Leptospira interrogans serovar *hardjo* Infection in Cattle in the South Okanagan District of British Columbia

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ABSTRACT

An outbreak of leptospirosis due to *Leptospira interrogans* serovar *hardjo* in the South Okanagan District of British Columbia was investigated. The infection was associated primarily with bulls, but serovar *hardjo* was isolated from both bulls and cows at slaughter. Kidney and cerebrospinal fluid were found to contain leptospires, independently of the presence and level of serum agglutinins. Treatment of a bull twice in six months with dihydrostreptomycin failed to diminish an agglutinin titer (1/200) which persisted for two years without reexposure of the bull. A serological survey of cull cows sold through a central auction mart revealed the presence of *hardjo* agglutinins in 15.4% of 1300 sera representing 163 herds in 20 locations. Thirty percent of these herds contained reactor cattle. The number of premises from which reactor cattle came in a given locality varied from 4% to 67.7%. Measures to control leptospirosis in the study are suggested.

Key words: Leptospirosis, infertility, *hardjo*, kidney, cerebrospinal fluid, Canada, British Columbia.

RÉSUMÉ

Infection des bovins par *Leptospira interrogans* serovar *hardjo*, dans le district d'Okanagan-Sud, en Colombie-Britannique

Cet article rapporte les résultats de l'investigation d'une éruption de leptospirose, imputable à *Leptospira interrogans* serovar *hardjo*, dans le district d'Okanagan-Sud, en Colombie Britannique. L'infection affectait surtout les taureaux, mais le sérotype *hardjo* se retrouva tant chez les taureaux que chez les vaches, à l'abattage. Les reins et le liquide céphalo-rachidien renfermaient des leptospires, indépendamment de la présence et du titre d'agglutinines sériques. L'administration de deux doses de dihydrostreptomycine à un taureau, en l'espace de six mois, ne contribua pas à faire baisser son titre d'agglutinines qui se situait à 1:200 et persista pendant deux ans, même en l'absence d'une nouvelle exposition au microorganisme. Un relevé sérologique des vaches réformées, vendues à un centre d'encan, révéla la présence d'agglutinines à l'endroit du sérotype *hardjo*, dans 15,4% des 1300 échantillons prélevés chez des sujets qui provenaient de 163 troupeaux et de 20 régions. Trente pour cent de ces troupeaux comptaient des sujets qui possédaient des agglutinines. Le nombre de fermes par région où l'on détecta des sujets porteurs d'agglutinines varia de 4% à 67,7%. L'auteur suggère des moyens susceptibles d'aider à contrôler la leptospirose.

Mots clés: leptospirose, infertilité, *hardjo*, reins, liquide céphalo-rachidien, Canada, Colombie-Britannique.

INTRODUCTION

Antibodies to *Leptospira interrogans* serovar *hardjo* occur in bovine sera associated with clinical disease in British Columbia (Greenfield J, Veterinary Laboratory, Abbotsford, B.C., written communication), and elsewhere in Canada (1,2). They were also found unrelated to disease in a B.C survey (3).

Failure of purebred bulls to qualify for entry into artificial insemination units was a costly result of serovar *hardjo* infection in 87 of 1584 bulls from Saskatchewan, Alberta and B.C. which were tested at the Animal Diseases Research Institute (A.D.R.I.), Lethbridge between 1981 and 1983. Such an event in a Shorthorn herd in the South Okanagan District of B.C. occasioned the study presented here. The purposes of this paper are to report the occurrence of infection by serovar *hardjo* in cattle with or without detectable agglutinins, to document the persistence of agglutinins in serum after treatment of a bull, to illustrate the pervasiveness of the infection in the South Okanagan District of B.C. and to suggest control measures.

