103. Studies on *Leptospira*. III
On the *Leptospira australis* A Strains Isolated
from Man and Animals in Japan

By Shutaro YAMAMOTO

Laboratory of Veterinary Pathology, Faculty of Agriculture,
University of Tokyo

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A new *Leptospira* type, *Leptospira hebdomadis* C, was reported by Shiozawa and Kubo¹⁾ in 1938. It had been isolated from a comparatively severe human case of Akiyami, or autumn fever, in an area along the Tenryu River in Shizuoka prefecture. In Japan, before the end of World War II, only one strain of this type, Hebdomadis C, was isolated and some researchers tried to find any case of infection with this type by serological examination of serum samples. In 1942 Shiozawa and Kubo²⁾ found one serum drawn from a human case of Sakushu fever in Okayama prefecture, which showed a titer of 3000 with the Hebdomadis C strain in the agglutination-lysis test.

In 1943 the author³⁾ found that this strain was serologically distinct from *L. hebdomadis* and other Japanese strains of leptospires and named it *L. tenryuensis*. After that he received from Dr. Mochtar the Ballico strain,⁴⁾ the type strain of *L. australis* A,⁵⁾ and demonstrated by Schüffner and Bohlander's agglutinin-absorption test that these 2 strains were serologically identical with each other. He claimed in 1944^{6,7)} that *L. australis* A Lumley 1937⁵⁾ be the legitimate name of this type.

In 1953, one strain of *L. australis* A, Kochi C strain, was isolated by Tsubosaki and Aoki⁸⁾ from the urine of a patient who was suffering from iritis in Kochi prefecture. This is the second strain of *L. australis* A isolated in Japan.

In the field of veterinary research, the author⁹⁾ examined, in the autumn of 1949, 24 bovine sera of hemoglobinuria in Hyogo prefecture, of which 6 sera reacted positively with the type strain of *L. australis* A, 15 sera with that of *L. autumnalis*, and 3 sera with *L. hebdomadis*. The presence of the bovine hemoglobinuria caused by leptospires was thus serologically demonstrated for the first time in Japan. In the autumn of 1950, two strains of *L. hebdomadis* were isolated by the author and Watanabe.¹⁰⁾ In the autumn of 1953, two strains of *L. autumnalis* were isolated by Yanagawa and his co-workers¹¹⁾ from the urine of diseased cattle in the same prefecture. In the autumn of 1955, Takamura and his co-workers¹²⁾ isolated one

strain of *L. australis* A, Kimura strain, from the urine of diseased cattle in the same prefecture. This may be the first strain of *L. australis* A isolated from cattle.

In the autumn of 1955 many strains were isolated from dogs in Ehime prefecture by the author and the research officers of the Ehime prefectural government working in co-operation.¹³⁾ Of these strains, 3 were closely related with the Ballico strain serologically.

In Europe, the München C 90 strain which was closely related with the Ballico strain was isolated by Rimpau from a human case of so-called field fever in Germany. Rimpau¹⁴⁾ considered this strain to be identical with the Ballico strain and named the disease caused by this type of *Leptospira* field fever C. Wolff and his co-workers¹⁵⁾ compared this strain with the Ballico in Schüffner and Bohlander's cross-absorption tests and confirmed the presence of a small difference between them. Then it became necessary to compare the Japanese strains of *L. australis* A derived from man and animals with the original strains Ballico and München C 90 in cross-absorption tests.

Materials and methods. The Ballico strain was received from Dr. Mochtar, of Batavia. The München C 90 strain was received from Professor Wolff in 1954 when the author was traveling in Europe. Of the Japanese strains, the Hebdomadis C and Kochi C were isolated from human cases, the Ehime 1, 4, and 5 strains from dogs in Ehime prefecture, and the Kimura strain was from a bovine case of hemoglobinuria. The serological technics applied were described in the previous paper¹⁶⁾¹⁷⁾ in detail.

Results. 1. *Agglutination-lysis tests between Japanese strains and rabbit immune sera against Ballico and München C 90.* The results are shown in Tables I and II by means of the author's notation system.¹⁶⁾ In these tables the Japanese strains are more closely related with the Ballico strain than the München C 90.

