

## スナネズミを用いた実験的レプトスピラ症の検討

誌名	日本大学農獣医学部学術研究報告
ISSN	00780839
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巻/号	45号
掲載ページ	p. 294-299
発行年月	1988年3月

## Further Studies on Experimental Leptospirosis in Mongolian Gerbils

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(Accepted Oct. 1, 1987)

**ABSTRACT.** Experimental infections of leptospirosis in mongolian gerbils (*Meriones unguiculatus*) with strains of various serovars of leptospire (*autumnalis*, *canicola*, *hebdomadis*, *icterohaemorrhagiae*, and *copenhageni*) were carried out to evaluate the suitability of mongolian gerbils in leptospiral study and the effects of the specific antiserum and procaine penicillin-G in treating leptospirosis. It was demonstrated that the animals inoculated with newly isolated strains resulted in a fatal infection associated with severe symptoms of leptospirosis. The suitability of mongolian gerbil as a test animal for the study of leptospirosis was accordingly concluded, and the positive effects of the specific antiserum and procaine penicillin-G were also confirmed.

**Key words:** Leptospira, Leptospirosis, *Meriones unguiculatus*, Mongolian gerbil

Guinea-pigs [1, 2], hamsters [3, 4, 5], and mice of 3-week-old [6, 7, 8] are known as the suitable animals for the study of leptospirosis of some serovars. Regarding mongolian gerbil, van der Hoeden [9] reported that *Meriones shawi* and *Meriones crassus sacramenti* were the excellent animals in leptospiral investigations because of their great susceptibility to several leptospiral serovars, *grippotyphosa*, *icterohaemorrhagiae*, *canicola*, *ballum*, *sejroe*, and *pomona*, but *Meriones guentheri* showed marked resistance to some of the serovars. Imamura *et al.* [10] also reported about the susceptibility of *Meriones unguiculatus* to the virulent serovars, *icterohaemorrhagiae* and *autumnalis*. Yukawa [11], in addition, demonstrated the suitability of *Meriones unguiculatus* in testing the potency of leptospiral vaccines.

This report deals with the susceptibility of mongolian gerbil to the experimental infections with strains of various serovars of leptospire including some new isolates, *autumnalis*, *canicola*, *hebdomadis*, *icterohaemorrhagiae*, and *copenhageni*, and with the suitability of the animal used for testing the potency of therapeutic antiserum as well as the effect of procaine penicillin-G in treating experimental leptospirosis.

### Materials and Methods

**Organisms:** The serovars and the strains of leptospire used were as follows: *autumnalis* strains MH-5 and Akiyami A; *canicola* strains TTD-262 and TTD-354; *hebdomadis* strains MH-1 and Akiyami B; *icterohaemorrhagiae* strains UR-157, UR-159, RGA, and Uchida; and *copenhageni* strains Shibaura and HR-101. Of the strains, TTD-262 and TTD-354 from dogs, UR-157, UR-159 and HR-101 from rats were all isolated by the authors and identified at the Leptospirosis Reference Laboratory (LRL) of the National Institute of Health, Japan. The rest of the strains were obtained from the LRL. Strain Shibaura is a virulent organism used to challenge the guinea pigs in the potency tests of leptospiral vaccines and antisera at the LRL. Strains MH-1 and MH-5 were isolated from patients in Okinawa, and strains Akiyami A, Akiyami B, RGA and Uchida have been passaged over a long period of time at the LRL. The organisms were cultured in Korthof's media [12] containing 10% of rabbit serum at 30°C for 7-10 days before use. The cell numbers of leptospire were counted by using Thoma's counting chamber (depth: 0.01 mm) modified for dark-field

illumination. Investigations on the susceptibility of gerbils to these strains were performed in 1979-1981. (Table 1)

Animals: The mongolian gerbils (*Meriones unguiculatus*), 57-65 gm in weight, were obtained from Prof. Y. Nakajima (Department of Parasitology, Yamanashi Medical College, Japan). They were cagebred at 17 to 28°C room temperature, and fed chow diet, CE-2 (Clea Japan Inc., Tokyo) with water add libitum. No nonspecific death or sign of illness was observed during the course of the experiments.

