서울의대잡지 : 제9권 제3호
The Seoul Journal of Medicine
Vol. 9, No. 3, September, 1968

Multiplication and Antibody Formation of Japanese Encephalitis Virus in Snakes*

—Ⅱ. Proliferation of the Virus—

뱀에서의 日本腦炎 바이러스의 增殖과 抗體形成에 關한 硏究

—Ⅱ. 바이러스의 增殖—

Ho Wang Lee, M.D., Ph.D.

Department of Microbiology, College of Medicine, Seoul National University, Seoul, Korea

Introduction

The ecology of Japanese encephalitis (JE) virus is not completely clear yet, especially about overwintering mechanism of this virus in the temperate zone. One of the hypothesis is that the virus overwinters in the hibernating cold-blooded animal.

There has been no report on the proliferation of JE virus and antibody formation in cold-blooded animal. It has reported in the progress report (1) that the antibody formation was observed in the non-poisonous snake collected in Korea when calf-serum and the virus were injected. And the author(2) has isolated 2 strains of JE virus from the snake caught in the field of Korea in 1966 and 1967.

The purpose of this experiment is to investigate the proliferation and antibody formation after JE virus inoculation into the snake. A part of results obtained in this experiment is reported hereafter.

Materials and Methods

Snakes:

The employed snakes are following 3 species of non-poisonous common snake caught in Korea. Their weight ranged between 30g and 60g, and the length from 50cm to 70cm. All snakes employed in this experiment are free from JE antibodies.

1) Natrix tigrina lateralis Berthold
2) Elaphe rufodorsata Cantor
3) Elaphe schrenkii Strauch

Viruses:

1) JEV M5/596 (3) 16th suckling mouse passage.
2) JEV S-6-182 3rd suckling mouse passage. This virus was isolated from the plasma of Elaphe rufodorsata Cantor in 1966.

A known amounts of the viruses were injected intradermally into snakes. The snakes were divided into three groups and they were kept at 20-25°C, in 4°C refrigerator and underground repectively. Viremia, hemagglutination-inhibition (HI) and neutralizing antibodies to JE virus were checked at a certain interval. The collected plasma for virus demonstration was kept in ampoule and preserved in -60°C and the plasma for antibody test was kept in -20°C until use. The blood was collected by cardiac puncture with 26 gaze needle.

Viremia and neutralizing antibody to JE virus were tested in primary chick embryo cell culture system (4).

Buescher et al's method (5) was employed in HI antibody test to JE virus.

* The research reported has been made possible through the support and sponsorship of the U.S. Army Research and Development Group (Far East), Department of the Army under Grant No. DA-CRD-AFE-S92-544-67-G78 and in part by PHS 5 R22 AI06103 from the National Institute of Allergy and Infectious Diseases, Public Health Service.

Results

Multiplication and antibody formation of JE virus in snake

As table 1 shows, JE virus employed in this experiment is M5/596 strain isolated from mosquito. Its laboratory history is 16th suckling mouse brain passage and lst swine kidney cell passage. 0.2ml of the virus was injected intradermally into 10 snakes and they were divided into two groups and kept at room temperature and at 4°C respectively. The appearance of viremia, neutralizing and HI antibodies to JE virus were checked at a certain interval thereafter. Viremia was proved in 3 snakes No. 295, 231, 248, out of 6 which were kept at room temperature during 35-44 days after virus inoculation. Only snake No. 170 out of 4 kept at 4°C showed viremia at day 9, however, it was died between 24 and 35 days after virus inoculation so that viremia could not be tested any more.

Another virus employed in the experiment was JE virus S-6-182 isolated from snake in 1966. Its laboratory history is 3rd suckling mouse brain passage. The virus was inoculated into 10 snakes and they were also divided into two groups and kept at room temperature and at 4°C. Viremia was existed in 3 snakes out of 6 kept at room temperature as in table 2. Snake No. 270 showed viremia at day 24, day 35 and day 44. And snake No. 26 at day 44, snake No. 191 at day 9 and day 44. Viremia appeared in three snakes out of 4 kept at 4°C, in snake No. 100 at day 9, No. 246 at day 13 and No. 276 at day 24. After that all the snakes were died, therefore examination can not be prolonged. In antibody test, HI antibody was demonstrated in day 44 and 65 plasma of snake No. 270 only, which had shown viremia. It is quite interesting that viremia and HI antibody demonstrated in day 44 blood at the same time.

