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MULTIPLICATION AND ANTIBODY FORMATION OF JAPANESE ENCEPHALITIS VIRUS IN SNAKES

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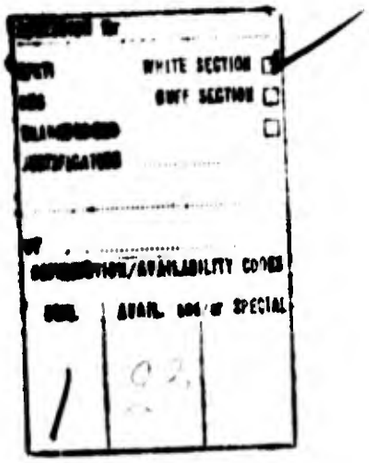

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MULTIPLICATION AND ANTIBODY FORMATION OF JAPANESE ENCEPHALITIS VIRUS IN SNAKES

by

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ABSTRACT

There is a hypothesis that Japanese encephalitis virus overwinters in the hibernating animals in the regions like Korea of which winter is so cold. The suther has recently reported that injection of Japanese encephalitis virus in cold-blooded animals, snakes, induced proliferation of the virus and antibody formation, though irregular. In 1967, a strain of encephalitis virus was isolated from the snakes caught in the nature, and as a result of serologic test with 535 snakes, 40% of them proved to contain hemagglutination inhibition antibodies to the virus. And the proportion was higher in the snakes collected during the epidemic season than other times. In hemagglutination inhibition antibody test, the proportion was higher when acetone extration was done three or four times than twice. In 1968, as a result of serologic test with 412 collected snakes, neutralizing antibody to encephalitis virus was detected from 9 snakes, 2% of the total snakes.

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Introduction

The ecology of Japanese encephalitis virus(JEV) is not completely [illegible], especially about overwintering mechanisms of the virus in the temperate zone like Korea. One of the hypotheses is that the virus overwinters in the hibernating cold-blooded animals.

The authors [illegible] recently reported that JEV can artificially overwinter in the snakes caught in Korea(1) and that injection of the virus [illegible] antibody formation and proliferation of the virus(2,3).

The author has isolated JEV from the plasma of the snakes collected in Korea in 1966 and 1967, and as a result of serologic test, high proportion of HI antibody to the virus was detected and the proportion is higher in the snakes caught during the epidemic season than other times. So those facts are reported hereafter.

Materials and Methods

Snakes:

The employed snakes were following 4 species of non-poisonous [illegible] snakes of Korea.

1) [illegible] CANTOR
2) [illegible] [illegible]
3) [illegible]
4) [illegible] CANTOR

Snakes were collected through the year, but were not caught from [illegible] to February and were caught from March to November. And the [illegible] collecting site was near the rice paddies.

Viruses:

The following 3 viruses were used for preparation of virus hemagglutinin and as controls for identification of the isolated viruses.

1) JEV [illegible](4), 17th suckling mouse passage.
2) [illegible] Virus, 2nd mouse passage in our laboratory.
3) Eastern equine encephalitis virus, 2nd mouse passage in our laboratory.

[illegible] and Eastern encephalitis viruses were kindly supplied from Dr. [illegible], [illegible], Seoul, Korea.

Isolation of Virus:

Blood of the snake was obtained by heart-puncture with 1 ml syringe containing heparin. The 1:10 dilution of plasma was used for plaque formation in primary chick embryo cell culture system(5).

Plaque neutralization test:

Plaque neutralizing antibody to JE virus was tested in primary

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chick embryo cells(5).

Hemagglutination inhibition(HI) test:

[illegible] method was employed(6). In the test performed in 1967, acetone extraction was done twice and since 1968 acetone extract was done three or four times.

Results

Isolation of Japanese encephalitis virus from the snakes caught in the field

In 1967, we collected 545 snakes and obtained plasma by heart puncture and the isolation of virus was performed in primary chick embryo cells with agar overlay.

As the table 1 shows, there were four different species in the collected snakes and among them, there were 510 Elaphe rufodorsata CANTOR. Japanese encephalitis virus was isolated from the snake S-7-2-3 caught at [illegible] near Seoul on July 16, 1967.

Identification of JE virus was confirmed by plaque neutralization test in primary chick embryo cells and as control, West Nile virus and Western equine encephalitis virus were employed. Isolation of virus from the snakes caught in 1968 and 1969 is still being carried on.

