NOTE

***Leptospira interrogans* serovar *hardjo* Infection in Cows of Hokkaido, Japan—Serological Evidence**

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Leptospira interrogans serovar *hardjo* has been isolated from cattle in many parts of the world [1, 4, 5, 6, 9, 10, 12, 13, 15, 16, 17] since the first isolation from cattle was made in 1960 in the United States [14]. The symptoms of *hardjo* infection in cattle are pyrexia, atypical mastitis (flaccidity of udders, secretion of abnormal milk) and abortion [6, 7, 13, 17, 18]. Inapparent infection of many cattle with *hardjo* has been observed [1, 3, 8, 11]. Clinical normal cattle is considered to act as a reservoir host [12]. Serovar *hardjo*, a member of the Sejroe serogroup, has been known to cross-react serologically with *hebdomadis* [2]. Serovar *hebdomadis* has been isolated from cattle of Japan. Neither the isolation of *hardjo* nor serological evidence of infection of cattle with *hardjo* has been reported in Japan. In 1982, in the northern part of Hokkaido, Japan, a cow died showing hemoglobinuria. Serological examination indicated that the cow had been infected with *hardjo*, and hence, the distribution of antibodies to *hardjo* in cows of Hokkaido was also studied.

In June 1982, a 6-year-old cow in Teshio-cho, Hokkaido, developed downer, and on the next day, it showed depression, inappetence, fever, anemia, he-

moglobinuria, and then died. The clinical signs led us to suspect that the cow had been infected with leptospira. Examinations for piroplasmiasis and bracken poisoning produced negative results. The serum antibody of the cow was negative in microscopic agglutination tests [19] to *icterohaemorrhagiae*, *autumnalis*, *canicola*, *australis*, *pyrogenes*, and *pomona*. With the exception of *pomona*, these serovars have been known to exist in Japan. The serum, on the other hand, showed a low level of positive reaction against *hebdomadis* (1:40). Hence, the serum of the diseased cow was tested with *hardjo*, with the result that the agglutination titer was 1:320 to *hardjo*. Histopathologically, focal necrosis in the liver was found. Urinary hemcast and parenchymatous degeneration in the kidneys were also found which were inconsistent with the lesions observed in the case of bovine leptospirosis due to *hebdomadis*, *autumnalis*, and *pomona*.

Paired sera were collected from 15 cows which were housed with the diseased cow in the same barn on 24 June, (11 days after the death of the diseased cow) and on 19 July, 1982 (Table 1). The sera of 9 of these agglutinated *hardjo* at 1:40 or more on 24 June. Of these, 5

developed a four-fold increase and 2 a two-fold increase in *hardjo* titer. The animals were negative to all the serovars described above at 1:10 serum dilution, except to *hebdomadis*, which was aggluti-

Table 1. Agglutination titers to *hardjo* of sera from 15 cows housed with the diseased cow in the same barn

Cow No.	24 June	19 July	rate of increase
1	80	160	×2
2	1280	1280	×1
3	—	—	
4	640	2560	×4
5	—	—	
6	—	—	
7	40	40	×1
8	—	—	
9	320	1280	×4
10	—	—	
11	80	320	×4
12	80	320	×4
13	320	640	×2
14	320	1280	×4
15	—	—	

A cow died on 13 June 1982 in the same barn showing hemoglobinuria and a titer of 1:320 to *hardjo*.

Titers are expressed as reciprocals of serum dilutions which caused 50% agglutination. Dashes indicate that the serum titer was less than 10.

nated by these sera at a dilution of 1:10–1:160 and the titers were 4–32 times lower than those to *hardjo*. The fact that the sera of the cows housed in the same barn increased the *hardjo* titer during the one-month period after the death of the diseased cow indicates that *hardjo* infection was already present in this herd. The cows of this herd were housed most of the time except a daily grazing for a few hours. The conditions of the housing and grazing were thought to be satisfactorily sanitary. No cows in this herd have been imported from abroad. All were born in northern Hokkaido. An attempt to isolate leptospiras from the kidneys and urine of the diseased cow by cultivation and inoculation into guinea pigs resulted in negative. However, the materials were obtained only after long preservation and were not suitable for isolation.

The sera of cows collected in Hokkaido from 1977 to 1983 were examined for the antibodies to *hardjo*. The number of sera which agglutinated *hardjo* at a dilution of 1:320 or more was shown in Table 2. The sera collected from apparently

Table 2. Distribution of *hardjo*-seropositive cattle in Hokkaido (1977–83)

Place		Ratio of positive sera collected from cows in the following year				
Town or city	Location in Hokkaido	1977	1979	1981	1982	1983
Teshio	North		0/10 (0%)		26/145 (18%)	
Horonobe	North			29/37 (78%)	3/8 (38%)	7/10 (70%)
Enbetsu						
Shosanbetsu	North		0/11 (0%)	0/12 (0%)	0/31 (0%)	
Haboro						
Tomamae	North		0/29 (0%)		3/30 (10%)	
Hokuryu	Center					0/11 (0%)
Hiroshima	Center	13/236* (5.5%)				
Ebetsu						
Sapporo	Center					0/16 (0%)
Chitose	Center					7/39 (18%)
Nakashibetsu	East					43/21 (14%)