Table I. Agglutination-lysis reactions between 8 strains and rabbit immune serum against the Ballico strain

Strain	Dilution of immune serum against Ballico									Control
	10	30	100	300	1000	3000	10000	30000	100000	
Ballico	30+++	3++	12++	12++	20++	70++	80—	80—	80—	80—
München C 90	3++	12++	20++	40++	40+	50+	60—	60—	60—	60—
Hebdomadis C	20+++	5++	12++	3++	5++	40++	70+	80—	80—	80—
Kochi C	24+++	16++	12++	20++	32++	70+	80—	80—	80—	80—
Kimura	30+++	15++	10++	18++	20++	100++	120—	120—	120—	120—
Ehime 1	12+++	3++	12++	4++	12++	90+	100—	100—	100—	100—
Ehime 4	40+++	15++	5++	12++	20++	70+	80—	80—	80—	80—
Ehime 5	20+++	12++	12++	12++	20++	70+	80—	80—	80—	80—

Table II. Agglutination-lysis reactions between 8 strains and rabbit immune serum against the München C 90 strain

Strain	Dilution of immune serum against München C 90									Control
	10	30	100	300	1000	3000	10000	30000	100000	
Ballico	18+++	8++	10++	60++	80—	80—	80—	80—	80—	80—
München C 90	15+++	10+++	5+++	3++	4++	10++	60++	80—	80—	80—
Hebdomadis C	6++	12++	80++	100++	110+	120—	120—	120—	120—	120—
Kochi C	9++	8++	24++	70++	90+	100—	100—	100—	100—	100—
Kimura	4++	16++	80++	100+	120—	120—	120—	120—	120—	120—
Ehime 1	2++	2++	60++	90++	100+	100—	100—	100—	100—	100—
Ehime 4	10+++	5++	50++	90++	100—	100—	100—	100—	100—	100—
Ehime 5	10+++	4++	24++	60++	80—	80—	80—	80—	80—	80—

2. *Comparison of strains in agglutinin-absorption tests.* Table III indicates the results of cross-absorption tests on the Ballico, München C 90, and 6 Japanese strains. From these results it is clear that the 6 Japanese strains have no other antigenic fractions than those of the Ballico and none of the fractions of the München C 90 which are not included in the Ballico.

Table III. Cross-absorption tests comparing the Ballico, München C 90, and 6 Japanese strains

Strain	Immune serum against			
	Ballico		München C 90	
	Before absorption	After absorption with München C 90	Before absorption	After absorption with Ballico
Ballico	3000	300	300	—
München C 90	1000	—	10000	1000
Hebdomadis C	3000	300	1000	—
Kochi C	3000	300	1000	—
Kimura	3000	300	300	—
Ehime 1	3000	300	1000	—
Ehime 4	3000	100	300	—
Ehime 5	3000	300	300	—

Tables IV to VII show the identification of 4 of the 6 Japanese strains with the Ballico. The results from the remaining 2 strains, Ehime 4 and 5, derived from dogs are not given, because they are essentially the same with those from the Ehime 1 strain in Table VII.

Table IV. Cross-absorption tests comparing the Ballico and Hebdomadis C strains

Strain	Immune serum against			
	Ballico		Hebdomadis C	
	Before absorption	After absorption with Hebdomadis C	Before absorption	After absorption with Ballico
Ballico	3000	—	1000	—
Hebdomadis C	3000	—	3000	—

Table V. Cross-absorption tests comparing the Ballico and Kochi C strains

Strain	Immune serum against			
	Ballico		Kochi C	
	Before absorption	After absorption with Kochi C	Before absorption	After absorption with Ballico
Ballico	3000	—	3000	—
Kochi C	3000	—	3000	—

Table VI. Cross-absorption tests comparing the Ballico and Kimura strains

Strain	Immune serum against			
	Ballico		Kimura	
	Before absorption	After absorption with Kimura	Before absorption	After absorption with Ballico
Ballico	3000	—	3000	—
Kimura	3000	—	3000	—

Table VII. Cross-absorption tests comparing the Ballico and Ehime 1 strains

Strain	Immune serum against			
	Ballico		Ehime 1	
	Before absorption	After absorption with Ehime 1	Before absorption	After absorption with Ballico
Ballico	3000	—	3000	—
Ehime 1	3000	—	3000	—

Summary. In Japan, one strain of *L. australis* A was isolated in 1937 from a patient of so-called autumn fever in Shizuoka prefecture. A second strain of this type was obtained in 1953 from a human case of iritis in Kochi prefecture. In 1955, isolation of a third strain was successful in a case of bovine hemoglobinuria in Hyogo prefecture and that of 3 more strains in dogs in Ehime prefecture. These 6 Japanese strains were found to be identical with the Ballico strain in agglutinin-absorption tests. Therefore, in Japan, the distribution of *L. australis* A in man, cattle, and dogs has been ascertained.

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