NOTES

Some Leptospira Agglutinins Detected in Domestic Animals in British Columbia

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ABSTRACT

During a period of six years 7,555 bovine sera, 421 canine sera, 251 porcine sera and 135 equine sera were tested for agglutinins to Leptospira interrogans serotypes canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona and sejroe. The bovine sera reacted predominantly with hardjo and/or sejroe at a rate of 15% compared to 3.5% with pomona. Breeding or abortion problems were associated with pomona but not with sejroe/hardjo agglutinins. The canine sera reacted to canicola (9.9%) and icterohemorrhagiae (5.4%), the porcine sera reacted to pomona (3.2%) and the equine sera reacted predominantly with canicola (8.9%) and icterohemorrhagiae (8.1%).

RÉSUMÉ

Au cours d'une période de six ans, les auteurs ont éprouvé 8,362 échantillons de sérum provenant de 7,555 bovins, 421 chiens, 251 porcs et 135 chevaux. Ils y recherchaient des agglutinines contre les sérotypes suivants de Leptospira interrogans: canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona et sejroe. Les échantillons des bovins réagirent dans une proportion de 15% avec hardjo et/ou sejroe, comparativement à 3.5% avec pomona. Les problèmes de reproduction et les avortements s'avérèrent reliés à la présence d'agglutinines spécifiques à pomona, mais non à sejroe ou hardjo. Les échantillons des chiens réagirent avec canicola (9.9%) et avec icterohemorrhagiae (5.4%); ceux des porcs réagirent avec pomona (3.2%); ceux des chevaux réagirent surtout avec canicola (8.9%) et avec icterohemorrhagiae (8.1%).

Bovine leptospirosis has been a disease reportable to the British Columbia Department of Agriculture (B.C.D.A.) since 1966. The disease was included in the legislative Acts[1] following a clinical outbreak of leptospirosis due to serotype *pomona* in eastern British Columbia but the overall incidence of the disease in B.C. was unknown. In 1969 a limited survey was initiated to determine the status of leptospirosis in some domestic animals and the testing of sera was continued on request as a routine service.

A total of 8,362 blood samples from 1,176 premises were collected. The total included 7,555 bovine samples from 643 premises, 421 canine samples from 387 premises, 251 porcine samples from 75 premises and 135 equine samples from 71 premises. Sample

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Submitted June 12, 1975.

[1]Contagious Diseases (Animals) Act and Veterinary Laboratories Act.

TABLE I. Leptospira Agglutinins Detected in Bovine Canine and Equine Sera in British Columbia[a]

Species	Number Tested	*Leptospira* Serotypes				
		canicola	*grippotyphosa*	*hardjo/sejroe*	*ictero-hemorrhagiae*	*pomona*
Bovine	7,555	Not tested	0[b]	1132 (15.0%)	Not tested	265 (3.5%)
Canine	421	42 (9.9%)	0	2 (0.5%)	23 (5.4%)	2 (0.5%)
Porcine	251	0	0	4 (1.6%)	0	8 (3.2%)
Equine	135	12 (8.9%)	7 (5.2%)	7 (5.2%)	11 (8.1%)	6 (4.4%)

[a]Tested by the microscopic agglutination method using live antigens: titers ⩾ 100 reported
[b]682 bovine sera tested

TABLE II. Leptospira Antibody Titers for Bovine, Canine, Equine and Porcine Sera Tested 1969-74 in British Columbia

Species	*Leptospira* Serotype	Number Tested	Total of Reactors (%)	Percent Reacting to Indicated Titers[a]			
				100	500	2,500	⩾12,500
Bovine	*hardjo*	7,555	12.7	40	37	12	11
	pomona	7,555	3.5	43	26	7	24
	sejroe	7,555	14.4	44	39	9	8
Canine	*canicola*	421	9.9	26	24	17	33
	hardjo	421	0.5	100	0	0	0
	icterohemorrhagiae	421	5.4	56	18	13	13
	pomona	421	0.5	100	0	0	0
	sejroe	421	0.5	50	50	0	0
Porcine	*hardjo*	251	0.8	100	0	0	0
	pomona	251	3.2	25	37	38	0
	sejroe	251	1.2	100	0	0	0
Equine	*canicola*	135	8.9	42	50	8	0
	grippotyphosa	135	5.2	86	0	0	14
	hardjo	135	1.5	100	0	0	0
	icterohemorrhagiae	135	8.1	91	0	0	9
	pomona	135	4.4	66	17	0	17
	sejroe	135	4.4	83	17	0	0

[a]Tested by the microscopic slide agglutination test using live antigens

sizes from premises ranged from one to 100. In many instances entire small herds of cattle were tested and they represented all common breeds kept in British Columbia.