Frequency of HI antibody and neutralizing antibody to JE virus in the snakes collected in the field

In 1967, we caught 535 snakes in the field and detected HI antibody to JE virus.

As the table 2 shows, there were four different species in the snakes, but most of them were E. rufodorsata CANTOR. As the results of HI test to JEV, we found that antibody containing proportion before June, pre-epidemic season, was 13% to 17% and during July, August, September, October, epidemic season, was 62% to 74% and, in November, when no mosquitoes out, was 26%.

In 1968, as the table 3 shows, 540 snakes were caught and among them were 525 E. rufodorsata CANTOR, and HI antibody to JE virus was detected. This higher proportion compared with previous years is a result of three times performance of acetone extraction, because we found that the proportion became higher when the acetone extraction was done three or four times than twice and we did so since 1968.

In 1968, neutralizing antibody test to JE virus was done and as the table 4 shows, neutralizing antibody was detected from 9 out of 412 snakes.

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Discussion

The authors have isolated one strain of JE virus from the snake collected in Korea in October, 1966 for the first time. At that time, JE antibody to the virus was proved to be in the plasma of the snake and the virus was isolated. So it became more interesting to study about the relationships between JE virus and snakes in the nature.

This project being carried, artificial injection of JE virus into the snakes caught in Korea induces antibody production and proliferation of the virus was proved by viremia, but the results were not so remarkable as in warm-blooded animals. And so, various factors affecting JE virus multiplication and antibody formation are still being studied. It seems to be silly to expect the same result as in warm-blooded animals because little is known about the physiology of cold-blooded animals and consequently there still lie many obstacles.

In 1967, plasma was obtained from 545 out of 500 snakes collected in the field by heart-puncture. And its dilution of 1:10 produced plaque formation in primary chick embryo cells. Among them, from the plasma of the rufodorsata Cantor which was caught in Jangyidong near Seoul, plaque of JE virus was obtained which was confirmed by neutralization.

Because the snake plasma is very toxic to cells(4), it can not be used to the cells as it is without dilution. So it must be, at least, diluted 1:10 to produce plaque formation in primary chick embryo cells. It is the feeling that if undiluted plasma is used, larger number of the virus plaque is expected to be produced. If another system of cells, which has a stronger resistance to toxicity of snake plasma and has better possibility of production plaque formation, or such a kind of method is discovered larger number of the virus can be isolated.

Virus isolation from plasma of the snakes caught in 1966 and 1967 is still going on. We studied about the proportion of JE antibody to the virus in snakes caught by months and the results are shown in the table 2. Antibody containing proportion in the plasma is relatively low before July, pre-epidemic season, about 60% - 70% during July, August, September. October, epidemic season, in November the proportion fallen down to 28%.

This phenomenon seems to have some relations with the fact that main vector mosquitoes of JE in Korea, Culex tritaeniorhynchus, appears after June, reaches to the peak in August and September, and disappears after November and that the main breeding site of the mosquitoes is rice-paddies, the same place where R. rufodorsata breeds, vector mosquitoes.

During the last two years, we tried to collect snakes during

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winter season but in vain.

In 1968, HI antibody test to JE virus was performed, and the result was that, as the table 3 indicates, 72% of T. ruffidorsata appeared to be positive.

After detecting the HI antibody to the virus in the snake plasma, to solve the question whether this antibody is non-specific or not, various methods were employed to remove non-specific inhibiters to JE virus hemagglutinin, and removal of acetone soluble non-specific lipids by repeated acetone extract increased HI antibody titer to JEV. So, in 1968, we performed three or four times of acetone extraction before the test and found that the amount of HI antibody to encephalitis virus increased two to four folds than when acetone extraction was done twice. So this higher proportion in the plasma of 1968 is due to three times performance of acetone extraction. In other words, this is one of the reason why the proportion has increased from 40% in 1967 to 72% in 1968. But this proportion does not equal to the incidential frequency of encephalitis cases in human. In 1967, 810 among 2,691 patients died and in 1968, 346 among 1,226 patients died.

In fact, the relationship between antibody containing proportion in the snakes and the epidemic in human is yet a problem to be studied from now on.

It is noticed that JE virus isolated from the snake is biologically different in many aspects from the virus isolated in the mosquitoes, but there has been no report about the difference in virulence so far.

In 1968, it is an astonishing fact that we've discovered 72% of antibody containing proportion in the snakes, and more experiments are required and some experiments should be repeated to support the results.