As table 3 indicates JE virus S-6-182 were used in this experiment. Its laboratory history is 2nd suckling mouse brain passage and 6th swine kidney cell passage. Virus was injected into 9

Table 1. Multiplication and antibody formation of JE virus (M5/596 SMP-16, PS-1*) in snakes

Group	Dose, route of inoculation	Snake No.	Viremia on days after virus inoculation					Antibody formation on days after virus inoculation										
								Neutralizing antibody						HI antibody				
			9	13	24	35	44	0	9	24	35	65	100	0	44	65	87	100
25°C	10^{-1} cell suspension 0.2ml I.D.	31	—	dead				—	—					<10				
		199	dead					—						<10				
		295	n.t.**	—		+	+	—	—	—	—	—	—	<10	<10	<10	<10	<10
	10^{-5} cell suspension 0.2ml I.D.	6	—	n.t.	—	dead		—	—	—				<10				
		231	—	n.t.		+	—	—	—	—	—			<10	<10			
		248	n.t.	—	—	—	+	—		—	—	—		<10	<10	<10		
4°C	10^{-1} cell suspension 0.2ml I.D.	170	+	n.t.	—	dead		—	—	—				<10				
		283	n.t.	—	dead			—						<10				
	10^{-5} cell suspension 0.2ml I.D.	229	—	n.t.	dead			—	—	—				<10				
		292	n.t.	—	—	dead		—	—					<10				

Elaphe rufodorsata Cantor ..... Snake Nos. 31, 199, 6, 248, 170, 283, 292
Natrix tigrina lateralis Berthold .. Snake Nos. 231, 229
Elaphe schrenckii Strauch .. .. Snake No. 295
*PS-1 ..... Procine kidney cell passage
**n.t. ..... 1st not tested

Table 2. Multiplication and antibody formation of JE virus (S-6-182 SMP-3) in snakes

Group	Dose, route of inoculation	Snake No.	Viremia on days after virus inoculation					Antibody formation on days after virus inoculation										
								Neutralizing antibody							HI antibody				
			9	13	24	35	44	0	9	24	35	44	65	100	0	44	65	87	100
25°C	10^{-3} SMB 0.2ml 1,420,000 PFU I.D.	108	dead					—	n.t.*						<10				
		144	—	n.t.	—	dead		—	—	±					<10				
		270	n.t.	—	+	+	+	—	n.t.	—	—			—	<10	20	20	<10	
	10^{-7} SMB 0.2ml 142 PFU I.D.	26	—	n.t.	—	—	+	—	—	—			—		<10	<10	<10		
		191	+	n.t.	—	—	+	—	—	—	—				<10	<10	<10		
		236	n.t.	—	—	—	—	—	n.t.	—	—	—	—		<10	<10	<10	<10	<10
4°C	10^{-3} SMB 0.2ml 1,420,000 PFU I.D.	100	+	n.t.	dead			—	—						<10				
		246	n.t.	+	dead			—							<10				
	10^{-7} SMB 0.2ml 142 PFU I.D.	146	—	n.t.	dead			—	—						<10				
		276	n.t.	—	+	dead		—	n.t.	—	—				<10				

Elaphe rufodorsata Cantor..... Snake Nos. 108, 144, 270, 191, 100, 246, 146, 276
Natrix tigrina lateralis Berthold .. Snake No. 236
Elaphe schrenckii Strauch ..... Snake No. 26
*n.t. ..... not tested

Table 3. Multiplication and antibody formation of JE virus (S-6-182 SMP-2, PS-6*) in snakes

Group	Dose, route of inoculation	Snake No.	Viremia on days after virus inoculation					Antibody formation on days after virus inoculation										
								Neutralizing antibody							HI antibody				
			9	13	24	35	44	0	9	24	35	65	100		0	44	65	87	100
25°C	10^{-1} cell suspension 0.2ml 30,000 PFU I.D	45	—	n.t.**	—	dead		—	—	—					<10				
		168	—	n.t.			—	—	—	+	+	+	+		<10	<10	<10	<10	<10
		230	n.t.	—	dead			—							<10				
	10^{-5} cell suspension 0.2ml 3 PFU I.D.	239	—	n.t.		—		—	—	—	—				<10				
		279	n.t.	—	—	—	+	—	n.t.	—		—			<10	<10	<10	<10	
4°C	10^{-1} cell suspension 0.2ml 30,000PFU I.D.	166	—	dead				—	—						<10				
		237	dead					—							<10				
	10^{-5} cell suspension 0.2ml 3 PFU I.D.	227	dead					—							<10				
		284	n.t.	—	—	dead		—							<10				

Elaphe rufodorsata Cantor..... Snake Nos. 45, 279, 284
Natrix tigrina lateralis Berthold .. Snake Nos. 230, 239, 237, 227
Elaphe schrenckii Strauch..... Snake Nos. 168, 166
*PS-6 ..... 6th porcine kidney cell passage
**n.t. ..... not tested

snakes and they were divided into two groups.

As a result of it, one out of 5 snakes kept at room temperature, snake No. 279 showed viremia at day 44. And those who kept at 4°C were died at once. Therefore they can not be checked. In antibody test, neutralizing antibody was found from day 24 to day 100 plasma after virus inoculation with snake No. 168 but HI antibody could not be proved.

Discussion

The viruses employed in this experiment were M5/596 isolated from mosquito and S-6-182 isolated from Korean snake.

In fact the snakes were kept 25°C, 4°C and underground after virus injection. In this report only former two groups are described because the snakes kept underground were overwintered, any results can not be reported till now.

As the results showed the viremia was proved in 4 out of 10 snakes inoculated with JE virus M5/596 but appearing time is not quite regular. Among the snakes kept at room temperature, viremia was proved comparatively late about one month after virus injection. Snake No. 231 showed that it could be possible to demonstrate viremia on day 35 but impossible on day 44.