MATERIALS AND METHODS

Serology

Serum antibodies were measured by the Microscopic Agglutination Test (MAT) for serovars *hardjo* and *pomona* in all sera, and for three additional serovars, *grippotyphosa, canicola* and *copenhageni*, in nine cattle tested at slaughter (Table I). Sera were diluted tenfold from 1/10, resulting in a final dilution of 1/20 etc. in the test. Results were recorded as positive or negative and the end titer was determined. The criterion for a positive test was the agglutination of 50% or more of the antigen by serum diluted at least 1/100 (final dilution 1/200). Titers were recorded as final dilution. Cattle whose sera produced a positive reaction were designated reactors and their herds of origin were denoted reactor herds.

Following detection of *hardjo* antibodies in a herd sire which was being tested for A.I. certification, bloods were collected from the bull three times in six months during which it was treated twice at a 30 day interval with dihydrostreptomycin (25 mg/kg). The following groups of cattle in the same herd were tested: in late fall, ten mature cows bred by the initial reactor bull the previous three summers; in early winter, the complete adult herd of 89 animals; and the following

TABLE I

SEROLOGICAL, CULTURAL AND HISTOLOGICAL FINDINGS IN SPECIMENS FROM CATTLE FROM A HERD INFECTED WITH *LEPTOSPIRA INTERROGANS* SEROVAR *HARDJO*

Subject	Agglutinins and Titer[a]		Specimen	Test Results		
	6 m preslaughter	At slaughter		Microscopic[b]	Cultural[c]	Histological
Bull, 2 yr	*hardjo* 1/200	*hardjo* 1/2,000	kidney	+	+ *hardjo*	Mild multifocal i.n.[d]; mononuclear leukocytes
			CSF[b]	–	–	
Bull, 2 yr	Negative	Negative	kidney	+	–	As above bull, but milder
			CSF	–	–	
Bull, 2 yr	Negative	Negative	kidney	+	+ *hardjo*	Severe extensive i.n.; mononuclear leukocytes
			CSF	–	–	
Bull, 3 yr	Not tested	*hardjo* 1/20,000	kidney	+	–	Severe i.n.; mononuclear and polymorphonuclear leukocytes
			CSF	+	–	
Mature cow	Not tested	*hardjo* 1/20	kidney	+	–	Numerous small foci of i.n.; edema and congestion
			CSF	–	–	
Mature cow	Negative	*hardjo* 1/20	kidney	+	–	Numerous small foci of i.n.; edema and congestion
			CSF	+	–	
Bull, 2 yr	*hardjo* 1/200	*hardjo* 1/2,000	kidney	+	+ *hardjo*	Mild focal i.n.; mononuclear leukocytes
			CSF	–	+ untyped	
Bull, 2 yr	*hardjo* 1/200	*hardjo* 1/200	kidney	–	+ *hardjo*	Mild focal i.n.; mononuclear leukocytes
			CSF	–	–	
Bull, 2 yr	*hardjo* 1/200	*hardjo* 1/2,000	kidney	–	+ *hardjo*	Mild focal i.n.; mononuclear leukocytes
			CSF	–	–	

[a]Agglutinins were measured by the Microscopic Agglutination Test in tenfold dilutions of serum from 1/20, against serovars *hardjo, pomona, grippotyphosa, canicola, icterohaemorrhagiae*. The criterion for reaction was 50% agglutination of antigen.
[b]+ Leptospires observed in kidney homogenates or cerebrospinal fluid (CSF) under 500X magnification, dark field illumination.
[c]+ = Leptospires isolated, serovar as shown.
[d]i.n. = Interstitial nephritis.

spring, eight bulls penned together including untreated reactors detected in the herd test. In the following spring, nine bulls and cows were blood tested at slaughter. One year after the first herd test, the 61 breeding cattle were retested.

In order to assess the prevalence of leptospiral infection in the vicinity of the herd under study, blood samples were obtained from all cows which were shipped for slaughter through the B.C. Livestock Cooperative at Okanagan Falls. Test intervals were May through August 1982 and December 1983 through April 1984. Blood was collected under the federal Brucellosis Control Program and shipped to A.D.R.I. for leptospirosis testing. A total of 1300 sera from cattle from 163 premises at 20 locations were tested.