Inoculations: Animals were intraperitoneally injected with 1.0 ml of inoculum, and were observed daily for signs of illness for 12 to 14 days. Animals survived 12 to 14 days were killed with chloroform, and examined for jaundice and hemorrhage at autopsy. Tubes of Flether's medium [13] were inoculated with small pieces of liver and kidney and examined microscopically for the growth of leptospire after 3-week incubation at 30°C.

Serological method: Microscopic agglutination test (MAT) was performed following the method described in the "Guidelines for the control of leptospirosis" [14].

The potency test of specific antiserum was performed following the method described in the "Minimum requirement of biological product (Ministry of Health and Welfare, Japan)" [15]. The therapeutic horse-antiserum and the antigen (strain Shibaura, Lot No. 39) were prepared at the Chemo-Sero-Therapeutic Research Institute in Kumamoto Japan.

Antibiotic: Aqueous procaine penicillin-G (300,000 units/ml) animal (Tamura Pharmaceutical Co., Tokyo) was injected intramuscularly to the test animals immediately prior to the leptospiral inoculation.

## Results

### *Susceptibility of mongolian gerbils to various strains of Leptospire.*

The results of the experimental infections are summarized in Table 2. All the gerbils inoculated with cultures at cell numbers,  $1.7 \times 10^4$ ,  $1.7 \times 10^6$  and  $1.7 \times 10^8$  of the strain MH-5, developed severe symptoms of leptospirosis and died within 4-7 days post inoculation. Generalized jaundice and hemorrhage were seen in subcutaneous tissues and lungs at autopsy. The lungs presented various-sized hemorrhagic foci, similar to the wing of a mottled butterfly. Strain Akiyami A subcultured over a long period of time was proved to be non-virulent and the inoculated gerbils survived the challenge two weeks without clinical sign.

Gerbils inoculated with cultures ( $2.7 \times 10^6$  and  $2.7 \times 10^8$  cells) of strain TTD-262 died from leptospirosis at the 5th and the 6th day post inoculation. Strain TTD-354 was also proved to be virulent to gerbils.

Gerbils infected with strain MH-1 died at the 4th day post inoculation, showing hemorrhage with or without jaundice. Strain Akiyami B was proved to be non-virulent to gerbils.

Strain UR-157 and UR-159 were virulent to gerbils which died with generalized jaundice and

Table 1. Serovars and strains of leptospire used in the study

Source	Origin	Serovar	Strain	Remark
Authors	Dog	<i>canicola</i>	TTD-262 TTD-354	Tokyo, 1977
	Rat	<i>icterohaemorrhagiae</i>	UR-157 UR-159	Tokyo, 1979
		<i>copenhageni</i>	HR-101	Hiratsuka, 1979
*L.R.L.		<i>icterohaemorrhagiae</i>	RGA Uchida	
		<i>autumnalis</i>	MH-5 Akiyami-A	Okinawa, 1976
		<i>hebdomadis</i>	MH-1 Akiyami-B	Okinawa, 1976
		<i>copenhageni</i>	Shibaura	challenge strain (virulent) at LRL

\* L.R.L.: Leptospirosis Reference Laboratory, National Institute of Health, Japan.

Table 2. Experimental infections of gerbils with various strains of leptospire

Serogroup	Leptospira			Gerbil			Day post inoculation		Autopsy		
	Serovar	Strain	Number of cell inoculated	Age (day)	Weight (g)	Sex	Died	Killed	Icterus	Hemorrhage	
Autumnalis	<i>autumnalis</i>	MH-5	$1.7 \times 10^8$	175	60	♂	4		+	+	
			$1.7 \times 10^6$	175	57	♂	6		+	+	
			$1.7 \times 10^4$	166	65	♀	7		+	+	
		Akiyami-A	$2.6 \times 10^8$	53	53	♂		14		-	-
			$2.6 \times 10^8$	82	49	♀		14		-	-
Canicola	<i>canicola</i>	TTD-262	$2.7 \times 10^8$	166	81	♂	5		-	+	
			$2.7 \times 10^6$	162	66	♀	6		-	+	
		TTD-354	$2.8 \times 10^8$	166	82	♂	5		-	+	
Hebdomadis	<i>hebdomadis</i>	MH-1	$2.1 \times 10^8$	70	43	♀	4		+	+	
			$2.1 \times 10^8$	70	63	♂	4		-	+	
		Akiyami-B	$2.8 \times 10^8$	53	39	♀		14		-	-
Ictero-haemorrhagiae	<i>ictero-haemorrhagiae</i>	UR-157	$2.1 \times 10^8$	357	67	♂	4		+	+	
			$1.9 \times 10^8$	175	86	♂	4		+	+	
		RGA	$1.1 \times 10^8$	39	45	♂		12		-	-
		Uchida	$2.8 \times 10^8$	90	59	♂		14		-	-
		Shibaura	$2.4 \times 10^8$	35	34	♂	3		+	+	
			$2.7 \times 10^8$	46	52	♂	5		+	+	
$2.7 \times 10^6$	42		44	♀	5		+	+			
HR-101	<i>copenhageni</i>	$2.8 \times 10^8$	183	75	♂	4		+	+		
		$2.8 \times 10^6$	183	63	♀	7		+	+		
		$2.8 \times 10^3$	167	63	♂	9		+	+		

