak of "dairy farm fever" (leptospirosis) reported from New Zealand (12) were similar to those reported here. Both *pomona* and a hebdomadis serovar (*hardjo*) were isolated from the anicteric patients (13).

The leptospiral serologic test results on dairy A patients were largely hebdomadis serogroup reactions; the highest titers were to *hardjo* antigen. Although their anicteric type of leptospirosis was not life threatening to the dairy workers, four were hospitalized. The serologic test results on the dairy A cows were similar to those of the dairy workers, largely hebdomadis group reactions, with highest titers to *hardjo*. The isolation of *hardjo* from urine specimens of two cows implicated *hardjo* in the outbreak, although attempts to isolate leptospire from the blood specimens and urine of dairy A workers were unsuccessful.

The owner of dairy A reported mastitis and occasional abortions in the herd; these conditions have been long known to be associated with *hardjo* infections (14-16). Serologic evidence of bovine infection with the hebdomadis serovar *sejroe* was reported in 1956 (17). More recently, titers to hebdomadis serogroup member *hardjo* have emerged as the most prevalent in cattle in the Southern United States (3-5). Serovar *hardjo* was first isolated from cattle in the United States in 1960 in Louisiana (18); since then, only a few isolations of *hardjo* from cattle have been reported (14,19)—none had been reported from human beings in the United States.

The serologic test results from the dairy B workers indicated that they had been infected with *hardjo* and *pomona* and possibly with *tarassovi*. The isolation of *pomona* from blood specimens of two patients probably was due to fortunate timing; however, *pomona* is much more readily isolated than the fastidious *hardjo*. One patient had a mixed infection—first with *hardjo*, during which *hardjo* antibodies were produced, and then with *pomona*. Serovar *pomona* was isolated during the bacteremic phase, before antibodies had developed.

The similarity of the serologic test results in the milking crew and cows in dairy B also was marked, indicating *pomona* and *hardjo* infections in both. The anicteric manifestations of infections with both serovars were similar in the patients. The presence of *tarassovi* antibodies in the serums of some of the milkers and cows may indicate that *tarassovi* is emerging as another important serovar in the United States.

Subclinical leptospiral infections may occur or persist in vaccinated cattle, and this appears to have happened in the two vaccinated herds.

Conditions for exposure and infection of the milkers

were optimal in the dairies because they worked in waist-deep pits in milking parlors where they were exposed to urine spray and droplets from the confined animals.

The results of this study indicate that leptospiral infection is an occupational hazard for dairy milkers and that preventive measures are needed. These measures should include boots and other protective clothing and perhaps protection from urine for the eyes and nose. Workers in certain high-risk agricultural occupations are being immunized with leptospiral bacterins in other countries, most recently in Israel (20,21). These bacterins are not available for such workers in the United States.

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## SYNOPSIS

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Leptospirosis was diagnosed in 11 milkers on 3 dairy farms in a Florida county. Serologic test results identi-

fied *Leptospira interrogans* serovar *hardjo* as the infecting organism in milkers and cows on one farm, and *hardjo* was isolated from two cows. On the second farm, serovars *hardjo* and *pomona* were implicated serologically in the cows and milkers, and *pomona* was isolated from two milkers. On the third farm, *hardjo* infection was identified by serologic tests in one milker, and *hardjo* was isolated from another.

This was the first isolation of *hardjo* from a human being reported in the United States. Leptospiral infection is an occupational hazard for dairy milkers in some areas of the United States. Thus, the authors recommend that preventive measures should be taken. These measures should include boots and other protective clothing and protection from urine spray for the eyes and nose.