Culture

Specimens for culture were obtained from a total of nine cattle culled on two occasions from the herd under study. The first group included three two year old bulls, one three year old bull and two mature cows. The second group consisted of three two year old bulls. They are listed in order in Table I. Cerebrospinal fluid (CSF) was collected in an open beaker at the time of decapitation and transferred to a capped tube. Kidneys were removed with care to preserve the capsule under the cover of perirenal fat. Specimens were transported to the laboratory within one hour.

Culture media included EM semisolid medium (EMss) (4) and SPL5X Leptospira Medium (Scientific Protein Laboratories, Waunakee, Wisconsin) (5X), a commercial liquid preparation of the Johnson-Harris modification of EM (5). The selective inhibitor, 5-fluorouracil (5FU) was added to the two types of media at 200 μg/mL. Kidneys were dissected free of perirenal fat and inspected for lesions. Lobes with small pale, hemorrhagic, or depressed lesions were selected for culture. The capsule was peeled back aseptically, or in the case of contaminated kidneys, the surface of the lobes was seared. Several samples of approximately 1 g of cortical and corticomedullary tissue were collected by probing with a broken Pasteur pipette and were ejected into transport medium (TM) (6). The resulting 1/10 suspensions were left at room temperature for 20 to 30 min, agitated on a Vortex mixer and diluted tenfold in TM to 1/1000. Dilutions 1/100 and 1/1000 were inoculated in 1 mL volumes to the media described above. Cerebrospinal fluid was inoculated in 1-2 drop volumes onto EMss, with and without 5FU, and in 1 mL volumes as three tenfold dilutions in TM to both culture media with and without 5FU. Suspensions were examined directly by dark field microscopy for the presence of leptospires.

Cultures were inspected at one week intervals by transillumination and microscopy. Transfers were made to fresh EMss or 5X liquid medium to obtain vigorous, contaminant-free cultures. Leptospires were tested for agglutination in reference sera as soon as cultures reached adequate density to obtain preliminary identification. They were then propagated to density 2×10^8 cells/mL in liquid medium and typed by cross absorption, according to World Health Organization standard procedure, at A.D.R.I., Lethbridge.

Pathology

Kidneys were inspected for evidence of focal or diffuse cortical lesions after the capsules were removed. Kidney sections including cortex and medulla were fixed in formalin, sectioned at 6 μ and stained with hematoxylin and eosin.

RESULTS

Serology and Culture (Herd Study)

Agglutinins to serovar *hardjo* only were detected in the herd under study. All reactors were bulls, with the exception of one cow which had been bred on range by a bull from another herd. The serum titer in the senior herd sire remained stationary at 1/200 despite two treatments with dihydrostreptomycin and this titer persisted one year later. Seroconversion occurred in a yearling bull which developed a titer of 1/20,000 during five months' confinement in a group of eight untreated bulls, three of which were reactors. After removal of seven bulls and two cows from the herd, and treatment of the remaining two year old bull, no seroconversion occurred in the cows during the next breeding and pasture season as shown by a herd test in late November, one year subsequent to the initial herd test.

Infection with leptospires was demonstrated by direct microscopy in each of the seven bulls and two cows which were sampled at slaughter. These observations were confirmed by culture in five animals (Table I). Serovar *hardjo* was isolated from five kidneys and one sample of CSF, obtained from animals with or without serum antibodies detectable by MAT. All isolates were agglutinated by antiserum to serovar *hardjo*. Three isolates were successfully maintained and propogated for serotyping. They were identified as *hardjo* strain *hardjoprajitno*.

