大韓微生物學會誌：第15卷 第1號 1980
J. Kor. Soc. Microbiol., Vol. 15, No. 1, 1980

A Study of the HI Antibody of the Koreans and Swine to Reovirus.

Yun Tai Lee & Chong Hoon Lee

Department of Microbiology, Catholic Medical College, Kyongwun-Dong, Chongro-Gu, Seoul, Korea

=국문초록=

한국인 및 가축(돼지)에 있어 *Reovirus*에 대한 HI 항체분석

李 淵 台·李 鍾 訓

가톨릭의대 微生物學敎室

본 연구는 한국인 및 한국 각지의 가축에 대한 reovirus 2형의 혈구응집저지항체의 분포를 알어보고저 실시된 연구이다.

사람의 피검물은 1979년 6월부터 12월까지 시내 종합병원에 래원한 신검대상자 총 614명과 한국각지의 가축(돼지) 총 877수를 대상으로 하였다. 돼지의 혈액은 1977년 6월부터 동년 9월까지 전국 25개 지역에서 채취했다. 이들에 대한 혈청을 분리하여 −20°C에 보관하면서 실험하였고, 혈구응집저지시험은 Rosen(1960b, 1974)의 수기를 따랐다. 항원(Reovirus 2형)은 이호왕(1975) 교수로부터 분양받어 본 교실에서 HeLa 세포에 계대하여 혈구응집가를 확인하여 사용했다.

실험결과는 다음과 같다.

1. 한국인 총 614명의 Reovirus 2형에 대한 10배 이상 HI항체 보유율은 73.29%로서 한국인이 높은 항체를 보유하고 있었다. 그리고 남녀비는 남자(76.47%)가 여자 65.12%)보다 약간 높은 경향을 보였다.

2. 연령별로 본 한국인의 항체 보유율은 전 연령군에 고루게 분포되어 있었으나 41세에서 50세군이 85.71%로 약간 높았다.

3. 월별 항체보유율은 6월이 93.22%로 높았고, 10월이 47.62%로 낮았다.

4. 한편 한국의 25개 지역의 총 877수의 돼지에 분포된 항체보유율은 61.80%(542수)였다. 이를 도별로 구분한 바 서울이 85.37%로 가장 높았고, 가장 낮은 지역은 충남으로 40%였다.

5. 월별로 검출된 돼지의 항체보유율은 6월(63.21%)부터 9월(72.25%)까지 사이에 점차 증가되어 가는 경향을 보였다.

6. 사람과 돼지에 분포한 HI 항체가는 그림 2와 같이 대부분이 1 : 20이내에서 1 : 80이내에 분포하였으며 상호 차가 있었다.

이상의 실험결과를 토대로 볼때 한국자연계에는 Reovirus 2형이 광범하게 분포되어 있음을 알 수 있었으며, 한국인 및 가축(돼지)에 있어서 높은 항체가를 보유하고 있다는 사실을 새로히 알 수 있었다.

Introduction

The reoviruses which are 60-80 nm in diameter possess two distinct capsid shells and 15 per cent of the virus particle weight of RNA, which contains double-strained RNA genome.

Though originally considered members of the ECHO

virus type 10 (Sabin, 1956) it was included in reoviridae at presnt.

There is little information available about the epidemiology of reoviruses in human beings and animals. Serum surveys show that antibodies are present in man and wild and domestic animals in all the parts of the world. Infected host excrets the viral particles into their stools (Rosen 1960 a and 1962, Stanley 1967). In human being, the virus infects the respiratory system or the gastrointestinal tract of children and causes the infected host to have febrile illness, upper respiratory infections and gastroenteritis. Not only all three reoviruses have been discovered from healthy children (Leers & Rozee 1966), but reovirus type 3 has been isolated from cases of Burkitt's lymphoma (Bell et al. 1964 and 1966).

The reoviruses fall into 3 genera on the basis of antigenicity (Rosen 1965a and 1962). To prove the different antigenicity, there are hemagglutination inhibition (HI), complement fixation, and neutralization tests in the methods of humoral antibody measusement.

Of all these tests, the hemagglutination inhibition test is the most economical, the correctest, and the fastest. Therefore, the HI tests is the most pracitcal.