Anotherwods, the appearance of viremia was not regular as that in the warm-blooded animal.

In the experiment JE virus S-6-182 was employed the viremia appeared in six out of nine snakes, as table 2 indicated. In this case viremia was easily demonstrated at day 35-44 with snakes kept at room temperature but in the snakes kept at 4°C viremia was proved in early stage and impossible to test further because of the death.

It is quite interesting that viremia as well as HI antibody were proved at the same time in the snake No. 270 but neutralizing antibody was not demonstrated. The phenomenon like this does not occur in the warm-blooded animal frequently. The more investigations are needed to explain this phenomenon.

Only one out of 8 snakes inoculated with S-6-182 strain, 6th swine kidney cell passage, showed viremia. This results could be seen in table 3. A specific thing is that neutralizing antibody was proved on day 24 after virus inoculation in snake No. 468 in which viremia and HI antibody could not be demonstrated.

Summing up, the experiments with snakes kept at 4°C was failed because of their death. Though the cause of death is not clear, it is probably because the temperature is lower than their optimum temperature in nature.

And according to the record of viremia examination, there are some variation depend on virus strains. The virus S-6-182 in mouse brain suspension gave the best results and virus M5/596 next. But the virus S-6-182 that has the laboratory history of 6th swine kidney cell passage did not give such good results as above strains.

It is difficult to explain the facts that in the virus inoculated snake sometimes viremia demonstration was impossible in early stage but the viremia appeared later, or that it appeared once and soon it disappeared. It may be a reason that the virus detector system used in our laboratory is not so sensitive that it might cause such results. It can be another reason that it may be caused by the unknown specific physiological function of the cold-blooded animal.

The author would like to recall that better results were obtained at low temperature in antibody test as described in the progress report(1). The reason why the results on antibody test was bad in this experiment may be that the antibodies could not be proved by ordinary method.

The definite conclusion can not be made because above mentioned experiments are limitted and preliminary one, further-more the results were irregular. More experiments are required and same experiments should be repeated to support the results.

Summary

1. JE virus was injected into snakes and they were kept at room temperature and 4°C. After that viremia was checked, virus multiplication could be demonstrated in some of the snakes but its appearance was irregular.
2. After injecting JE virus into the snake, it was difficult to demonstrate antibodies but there were traces that implied antibody formation.

ABSTRACT

There is a hypothesis that Japanese encephalitis(JE) virus overwinters in the body of hibernating cold-blooded animal in the temperate zone. It is not known that JE virus multiplies and produces antibodies in the snake, however the author succeded in demonstrating the HI and neutralizing antibodies to JE virus in the plasma of non-poisonous common snake caught in Korea. This experiment was designed to explore that JE virus multiplies and produces specific antibodies in the snake after the virus inoculation. Two strains of JE virus isolated from mosquito and snake were used. Varying known amounts of virus were injected into snake intradermally and the snakes are kept at various degrees of temperature. After that viremia and antibody were checked at a certain interval. According to the results, viremia could be demonstrated but it was irregular. The demonstration of antibodies was difficult but HI and neutralizing antibody to the virus could be demonstrated in only 2 out of 30 snakes. Though it is not definite one the above results support strongly the facts that virus multiplies and produces the specific antibody in the body of snake.

國 文 抄 錄

뱀에서의 日本腦炎 바이러스의 增殖과 抗體形成에 關한 硏究

서울大學校 醫科大學 微生物學敎室

李 鎬 汪

日本腦炎 바이러스가 冷血動物 體內에서 越冬한다는 學說은 오래前부터 있으나 腦炎바이러스가 뱀의 體內에서 增殖한다든지 抗體를 形成한다는 報告는 아직 없다.

著者는 最近에 뱀의 血淸中에서 腦炎바이러스에 對한 抗體를 證明한 바 있다.

이 實驗에서는 뱀과 모기에서 分離한 腦炎바이러스를 뱀의 皮內에 注射後 各種 溫度에 保存하여 바이러스의 增殖과 抗體形成을 調査하였다.

그 結果 바이러스의 增殖을 證明할 수 있었고, 血液中에 나타나는 率은 不規則하며 抗體는 30마리의 뱀中 2마리에서 證明할 수 있었다.

REFERENCES

1) Lee, H. W. : *Progress report No. FE-343-1, December, 1967.*
2) Lee, H. W. : *Unpulished data.*
3) Lee, H. W., and Scherer, W. F. : *The anamnestic antibody response to Japanese encephalitis virus in monkeys and its implication concerning naturally acquired immunity in man. J. Immunol. 86 : 151, 1961.*
4) Lee, H. W. : *Plaque assay of JE virus in chick embryo cells and porcine kidney cells. Korean Central J. Med., 11 : 583, 1966.*
5) Buescher, E. L., Scherer, W. F., Grossberg, S. E., Chanock, R. M., and Philpot, V. B. Jr. : *Immunologic studies of Japanese encephalitis virus in Japan. I. Antibody responses following overt infection of man. J. Immunol., 86 : 582, 1959.*