Pathology (Herd Study)

Gross lesions were present but inconspicuous in the kidneys examined. Microscopic foci of interstitial nephritis of varying severity and extent were seen in every kidney. The main inflammatory cell type was the mononuclear leukocyte, but in the bull with a titer of 1/20,000 a marked infiltration of polymorphonuclear leukocytes was found. The kidneys of two cows with only trace agglutinin titers contained foci of edema and congestion as well as numerous small foci of interstitial nephritis. The relation of histological to cultural and serological findings is presented in Table I and discussed below.

Regional Serological Survey

Sampling of culled cows at Okanagan Falls Livestock Cooperative yielded 1300 sera, which were derived from 163 herds in 20 locations within the South Okanagan district. The distribution of sampling and the 14 locations from which a total of 200 reactors came are shown on the map in Figure 1. Sera reacted only to *hardjo* antigen, with the exception of a single *pomona* reactor which had no *hardjo* titer. The overall reactor rate in culled cows from herds throughout the district was 15.4%, with the highest cattle and herd reactor rates at Westbridge and Princeton (47.5 and 54.4%, 25.4 and 52.9% respectively). By comparison, cattle and herd reactor rates for Rock Creek, the location of the study herd were 7.2 and 36.4%, respectively. Table II contains the survey data.

Reactor cattle were present in each of five shipments of cattle from one premises in the Princeton area. A total of 55 culled cattle from this premises contained 18 reactors. Similarly, five shipments from two related herds in the Westbridge area all contained reactors, totalling 25 out of 47 cows culled.

DISCUSSION

The problem of antibody to *hardjo* which persists after renal infection is no longer detectable in bulls, prompted the search for a focus of infection other than kidney. The decision was made to culture CSF because leptospires have been isolated regularly from hamster brain during acute infection (7). The isolation of leptospires from CSF of the bull reported here was associated with a chronic infection, as indicated by antibody titers. The acute phase had occurred five months before slaughter. In addition, leptospires were observed by direct microscopy in the cerebrospinal fluid sample from a cow which was infected at least nine months previously. Although renal leptospirosis was also demonstrated in these cattle, the verification of infection of the central nervous system long after serological evidence of initial infection supports the theory that leptospires may localize elsewhere than in kidney. An alternative possibility is that reinfection occurred in the interval between blood tests. Sera were not tested for the presence of protective antibody, which is not detectable by the MAT.

More isolations were made from the second group of cattle slaughtered

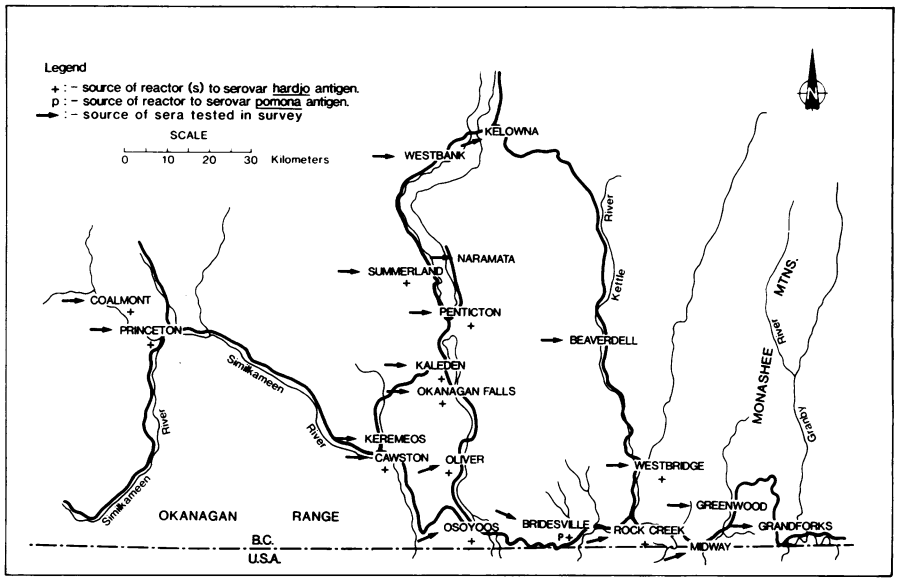

FIGURE 1. Sources of cattle tested serologically for leptospirosis in South Okanagan district of British Columbia, 1982-84.