The purpose of this paper is to study the indicence of humral antibody about reovirus type 2 in sera from the Koreans at age, sex, and month, in 1979 and swine in 1977, respectively.

Materials and Methods

1. Specimen

The total of 614 cases of human specimens (442 cases in male and 171 cases in female) was collected from the persons through a physical examination in the National Seoul Hospital on June, 1979 to December and the swine blood were collected from 25 different areas of Korea from the Ist of June to 30th of Sept. in 1977. Age of the swine was around a year and the were separated from the 10 ml of human blood and then kept at -20.C before use.

2. Antigen

The reovirus type 2 isolated from rodents by Lee (1975) was made a use of in this study. In our laboratory, this virus was subcultured on HeLa cell monolayer six times at the interval of 7 days, and was frozen and thawed three times at −85.C and centrifuged at 3,000 rpm for ½ hour. This harvested suspension was used in the hemagglutination (HA) test and the units were 8 units/ml.

3. Sera

To remove all kinds of non-specific serum inhibitor of hemagglutination; the 0.8 ml of 25% Kaolin was added to the 0.2 ml of the serum. The treated serum was kept at the room temperature for 25 minutes and then was centrifuged at 3,000 rpm for 20 minutes. The suspenatant was harvested and the 0.1ml of human blood O type washed three times was added to this suspension. The treated suspension was kept at 4.C for 15 minutes and centrifuged at 1,500 rpm for 10 minutes.

4. Erythrocytes

The 20 ml of healthy human blood O type was washed three times with dextrose-gelatin veronal (DGV) buffer and diluted to 0.33 % solution with normal saline.

5. Hemagglutination inhibition test

This test was based on the Rosen's method (1960a and 1974) in principle.

Result

A. Human

1. Sexual distribution

Table 1. contains the distribution of hemagglutination inhibition antibody titer of the 614 human sera against reovirus type 2. The positive rate of HI antibody showed the 73.29% (450 out of 614 cases). It seemed that a sexual analysis showed a little significant difference between male and female the 76.47% in male and the 65.12% in female respectively appeared(figure 1).

2. The distribution at age

Table 2. is compared with the results of HI antibody titer at age. The positive appeared widely in the each age group. However, the 31 to the 50 old age groups known as the active age showed a little higher positive percentage than any other age groups.

Table 1. The incidence of antibodies to reovirus type 2 in sera by sex from koreans, 1979.

Sex	HI antibody titers									Positive. /Total (%)	
	NG	10	20	40	80	160	320	640	1280		
Male	104	17	67	96	75	45	27	9	2	338/442(76.47)	450/614(73.29)
Female	60	10	16	23	33	14	14	2	0	112/172(65.12)	
Total		27 (6.0)	83 (18.44)	119 (26.44)	108 (24.0)	59 (13.11)	41 (9.11)	11 (2.44)	2 (0.44)	450/614 (73.29)	

NG : negative cases

Table 2. The incidence of antibodies to reovirus type 2 in sera by age groups from koreans, 1979

Age	Sex	HI antibody titers									Positive. /Total (%)	
		NG	10	20	40	80	160	320	640	1280		
11-20	M	35	7	15	21	26	10	6	2	0	87/122(71.31)	118/186(63.44)
	F	33	4	6	6	9	3	2	1	0	31/64 (48.44)	
21-30	M	48	6	38	49	32	27	13	5	1	172/219(78.08)	226/296(76.35)
	F	22	5	7	12	13	9	8	1	0	55/77 (71/43)	
31-40	M	15	3	10	15	15	6	4	2	0	55/70 (78 57)	74/94 (78.72)
	F	5	0	2	3	9	2	3	0	0	19/24 (79.17)	
41-50	M	4	1	2	8	2	2	3	0	1	19/23 (82.61)	24/28 (85.71)
	F	0	0	1	2	1	0	1	0	0	5/ 5 (100)	
51-60	M	2	0	2	2	0	0	1	0	0	5/ 7 (71.43)	6/ 8 (75.00)
	F	0	0	0	0	1	0	0	0	0	1/ 1 (100)	
>61	M	0	0	0	1	0	0	0	0	0	1/ 1 (100)	2/ 2 (100)
	F	0	1	0	0	0	0	0	0	0	1/ 1 (100)	

