堺市・高知市および福井市における野犬のレプトスピラ保有状況について

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Researches for Leptospirosis of the Stray Dogs in Sakai-, Kôchi- and Fukui-City

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Canine leptospirosis appears to have world-wide distribution and to be important not only from the stand point of the disease as manifested in dogs, but also from the public health as dogs play an essential rôle in the human infection of canicola fever. In our country, the incidence of canine leptospirosis has established chiefly in Tôkyô[4,6,19] and Kyûsyû[5,7,8,10,15,20], lacking in the survey in other districts. As the geographical distribution of the disease is very significant, I intended to survey its incidence in Sakai-, Kôchi- and Fukui-city, and following results were obtained.

Materials and methods

The materials are as follows: 471 stray dogs captured at Sakai-city from Dec. 1952 to Nov. 1953, 11 at Kôchi-city Dec. 1953 and 23 at Fukui-city Nov. 1954. All of the 505 serum samples were tested by Schüffner-Mochtar agglutinationlysis test (S–M test) and the positive titer was settled on 1 : 300 or more. The isolation of leptospira was attempted from the kidneys by means of culture in Korthof's medium at 27°C after the examination under dark-field microscopy.

After noting the gross changes of the kidney, a wedge of tissue was fixed in 10% formalin, and following stains were used: hematoxlin-eosin, elastica Van Gieson, silver impregnation of TATEDA and Levaditi.

The standard strains of leptospira and standard serum samples were donated by Dr. S. YAMAMOTO, Professor of Tôkyô University.

Results

1. Culture of the kidney. Fortyone strains were isolated, of which 26 strains were identified as *L. canicola* after S–M test. As the remaining 15 were missed on account of the insufficient growth or contamination, they were identified by host serum, of which 11 were positive *L. canicola* and 4 were *L. icterohaemorrhagiae* (*L. ictero.*).

Three strains were isolated in Kôchi, and 2 in Fukui and all of them were positive *L. canicola.*

2. Serological examination. Out of the 471 serum samples of Sakai, 227 (48.2%) were positive with titers 1 : 300 or more, of which 202 were positive with *L. canicola*, 14 with *L. ictero.* and 11 showed same titers with *L. canicola* and *L. ictero.*.

Each 4 samples of Kôchi and Fukui were positive with *L. canicola*, and remaining one of Kôchi with *L. ictero.*.

In regard to the incidence of the leptospira carriers and the serological positive cases in Sakai, the monthly and seasonal fluctuation was shown in Table 1. The incidence of the former was high in Dec. 1952 (18.7%), Feb. 1953 (16.1%) and May. 1953 (13.4%), and seasonally it was high in winter (13.4%) and low in summer (2.8%). The incidence of the latter was rather constant (from 39.5% to 58.7%) in each month, and somewhat high in winter (53.7%).

Table 1. Incidence of canine leptospirosis.