Because HI antibody to JEV was considered non-specific on account of cross-reaction in the group, however JEV is the virus that exists only in Korea so far, snake plasma collected in 1968 was kept at 56°C for 30 minutes, and then neutralizing antibody test to the virus was performed. The result of neutralizing antibody test to encephalitis virus in the plasma from 412 snakes diluted 1:10 was proved to be positive in 9 snakes, and this corresponds only to 2%.

The results of the experiments about the proliferation of JE virus and antibody production(2,3) have clarified that HI and neutralizing antibodies do not exist at the same time in the snake as do exist in the warm-blooded animals usually and their existing time is irregular and the number of snakes that produce antibodies are very few after artificial inoculation of the virus at 21°C and 4°C.

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According to these facts, in the snakes of the nature that have been inoculated with the virus, HI antibody production seems to occur easily and neutralizing antibody production seems to be very difficult, with some unknown reasons.

As a matter of fact, present data were obtained by the processes used in antibody test of warm-blooded animals. So if we employ new methods which can prove antibodies of cold-blooded animals, better results are expected to be obtained. Now, the new techniques for the antibody test are still being studied.

Summary

1. In 1967, a strain of JE virus was isolated from the plasma of E. rodorata COLUBER caught in the rural area near Seoul on July 16.

2. In HI antibody test to the virus with the plasma of 535 collected snakes in 1967, 40% proved to be positive. And it was found that snakes caught during epidemic season resulted in higher proportion of HI antibody.

3. According to the results of serological test of 140 collected snakes in 1968, 72% of them were proved to have HI antibody to JE virus. This proportion is higher than that of 1967 and it may due to the more removal of non-specific inhibitors of snake plasma by three or four times performance of acetone extraction.

4. In 1968, the result of neutralizing antibody test to JE virus in the plasma from 412 snakes proved to be positive in 7 snakes, 2% of total snakes.

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Table 1

Isolation of JE virus from Non-poisonous Snakes of Korea Collected in 1967

Species of snakes	No. of JEV isolation / Total No. tested
Elaphe rufodorsata GRAY	1* / 20
Natrix tigrina lateralis BERGHOLD	0 / 24
Elaphe schrenckii STRAUCH	0 / 10
Rhabdon rufozonatum rufozonatum CANTOR	0 / 1

*: JEV S-7-283 (7/16/67 Chunchon, Seoul)

Table 2

Occurrence of CF antibody to CF Virus in human sera collected in Iowa in 1947

$$\frac{\text{No. of positive}}{\text{Total No. tested}} = \frac{215}{330} = \text{\% of total} = 65$$

Species of animal	CF titer	March	April	May	June	July	August	September	October	November	No. of animals
H. infecundus Linnaeus	<10		103	30	4	8	5	41	2	24	>500
	10		11	10		3	2	21	13	14	
	20		6	13		11		33	31	7	
	40			2	1	11	3	14	5	1	
	80		1			2					
M. tuberculosis intracellularis Schroeder	<10	1	1	12		2		4	1	1	24
	10										
	20			1		1					
T. schwenkfeldi Ciferri	<10		3	1	1			1		1	15
L.M. tuberculosis Schroeder	<10		1								1
No. of positive / No. tested		0/1	25/155 (13%)	26/85 (31%)	1/6 (11%)	26/76 (74%)	5/7 (71%)	57/103 (55%)	43/73 (61%)	32/78 (41%)	
Total No. tested											330

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Table 3

Occurrence of HI Antibodies to JE Virus in Amphibious Snakes of Korea Collected in 1968

$$\frac{\text{No. of Positive}}{\text{Total No. tested}} = \frac{400}{540} = 72\%$$

Species of snake	No. of HI positive / No. of tested	HI titer to JEV	No. of snake	% of HI positive
Elaphe rufodorsata CANTOR	399/525	< 10	146	72%
		10	111	
		20	142	
		40	87	
		80	39	
Natrix tigrina LATERALIS BOULENGER	0/8	< 10	8	0
Rh. ac. schrenckii GIRARD	1/7	< 10	6	14%
		10	1	

Table 4

Occurrence of Plaque Neutralizing Antibody to JE Virus in Snakes of Korea Collected in 1968

$$\frac{\text{No. of Positive}}{\text{Total No. tested}} = \frac{9}{412} = 2\%$$

Species of snake	No. of neutralizing antibody positive / No. of tested
Elaphe rufodorsata CANTOR	9/399
Natrix tigrina lateralis BOULENGER	0/7
Rh. ac. schrenckii GIRARD	0/6

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