Table 3. Effect of specific antiserum on leptospiral infection in gerbils

Gerbil			Leptospire			Day post inoculation		Autopsy			
Age (day)	Weight (g)	Sex	Serovar	Strain	Number of cell inoculated	Dilution of antiserum	Died	Killed	Icterus	Hemorrhage	
180	62	♂	<i>copenhageni</i>	Shibaura	$1.2 \times 10^5$	1 : 300		14	-	-	
180	47	♀			$1.2 \times 10^3$			14	-	-	
184	66	♂			$1.2 \times 10^5$	—	5		+	+	
180	61	♀			$1.2 \times 10^3$	—	7		+	+	
215	69	♂			$1.6 \times 10^5$	1 : 1000		14	-	-	
200	76	♂			$1.6 \times 10^3$			14	-	-	
215	67	♂			$1.6 \times 10^5$	—	5		+	+	
200	68	♀			$1.6 \times 10^3$	—	6		+	+	
150	63	♂			$0.9 \times 10^5$	1 : 3000		8		+	+
147	49	♀			$0.9 \times 10^3$			14	-	-	
150	59	♀			$0.9 \times 10^5$	—	6		+	+	
147	50	♀			$0.9 \times 10^3$	—	8		+	+	

hemorrhage at the 4th day post inoculation, but strains RGA and Uchida showed no virulence.

Strain Shibaura and HR-101 were virulent to gerbils that showed generalized jaundice and hemorrhage.

Leptospire were reisolated from livers and kidneys of all gerbils died.

#### *Effect of specific antiserum in leptospiral infection.*

Cultures of the strain Shibaura were mixed with equal volums of 300-, 1,000- and 3,000- fold dilutions of antisera respectively. The mixtures were incubated at 37°C for 30 minutes, and then administered intraperitoneally 1.0 ml to the animals. The results are shown in Table 3. No death occurred among the gerbils injected with antisera of 300- and 1,000- fold dilutions. A fatal infection occurred in the gerbils injected with  $0.9 \times 10^5$  cells and 3,000- fold diluted antiserum, but those injected with  $0.9 \times 10^3$  cells and the same dilution of the antiserum survived 14 days. All the control gerbils challenged without the assistance of antiserum died between 5 to 8 days post inoculation. The MAT titer of the antiserum against strain Shibaura was 1:800.

#### *Effect of antibiotic in leptospiral infection.*

One ml or 0.1 ml of aqueous procaine penicillin-G (300,000 unit/ml) was injected intramuscularly in gerbils immediately prior to the inoculation of the cultures ( $3.1 \times 10^7$  cells) of the strain Shibaura. The treated gerbils survived without any sign of leptospirosis but the control animals died at the 8th day showing icterus and hemorrhage. The results are shown in Table 4.

## Discussion

To select a suitable animal is an important matter for the study of leptospirosis. Of the animals having been studied for the susceptibility to the leptospiral infection, guinea-pig and hamster are the most widely used laboratory animals for the study of the disease after the previous reports [1, 2, 3, 4, 5]. However, it has been known that only the serogroups of Icterohaemorrhagiae and Autumnalis in guinea-pig, and Icterohaemorrhagiae and Canicola in golden hamster cause them a severe and fatal infection.