City	Month	Number tested	Culture Positive *L.c.* S-M test on the isolated strain	Culture Positive *L.i.* S-M test on the isolated strain	Culture Positive *L.c.* S-M test on the host serum	Culture Positive *L.i.* S-M test on the host serum	Total	Percent	Seasonal incidence (%)	Serological Positive *L.c.* Number of dog with antibodies	Serological Positive *L.c.* & *L.i.* Number of dog with antibodies	Serological Positive *L.i.* Number of dog with antibodies	Total	Percent	Seasonal incidence (%)	Nephritis Posivite	Percent
Sakai	1952 Dec.	64	9	0	1	2	12	18.7	18/134 (13.4%)	30	2	4	36	56.3	72/134 (53.7%)	33	54.1
	1953 Jan.	39	1	0	0	0	1	2.6		19	1	1	21	53.8		28	71.8
	Feb.	31	4	0	1	0	5	16.1		14	1	0	15	48.8		15	48.1
	Mar.	41	2	0	1	0	3	7.3	11/108 (10.2%)	18	0	1	19	46.3	50/108 (46.3%)	21	51.2
	Apr.	29	1	0	2	0	3	10.4		12	1	0	13	44.4		14	48.3
	May.	38	3	0	1	1	5	13.2		15	1	2	18	47.4		21	55.3
	June	43	1	0	0	0	1	2.4	3/107 (2.8%)	16	1	0	17	39.5	46/107 (43.0%)	17	39.5
	July	34	0	0	0	0	0	0		16	0	0	16	47.5		15	44.1
	Aug.	30	0	0	2	0	2	6.7		12	0	1	13	43.3		11	36.7
	Sep.	38	1	0	1	0	2	5.3	9/122 (7.4%)	13	1	1	15	39.5	59/122 (48.4%)	18	48.4
	Oct.	38	3	0	0	0	3	7.9		14	1	2	17	44.7		17	44.7
	Nov.	46	1	0	2	1	4	8.7		23	2	2	27	58.7		25	54.3
	Total	471	26	0	11	4	41	8.7		202	11	14	227	48.2		235	49.7
Kôchi	1953 Dec.	11	3	0	0	0	3	27.3		4	0	0	4	36.4		7	63.6
Fukui	1954 Nov.	23	2	0	0	0	2	8.7		4	0	1	5	21.7		5	22.7

Note: *L.c.*—*Leptospira canicola*, *L.i.*—*L. icterohaemorrhagiae*.

3. Pathological findings of the kidneys. As it has been well established that canine leptospirosis is the most important etiologic factor of interstitial nephritis of the dog, I gave my best attention to this change. Out of the 505 kidneys, 247 (48.9%) had various lesions of interstitial nephritis, which were classified in following 4 types according to the pathological process.

I-type (acute, slight)—Macroscopically most of the kidneys showed no lesions, and the capsules stripped easily, but in some cases there were slight cloudy swelling. Microscopically, the very small foci or diffuse lesions of infiltration of lymphocytes were recognized in the interstitial tissue, where few plasma cells, histiocytes were present in some cases, but no fibroblasts. Slight degeneration of tubules and increased cellularity of glomeruli were seen sometimes.

II-type (acute, sever)—Macroscopically, the kidneys were often enlarged and pale and the capsules were not adherent. This type was characterized by the presence of scattered

small foci or diffuse areas of gray-white colour chiefly in the deeper parts of the cortex. Microscopically, the cellular infiltrations were quite remarkable, which were intertubular, perivascular and periglomerular in distribution. The infiltrating cells were principally lymphocytic-type cells which were accompanied by variable numbers of plasma cells, histiocytes and neutrophils, and the fibroblasts began to appear in the lesions.

III-type (subacute)—The kidneys were enlarged slightly, pale mottled with grayish scars, and somewhat firm on palpation. The capsule stripped not easily. Microscopically, fibroblasts increased in the areas of cellular infiltration with subsequent proliferation of fibrous connective tissue. The tubules in the areas were often degenerate or even destroyed and hyaline casts were often seen.

IV-type (chronic)—The kidneys were shrunken, contracted and very firm, and showed nodular surface. The cortex was decreased in size. Connective tissue proliferation with hyalinization was extensive in all instances, and corticomedullary zone was more involved. Marked tubular changes as atrophy, disappearance, and cystic dilatation some with atrophic flattened epithelium and others with hyperplastic epithelium were commonly observed.

As can seen in Table 2, interstitial nephritis was observed in all of the 46 kidneys from which leptospira was isolated, and 212 (89.8%) of the 236 serologically positive cases, whereas it was seen in only 35 (13.0%) of 269 serologically negative ones.

Table 2. Correlation of leptospira isolation, antibodies and interstitial nephritis.

Interstitial nephritis	Isolation of lept.	Serological		Total
		Positive	Negative	
Without lesion	0	24 (10.2%)	234 (87.0%)	258 (51.1%)
With lesions				
I-type	13	52	15	67
II-type	13	66	8	74
III-type	15	60	8	68
IV-type	5	34	4	38
	46 (100%)	212 (89.8%)	35 (13.0%)	247 (48.9%)
Total	46	236	269	505

All dogs from Sakai, Kôchi and Fukui were summed up.