TABLE II

RESULTS OF SURVEY FOR AGGLUTININS[a] TO *LEPTOSPIRA INTERROGANS* SEROVARS *HARDJO* AND *POMONA* IN SERA OF CULLED COWS SOLD AT OKANAGAN FALLS, BRITISH COLUMBIA, MAY TO AUGUST/82 AND DECEMBER/83 TO MARCH/84

Locality of Origin	No. of Cattle Tested	No. of Herds Represented	Reactor[b] Cattle		Reactor Herds[c]	
			No.	%	No.	%
Beaverdell	4	2	0	—	0	—
Bridesville	58	9	3[b]	5.2	3	33.3
Cawston	82	10	8	9.8	3	30
Coalmont	9	1	3	NA	1	NA
Grandforks	142	15	31	21.8	6	40
Greenwood	33	4	0	—	0	—
Kaleden	11	2	2	18.2	1	50
Kelowna	3	3	0	—	0	—
Keremeos	164	23	15	9.1	5	21.7
Midway	22	10	0	—	0	—
Naramata	1	1	0	—	0	—
Okanagan Falls	95	8	19	20	2	25
Oliver	133	18	5	3.8	4	4
Osoyoos	33	6	1	3.0	1	16.7
Penticton	60	6	4	7.0	2	16.7
Princeton	228	17	58	25.4	9	52.9
Rock Creek	97	11	7	7.2	4	36.4
Summerland	28	3	6	21.4	2	67.7
Westbank	17	3	0	—	0	—
Westbridge	80	11	38	47.5	6	54.5
Total	1,300	163	200	15.4	49	30.1

[a]Measured by the Microscopic Agglutination Test using a tenfold dilution scheme and a criterion of 50% or greater agglutination of antigen at 1/200 final serum dilution to denote a reactor.
[b]All reactions were to *hardjo* antigen, except for one *pomona* reaction from a Bridesville cow.
[c]Herds from which reactor cattle came.

than from the first, despite direct microscopic evidence of leptospires in both groups. The discrepancy may be due to the use of an inferior batch of medium for the first group of cultures. It was noted that other *hardjo* field isolates in the laboratory failed to survive two transfers in this medium. Thiermann (8) has shown that deficiency in a medium can be demonstrated by its inability to support growth of a field stain of *hardjo* in subculture. It is therefore probable that more direct observations would have been confirmed by culture if all the growth medium used had been equally capable of supporting the growth of *hardjo*.

It is debatable if serology can be used to detect individual cattle infected with *hardjo*. Individual cattle, naturally and experimentally infected, vary widely in their antibody response to *hardjo* infection (9,10,11). In the current study (Table I) the demonstration of leptospires in kidneys of cattle whose agglutinin levels ranged from zero to 1/20,000 lends weight to the argument that negative serology in cases of suspected *hardjo* infection is valid only on a herd basis. On the other hand, serological testing of culled cows disclosed herds in which repeated shipments contained a high proportion of *hardjo* reactors. In such cases, leptospirosis could rationally be included among possible causes for infertility leading to culling and reproductive tracts should be examined for the presence of leptospires.

The source of *hardjo* infection in the study herd was not definitely established. Initial serology suggested that active infection was confined to the bulls and that therefore it had entered the herd after the last breeding season prior to the study. The single reactor cow which was detected had been brought into the herd four years previously from Prince George, far north of the study area. She had also been bred by an outside bull on range. Prolonged shedding of *hardjo* by cows has been recorded (10). This cow could therefore have been a source of infection to a herd bull, who then transmitted it to the other bulls confined together after the breeding season. This explanation is inconsistent with the finding that the cow had an ascending titer on retest a month later, suggesting very recent infection or reexposure which prompted an anamnestic response. These conflicting alternatives illustrate the limitations of the MAT as an indicator of a point in the course of leptospiral infection in individual cattle.