NG : Negative, M : male, F : female

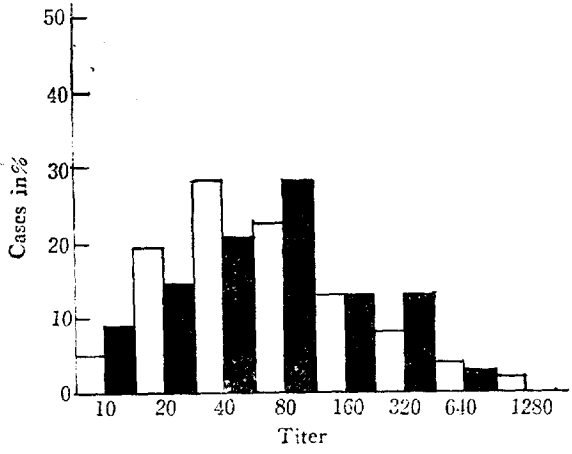

Fig. 1. The incidence of antibodies to reovirus type 2 in sera from Korean, 1979.
□ : male, ■ : female

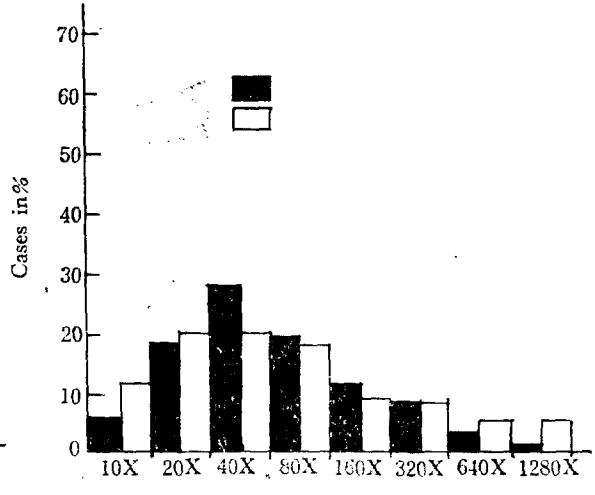

Fig. 2. The various antibody titers between the human beings and swine.

Table 3. The incidence of antibodies to reovirus type 2 in sera by month from koreans, 1979.

Month	Sex	HI titers									Positive. /Total (%)	
		NG	10	20	40	80	160	320	640	1280		
Jun.	M	4	3	7	12	14	6	7	0	1	50/54 (92.59)	55/59 (93.22)
	F	0	1	0	0	1	0	2	1	0	5/ 5 (100)	
Jul.	M	4	2	8	17	15	13	6	6	1	68/72 (94.44)	71/79 (89.87)
	F	4	0	2	0	1	0	0	0	0	3/ 7 (42.86)	
Aug.	M	9	4	8	35	26	16	8	3	0	100/109(91.74)	159/176(90.34)
	F	8	2	6	10	20	9	11	1	0	59/67 (88.10)	
Sep.	M	16	4	7	5	6	1	3	0	0	26/42 (61.90)	47/87 (54.02)
	F	24	3	3	5	5	4	1	0	0	21/45 (46.67)	
Oct.	M	25	0	5	7	6	3	1	0	0	22/47 (46.81)	30/63 (47.62)
	F	8	3	1	1	3	0	0	0	0	8/16 (50.00)	
Nov.	M	33	4	23	15	6	5	2	0	0	55/88 (62.50)	60/100(60.00)
	F	7	0	0	2	2	1	0	0	0	5/12 (41.66)	
Dec.	M	13	0	9	5	2	1	0	0	0	17/30 (56.67)	28/50 (56.00)
	F	4	1	4	5	1	0	0	0	0	11/20 (55.00)	

NG : Negative

Table 4. Areal distribution of HI antibody titer of swine in Korea, 1977.