Discussion

Isolation of leptospira. The ratio of the isolation of leptospira was 8.7% in Sakai, 27.3% in Kôchi and 8.7% in Fukui. Except in Kôchi, these ratios were in the range of those in past reports of our country, i.e. KITAOKA[4] 2.5% in Tôkyô, MAJIMA[7] 4.4% in Kita-Kyûsyû, KUBO[6] 6.3% in Tôkyô, YAMAMOTO[19] 6.4% in Tôkyô, MISAO et al.[10,11] 6.6% in Fukuoka-city, OTSUKA[15] 7.4% in Fukuoka prefecture and MANAKO et al.[8] 10.4% in Yamaguchi. Thus a certain number of the stray dogs were proved to carry leptospira, and to serve as the source of canine and human infection, though the year and the district of survey were different.

An unusual high ratio of leptospira isolation (27.3%, 21.4%) was reported by KOBAYASHI[5] on the stray dogs captured at the village where human canicola fever had been prevalent. The reason why such a high incidence was obtained in my survey in Kôchi remained unclear, as the number of the dogs examined were insufficient to draw conclusions.

Most of the isolated strains belonged to *L. canicola*, agreeing with the past reports in our country which indicated only a few incidence of *L. ictero.* : KITAOKA[4] 2 strains

from stray dogs, YAMAMOTO[18] 3 strains from an icteric dog and two subclinical dogs and NODA et al.[12] 1 strain from an icteric dog.

Serological reaction. I observed 48.2% of serologically positive cases in Sakai, which was higher than that of TSUBOSAKI et al.[17] (13.4% in Yamaguchi), OKADA[13] (17.8% in Matsuyama), MISAO et al.[10] (23.6% in Fukuoka), YOSHIDA et al.[20] (31.9% in Nagasaki), TSUBOSAKI et al.[16] (34.1% in Kôchi), KITAOKA[4] (35.7% in Tôkyô) and MAJIMA[7] (36.1% in Kita-Kyûsyû), resembling to YAMAMOTO[19] (45% in Tôkyô) and MANAKO et al.[8] (45.5% in Fukuoka) and lower than OTSUKA[14] (69.1% in Fukuoka) and KOBAYASHI[5] (54.5% and 62.5% in Fukuoka) who surveyed at the districts where human canicola fever had been prevalent.

Concerning to the serological type, a large majority (89.0%) of the positive samples of Sakai had antibodies against *L. canicola* and only 6.2% against *L. ictero.*, showing same tendency as the isolated strains. The incidence of the *L. ictero.* was somewhat lower than that of YAMAMOTO[19] (17 in 162—10.5%), OKADA[13] (4 in 37—10.8%), MANAKO et al.[8] (24 in 215—11.2%) YOSHIDA et al.[20] (8 in 68—11.8%) and MISAO et al.[10] (16 in 108—14.8%), and considerable lower than that of TSUBOSAKI et al.[16] (20 in 73—27.8%) and KITAOKA[4] (72 in 162—44.4%).

Pathological findings. As the result of the pathological examination of the kidneys, interstitial nephritis was observed very commonly (48.9%) which were classified in 4 types according to the pathological process. YAMAMOTO[19] classified it in 3 types, OTSUKA[14] in 4 types and MCINTYRE et al.[9] and BLOOM[1,2] in actue, subacute and chronic types. It is appropriate that these types are related to each other and transform from acute type of chronic type in turn. It was proved that the intimate relationship exists between the leptospira infection and interstitial nephritis, and the former is the most important etiological facter of the latter in the stray dogs of our country. These results agreed with YAMAMOTO[18] and OTSUKA[14], but somewhat differed from FREUDIGER[3] who observed no pathological difference between serological positive and negative dogs.