Another possible source of infection to the herd was a young bull which had been purchased in Saskatchewan and exposed only to the other bulls after the breeding season. Acquisition of infection by cows or steers on summer range was a distinct possibility, in view of the foci of infection found in the district survey. Natural transmission of *hardjo* is a complex process in which host susceptibility, behaviour and environment all interplay (12).

The value of the survey data for five localities listed in Table II is limited by low numbers of tests. These data have been included as supportive information for the map, but reactor percentages have been omitted. Deletion of these data from survey totals raises the cattle and herd reactor rates by 0.2 and 2%, respectively.

Prevention of leptospirosis in most cattle ranging in enzootic areas could be achieved by annual vaccination against indigenous serovars. Enzootic areas could be identified by appropriately designed surveys of market cows, sera from which are obtained for the federal Brucellosis Control Program in Canada. Information on leptospirosis prevalence in each major livestock producing area in a province thus would become available to veterinary practitioners with a minimum of effort.

In purebred herds, treatment of infected bulls with a single injection of dihydrostreptomycin at a dose of 25 mg/kg body weight (13) will reduce urinary shedding. However, antibody may persist at a detectable level for years, preventing certification. Similarly vaccination may render cattle uncertifiable for several months. Therefore owners of purebred herds should be urged to undertake a program to eliminate leptospirosis from the herd and to prevent its reentry. The following suggestions are based on general knowledge of the disease as it is manifested in Canadian cattle.

1. Determine the level and location of infection present in the herd by blood testing 10-20% of the cattle in each segment of the herd. Currently the MAT can be conducted for western Canada by arrangement with the federal laboratory at Lethbridge.
2. Vaccinate all cattle in enzootic areas over six to nine months of age for which a negative MAT for certification will not be required within three months. Vaccination should be continued for two to three years to minimize the number of susceptible cattle until no long-term shedders of leptospires remain in the herd.
3. Treat all bulls which have not been vaccinated, if history and serological test results indicate that they have been exposed to infection. Bulls suspected of spreading infection should be treated to reduce the level of urinary shedding regardless of subsequent vaccination.
4. Purchase cattle preferably from tested negative herds, or treat all introduced cattle in isolation. A negative serological test on one animal is no guarantee that it is not shedding leptospires.
5. Avoid massive exposure of cattle to herds heavily infected with leptospirosis, for example, on communal grazing leases.
6. Monitor the herd periodically for leptospirosis, coincident with other serological testing.

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Perhaps the most striking feature of this book is that it is extremely well illustrated with about 385 figures, most of which are photographs and photomicrographs. Many of the figures have multiple illustrations showing diagnostic or other features of similar or comparable organisms.

Practitioners who are involved with poultry or who are called upon to offer assistance in the control of parasitism in birds will find this book wanting. Parasitism in birds has been excluded. This was surprising since the senior author stated that the objective for preparing it was to introduce veterinary students to those aspects of parasitology that they will find useful in their careers and secondly, to provide them, as veterinarians, with a practical reference.

The book is not without its vernacular expressions. For example, we read of "unfortunates" presumably referring to those animals unfortunate enough to be parasitized. The eggs of *Hymenolepis diminuta* are described as handsome. We also read that the ram Violet made famous by Dr. Whitlock in his breeding trials on inherited resistance to trichonstrongylidosis was blown to his glory one dark and stormy night when the electric transmission lines unfortunately fell upon him.

This book is a practical type of book dealing with the clinical diagnoses, treatment and control of parasitism in livestock and pet animals. Veterinarians should find it a very useful and handy reference to refresh and update their basic knowledge on specific parasitological problems as they encounter them.

H. J. Smith.