Area	Positive cases	No. of examine
Seoul City	35 (85.37)	41
Gyung Ki Do	46 (77.97)	59
Kang Won Do	67 (46.85)	143
Chung Chong Nam Do	4 (40.0)	10
Kyung Sang Puk Do	65 (62.5)	104
Kyung Sang Nam Do	38 (71.70)	53
Chol La Puk Do	139 (64.06)	217
Chol La Nam Do	67 (62.04)	108
Total	542 (61.80)	877

3. Mothly distribution

The monthly distribution of the HI antibody titers of Koreans against reovirus type 2 is summarized in table 3. The HI antibody appeared in each monthly group. The positive rates of HI antibody were more or less higher in June, July, and August; 93.22% in June, 89.87% in July, 90.34% in August, and 47.62% to 60.00% in September to December.

B. Swine

Table 4 and 5 showed the distribution of HI antibody titer of the 877 swine sera which were to reovirus type 2. The positive rate of antibody was the 61.8 % (542 out of 877 cases). The area of province appeared varying antibody titers, the rate of positive cases showed from the 40.0%(Chung Chong Nam Do) to the 85.37 % (Seoul city) and the other were intermediate.

The monthly distribution of positive cases were shown as **table 6** which was from June to September. The positive rate of antibody showed such following results as June (63.21%), July (61.53%). August (65.77%) and September (72.25%) in respectively. This seems to be a gradualy higher titer.

Table 5. HI antibody titer of reovirus type 2 in swine from the various areas in Korea, 1977.

Province	Areas	H.I. titer 10 x	20 x	40 x	80 x	160 x	320 x	640 x	1280 x	Positive case	No. of examine
Seoul city	Sang Ke Dong	4(11.43)	9(25.71)	6(17.14)	4(11.43)	1(2.86)	3(8.57)	6(17.14)	2(5.71)	35(85.37)	41
Gyung Ki Do	Yong In	—	—	—	1	8	2	2	3	16(66.67)	24
	So Sa	2	7	6	2	1	1	—	—	19(54.29)	35
	Gal Mae Ri	2	7	8	3	1	—	—	—	21(56.76)	37
	Pa Ju	—	—	3	2	—	2	2	1	10(71.43)	14
	Go Yang	—	—	3	2	—	—	—	—	5(71.43)	7
	Dong Du Chun	2	7	12	14	6	5	—	—	46(77.97)	59
	Ma suck	1	—	2	3	1	3	—	—	10(52)	25
	Total	7(5.51)	21(16.54)	34(26.77)	27(21.26)	17(13.39)	13(10.24)	4(3.15)	4(3.15)	127(63.18)	201
Chung Nam	On Yang	—	1(25)	1(25)	—	2(50)	—	—	—	4(40)	10
Kang Won Do	Kang Lung	6	6	16	6	6	3	—	—	43(69.35)	62
	Sam Chuck	—	1	2	—	1	2	—	—	6(18.75)	32
	Sock Cho	—	2	1	1	1	1	—	1	7(43.75)	16
	Mon Ju	—	5	2	2	—	—	1	1	11(33.33)	33
	Total	6(8.96)	14(20.90)	21(31.34)	9(13.43)	8(11.94)	6(8.96)	1(1.49)	2(99)	67(46.85)	143
Kyung Sang Pug Do	An Dong	—	—	—	1	2	—	2	2	7(50)	14
	Sana Jow	—	1	—	1	1	3	5	8	19(47.5)	40
	Yung Chun	2	7	3	5	2	6	2	12	39(78)	50
	Total	2(3.08)	8(12.31)	3(4.62)	7(10.77)	5(7.69)	9(13.85)	9(13.85)	22	65(62.5)	104
Kyung Sang Nam Do	Ku Chang	—	—	2	2	1	—	1	—	6(46.15)	13
	Ham Yang	5	6	6	8	5	2	—	—	32(80)	40
	Total	5(13.16)	6(2.63)	8(21.05)	10(26.32)	6(15.79)	2(5.26)	1(2.63)	—	38(71.70)	53
Chol La Puk Do	Nam Won	8	2	2	3	4	1	1	—	21(48.84)	43
	Ko chang	7	12	9	3	—	2	—	—	33(82.5)	50
	Im Sil	4	3	2	3	—	—	—	—	12(70.59)	17
	Bu An	12	20	15	14	4	5	3	—	72(62.39)	117
	Total	31(22.30)	37(94.87)	28(20.14)	23(16.55)	8(5.76)	8(5.76)	4(2.88)	—	139(64.06)	217
Chol La Nam Do	Bul Kyo	9	10	6	8	1	1	1	—	36(65.45)	55
	Dam Yang	3	3	1	3	1	1	2	—	14(53.85)	26
	Jin Do	—	3	4	2	—	3	4	1	17(62.96)	27
	Total	12(17.91)	16(23.88)	11(16.42)	13(19.40)	2(2.99)	5(7.46)	7(10.45)	1(1.49)	67(62.04)	108
	Total	67(12.36)	112(20.66)	112(20.66)	93(17.16)	49(9.04)	46(8.49)	32(5.90)	31(5.72)	542(61.80)	877