The susceptibility of mice of 3-week-old to leptospiral infection was reported to limit at 3-week-old ones [6, 7], but it was also reported that the inoculated mice younger than 3 weeks exhibited no sign of leptospirosis [8]. Besides, American deer mice, which are not available in Japan, were reported susceptible to various serovars of leptospire.

Early in 1934, van der Hoeden [9] reported that *Meriones shawi* and *Meriones crassus sacramento* were the animals suitable for the studies of leptospirosis and that gerbils were susceptible to several serovars of leptospire, such as *grippotyphosa*, *icterohaemorrhagiae*, *canicola*, *sejoe*, *ballum* and *pomona*. Levis and Gray [16] reported the susceptibility of *Meriones unguiculatus* to serovars *pomona* and *icterohaemorrhagiae*. Imamura et al. [10] previously reported that strain Akiyami A (serovar *autumnalis*) caused fatal infections with frank signs of leptospirosis on *Meriones unguiculatus*. Tripathy and Hanson [17, 18] reported the experimental infection of serovar *grippotyphosa* in gerbils. Recently, Yukawa [11] reported that *Meriones unguiculatus*

Table 4. Effect of Penicillin on leptospiral infection in gerbils

Gerbil			Leptospire			Day post inoculation			Autopsy	
Age (day)	Weight (g)	Sex	Serovar	Strain	Number of cell inoculated	*Pc-G (ml)	Died	Killed	Icterus	Hemorrhage
	118	♂	<i>copenhageni</i>	Shibaura	$31. \times 10^7$	1.0		14	—	—
	85	♂						14	—	—
145	70	♂					14	—	—	
	91	♂					14	—	—	
	107	♂					—	8	+	+
	89	♂					—	8	+	+

\* Pc-G: Aqueous procaine penicillin-G (300,000 units/ml).

is a suitable test animal for the potency test of leptospiral vaccines.

In this study, the authors further investigated the susceptibility of mongolian gerbils to leptospire by experimental infection with various strains of leptospire and observed that animals infected with newly isolated strains demonstrated a severe infection of leptospirosis and died within 4-11 days post inoculation. Furthermore, autopsy revealed hemorrhage in tissues with or without jaundice.

Experiments were also performed to test the effect of the antiserum and procaine penicillin-G on leptospiral infection. The results concluded that gerbils are the animals suitable for the potency test of antiserum and antibiotic on leptospirosis.

#### Acknowledgments

The authors wish to thank Prof. Y. Nakajima of Yamanashi Medical College for his supply of the mongolian gerbils, and Dr. M. Mori and Dr. Y. Arimitsu for their provision of the strains of leptospire to us.

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スナネズミを用いた実験的レプトスピラ症の検討：湯川真嘉・今村 晋\*・長崎弘美\*・望月公子

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レプトスピラ症の研究には、モルモット、ハムスターあるいは特定の系統のマウスが用いられてきたが、何れも限られたレプトスピラ菌株のみにしか感受性が認められず、本症研究の大きな阻害要因となっており、多くのレプトスピラ菌株に感受性をもった実験動物の開発が切望されていた。著者らはこのような観点から多くの実験を重ねてきたところ、スナネズミがこの目的に適合したものであることを知った。そこでこれらを更に明らかにするため、スナネズミにネズミおよびイヌより分離したレプトスピラ株ならびに国立予防衛生研究所より分与された株を用い、その感受性および抗血清・抗生物質の効果につき詳細な検討を行った。

その結果、スナネズミの各種菌株に対する感受性は、長期保存株では全ての株に対して感受性をもたないが、新鮮分離株に対しては何れも強い感受性が認められた。発症、死亡例は何れも肺出血巣が著明であり、また黄疸、皮下出血も高度に出現した。また、動物に同時にウイルス病治療血清、*copenhageni* Shibaura 株を投与した場合、治療血清の効果が明らかであった。尚 *Icterohaemorrhagiae* 群菌株に対しても特異的な効果が示された。抗生物質の治療効果に対しても、生存、死亡を指標とした実験で投与群と非投与群間に明らかな差が示された。

これらから、スナネズミが従来行い得なかった感染実験による発症、死亡を指標とした、ワクチン検定などにも、極めて有用であることが示された。