* Numerators indicate number of cows which had titers of 1:320 or more, while denominators denote number of cows tested.

healthy cows from Teshio in 1979 were all negative (0/10, 0%), and the sera collected in 1982 were positive at a ratio of 26/145 (18%). The latter included 7/15 (47%) of the herd where the diseased cow was found, as shown in Table 1. In Horonobe, where a cow died on 13 July 1982 showing hemoglobinuria, 7 positive reactors were found 5 months later, in 1983 in 10 cows tested (70%) in the same herd. It was found in the same town that a positive ratio of the sera randomly collected from apparently healthy cows was 29/37 (78%) in 1981 and 3/8 (38%) in 1982. The ratio of positive reactors found in the Horonobe cows was thus considered to be high. No positive reactors were found in the apparently healthy cows of Ebetsu, Shosanbetsu, and Haboro in 1979–1982, and in Hokuryu, Sapporo and Ebetsu in 1983. However, positive reactors were found in apparently healthy cows randomly collected from Hiroshima in 1977, Tomamae in 1982, and Chitose and Nakashibetsu in 1983 at a ratio ranging from 5.5 to 18%. The results thus revealed that *hardjo* infection of cattle had already occurred in Hokkaido from the year 1977. The kidneys of the serologically positive 9 cows from other herds of Teshio were examined when slaughtered. Isolation of leptospiras was negative.

Although histopathological examination of the 2 diseased cows in Teshio and Horonobe did not show sufficient evidence of leptospirosis, the occurrence of *hardjo* infection of cattle in Hokkaido was indicated by the present seroepidemiological investigation, including the examination of the paired sera. This is the first evidence of *hardjo* infection of cattle in Japan. Isolation of the causative agent, signs of illness and loss due to *hardjo* infection of cattle in Japan should be studied in future.

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REFERENCES

1. Collins, M. T., Gallegos, T. A., Reif, J. S., and Adrian, W. T. (1981). J. Am. Vet. Med. Assoc. **179**, 1136–1139.
2. Dikken, H., and Kmety, E. (1978). Methods in Microbiology **11**, 259–308.
3. Elder, J. K., and Ward, W. H. (1978). Aust. Vet. J. **54**, 297–300.
4. Gordon, L. M. (1977). Aust. Vet. J. **53**, 227–229.
5. Hathaway, S. C., Little, T. W. A., and Stevens, A. E. (1982). Vet. Rec. **111**, 58.
6. Higgins, R. J., Harbourne, J. F., Little, T. W. A., and Stevens, A. E. (1980). Vet. Rec. **107**, 307–310.
7. Hoare, R. J., and Claxton, P. D. (1972). Aust. Vet. J. **48**, 228–232.
8. Hussain, B., Gbadamosi, S. G., and Siddique, I. H. (1978). Can. J. Comp. Med. **42**, 373–375.
9. Lake, D. E. (1973). N. Z. Vet. J. **21**, 52.
10. Michna, S. W., Ellis, W., and Dikken, H. (1974). Res. Vet. Sci. **17**, 133–135.
11. Milner, A. R., Wilks, C. R., and Calvert, K. (1980). Aust. Vet. J. **56**, 327–330.
12. Orr, H. S., and Little, T. W. A. (1979). Res. Vet. Sci. **27**, 343–346.
13. Robertson, A., Boulanger, P., and Mitchell, D. (1964). Can. J. Comp. Med. Vet. Sci. **28**, 13–18.
14. Roth, E. E., and Galton, M. M. (1960). Amer. J. Vet. Res. **21**, 422–427.
15. Stoenner, H. G. (1967). J. Am. Vet. Med. Assoc. **151**, 1087–1090.
16. Sullivan, N. D., and Stallman, N. D. (1969). Aust. Vet. J. **45**, 281–283.
17. Sullivan, N. D., and Callan, D. P. (1970). Aust. Vet. J. **46**, 537–539.
18. Sulzer, C. R., Shotts, E. B. Jr., Olsen, C. D., Galton, M. M., and Stewart, M. A. (1964). J. Am. Vet. Med. Assoc. **144**, 888–890.
19. WHO Expert Group (1967). WHO Tech. Rep. Ser. **380**.

要 約

北海道の牛における *Leptospira interrogans* serovar *hardjo* の感染——血清学的検討（短報）：土本まゆみ・喜田 宏・梁川 良・乾 純夫¹⁾（北海道大学獣医学部家畜衛生学講座,¹⁾家畜衛生試験場）——北海道天塩町で乳牛1頭が血色素尿を示して突然死亡した。病理組織学的にはわが国の牛レプトスピラ病および *pomona* による本病の所見とは一致しなかったが、その血清は *hardjo* に対し1:320の凝集価を示し、同居牛15頭中7頭のペア血清でも抗体価の上昇が認められた。これを機に北海道の牛の *hardjo* に対する抗体調査を行なった。その結果、1977年以降道内各地で抗体陽性牛が認められ、特に隣接の幌延町で多くの抗体陽性牛が認められた。