Bovine samples were obtained from inspected slaughter houses, veterinary practitioners, herds studied under the B.C.D.A. Contagious Diseases Act and from Canada Department of Agriculture Health of Animals Branch, B.C. Canine blood samples were obtained from local animal hospitals on a survey, not a clinical basis, porcine samples from farms and equine samples were mostly those submitted to the laboratory for pregnancy testing.

Serum was harvested from blood and stored at refrigerator temperature, and was usually tested within a few days.

Live cultures of the following *Leptospira interrogans* serotypes were maintained at 29°C in Fletcher Medium[2] with 10% rabbit serum added: *canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona* and *sejroe*. Subcultures were made weekly. The microscopic agglutination-lysis test using live antigens was used to detect antibodies. Antigens were used after five days growth in Leptospira Medium EMJH[3] with 10% Leptospira Enrichment EMJH[3]. Four dilutions of serum were prepared using phosphate buffered saline at pH 7.4 (1:50, 1:250, 1:1,250, 1:6,250). One standard drop of each dilution was placed into individual wells of a porcelain spot plate and one drop of a live antigen added to each well. This made the final serum dilutions 1:100,

[2]BBL, Cockeysville, Maryland.

[3]Difco Labs., Detroit, Michigan.

1:500, 1:2,500, 1:12,500. The live leptospira cultures were not pipetted by mouth. The serum/antigen mixtures were incubated at 29°C for three hours in a humid chamber, then a loopful from each well was placed on a clean glass slide without a coverslip and examined by dark-field illumination microscopy at X100 magnification. If 50% or more leptospires were agglutinated in a given dilution of serum then the agglutination was considered to be a positive reaction. A serum giving 50% agglutination in the 1:100 dilution was considered to be a reactor.

The results of the serological tests conducted throughout a six-year period are shown in Table I. Table II indicates the bovine, canine, porcine and equine titers to the six *Leptospira* serotypes investigated.

Although serotypes *hardjo* and *sejroe* were used separately in the tests, reactions to either are presented as one in Table I. These serotypes share antigens within the Hebdomadis group so that cross agglutination is usual and the infecting serotype cannot be identified from the antibody reaction of the host. The findings showed a prevalence of *hardjo/sejros* serotypes in the bovine samples but this was not coincident with breeding or abortion problems which Michna (5) associated with *sejroe* infections. Two sera from Beefalo (half Buffalo) cattle with no clinical signs of leptospirosis had titers of 500 with *sejroe* only. In this study the detection of *pomona* antibodies in cattle was always concurrent with abortion problems.

Canicola, a serotype found commonly in dogs (1), was found by Chernesky (3) in British Columbia in 10% of the dogs from Vancouver and in 2.6% of the dogs from a more rural area. In the study presented, sera came from dogs in Vancouver and environs and the *canicola* reactor rate of 9.9% compares closely with Chernesky's findings. *Icterohemorrhagiae*, a serotype commonly associated with dogs (1, 3, 4) was detected in 5.4% of the canine samples.

In horse sera, as with canine sera, reactions to *canicola* and *icterohemorrhagiae* predominated. Most equine samples came from suburban companion-stock which are often in close contact with canine pets, and perhaps household dogs infected the horses. Stable rats and mice were not tested.

The predominance of *pomona* in swine is consistent with other reports (1, 4), but the incidence was low.

A rising leptospira titer may be a useful indication of active infection but in this study insufficient sequential samples were received to enable any attempt to survey the province for active infections. The results indicate an exposure of certain hosts to some leptospires. This laboratory defines a "positive" reactor as one with a titer of 500 or higher but leptospires have been isolated from cases where the titers were lower than 100.

The first 4,636 bovine sera constituted a first part to this survey and considering these sera, an attempt was made to relate serological reactors to geographic regions of the province. The greatest incidence occurred in the southwest (Fraser River Valley area) and no specific reason for this could be found. The climate of the Valley is milder and moister than most other regions from which samples were received and there is a denser cattle population in this dairy farm area. The soil is not predominantly alkaline, a factor which has been related to the survival of leptospires in nature (2).

ACKNOWLEDGMENTS

The authors thank Dr. P. J. Quinn and Miss Nettie Guthrie, Ontario Veterinary College, Guelph for supplying the live *Leptospira* serotypes and technical advice.

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