Summary

Researches for canine lepotspirosis were carried out on the 471 stray dogs captured at Sakai-city, 11 at Kôchi-city and 23 at Fukui-city and following results were obtained.

1. The 41 strains of leptospira were isolated from the stray dogs at Sakai, of which 26 were proved to be *L. canicola*. Remaining 15 were identified indirectly by host serum, of which 11 were *L. canicola* and 4 were *L. icterohaemorrhagiae*. Three strains were isolated in Kôchi and 2 in Fukui and all of them were *L. canicola*. All cases carrying leptospira in their kidneys were serologically positive.

2. Out of the 471 serum samples of Sakai, 227 (48.2%) were positive by serological examination, of which 202 were positive with *L. canicola*, 14 with *L. icterohaemorrhagiae* and 11 showed same titers with *L. canicola* and *L. icterohaemorrhagiae*. Each 4 samples of Kôchi and Fukui were positive with *L. canicola* and remaining one of Kôchi with *L. icterohaemorrhagiae*.

3. Out of the 505 kidneys of Sakai, Kôchi and Fukui, 247 (48.9%) had various lesions of interstitial nephritis which were classified in the following 4 types according to the pathological process, i.e. I-type (acute, slight), II-type (acute, severe), III-type (subacute) and IV-type (chronic). Interstitial nephritis was observed in all of the 46 kidneys carrying leptospira and also in 212 (89.8%) of the 236 serologically posive cases, whereas in only 35 (13.0%) of 269 serologically negative ones. Thus the considerable correlations between leptospira isolation, antibodies and interstitial nephritis were demonstrated.

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References

1) BLOOM, F.: Arch. Path., **28**: 236, 1939.
2) BLOOM, F.: J. Amer. Vet. Med. Ass., **91**: 679, 1937.
3) FREUDIGER, U.: Inaug. Diss. Vet. Med. Univ. Bern, 1952.
4) KITAOKA, M.: Jikken-igaku-zasshi, **24**: 861, 1940.
5) KOBAYASHI, U.: J. Jap. Soc. Intern. Med., **44**: 1032, 1955.
6) KUBO, I.: Mitt. Med. Ges. Tôkyô, **52**: 13, 1938.
7) MAJIMA, T.: J. Jap. Ass. Inf. Dis., **30**: 366, 1956.
8) MANAKO, K., OTSUKA, S., TSUJIKAWA, H., NISHIKAWA, N. and KATSUKI, H.: Med. Biol., **45**: 183, 1957.
9) MCINTYRE, W. I. M. and MONTGOMERY, G. L.: J. Path. Bact., **64**: 145, 1952.
10) MISAO, T., HIROYOSHI, S., KATSUTA, K., NISHIHARA, Y., KOBAYASHI, Y. and KUWAJIMA, K.: J. Jap. Soc. Intern. Med., **42**: 101, 1953.
11) MISAO, T., KATSUTA, K., NISHIHARA, Y., KOBAYASHI, Y. and KUWAJIMA, K.: ibid., **43**: 701, 1954.
12) NODA, S., OKA, T. and TAKAOKA, H.: J. Vet. Med., **199**: 1085, 1956.
13) OKADA, A.: J. Jap. Vet. Med. Ass., **11**: 210, 1958.
14) OTSUKA, S.: Fukuoka Acta Med., **45**: 703, 1954.
15) OTSUKA, S.: Jap. J. Vet Sci., **15**: 47, 1954.
16) TUBOSAKI, H. and AOKI, T.: Jap. J. Bact., **10**: 395, 1955.
17) TSUBOSAKI, H., AOKI, T., YAMAMOTO, Y., ÔISHI, S. and HASEGAWA, B.: ibid., **11**: 179, 1956.
18) YAMAMOTO, S.: Jap. J. Vet. Sci., **2**: 39, 1940.
19) YAMAMOTO, S.: ibid., **5**: 45, 1943.
20) YOSHIDA, S. and NISHIDA, K.: Nagasaki Med. J., **30**: 1485, 1955.