Table 6. Monthly distribution of HI antibody titer against swine from Korea, 1977.

Month	HI titers								Positive cases (%)	No. of test
	10x	20x	40x	80x	160x	320x	640x	1280x		
Jun.	35	47	43	33	21	16	22	15	232(63.21)	367
Jul.	2	9	10	7	4	10	6	2	50(61.53)	130
Aug.	13	29	26	33	16	16	2	13	148(65.77)	225
Sept.	18	30	32	18	9	4	1	—	112(72.25)	155
Total	68 12.54	115 21.2	111 20.47	91 16.78	50 9.2	46 8.48	31 5.7	30 5.5	542 61.80	

Discussion

The term, reovirus (Rerpiratory Entero Orphan Virus), was proposed in 1959 (Sabin, Rosen, 1960a) as identical with, or related to ECHO type 10 virus (Sabin, 1956, Ramos et al 1958), but belongs to reoviridae now a days. A very wide natural host range is distributed in the reoviruses infections occuring in a variety of wild and domestic animals in addition to man (Rosen, 1968). The etiologic role of the reoviruses in human disease is still uncertain, although they have been isolated from a variety of illnesses, but the reoviruses have usually been isolated from the feces and respiratory secretions of hesalthy persons as well as from the patients with a variety of clinical illnesses most commonly been related to defined upper and low respiratory infection; exanthems, steatorrheas, central nervous system infections, and hepatitis. Additionally, in mice, reovirus 3 produces hepatitis and encephalomyelitis and the type 3 virus has been isolated from the cases of Burkitt's lymphoma (Beel et al. 1964, 1966, 1967). The reovirus contains RNA (15%) and protein (85%), and viral genome is double stranded with MW estimated from the percentage RNA pervirion as 17×10^6 to 22×10^6. The reovirus protein synthesis in the cytoplasm and localized within intracytoplasmic structures.

Messenger RNA such as molecules is used to synthesize progeny double stranded RNA. A virus induced RNA-dependent RNA polymerase. Three immunological types fo reovirus can be distinguished by neutralization, complement fixation and hemagglutination inhibition tests. All the three immunlogical types agglutinate human group 0 cells, but type 3 virus agglutinates ox red blood cells (Brubaker et al. 1964). The hemagglutination inhibition test is the most economical, the correctest, and the fastest.

However, most of diagnostic serology of reovirus infections have been done with the HI test (Rosen 1960 a, b and 1974). A strain of simian origin (SV_{12}) discovered the first reovirus was reported (Hull et al. 1956) to hemagglutinate. The reoviruses of all the same titer at 4°C, 23°C and 37°C and no effect of pH is seen in the ranges from 6.0 to 8.0 (Halomen, 1961). In this investigation, the hemagglutination-inhibition test is the method to determine the serotypes. This is easily done by using human erythrocytes, preferably to type 0, and collected human and swine sera.

There is little information available about the epidemiology of reoviruses in human beings and animals. The serum survey shows that antibodies are present in man as mell as in wild and domestic animals in all the parts of the world (Rosen 1968, Rosen 1974). Reovirus antibodies have been found in wild domestic animals and human beings. Whang et al. (1975) reported that HI antibodies resulted from the out patients of the hospital.

They showed 65.2 % from 510 patients. In this experiment, the table 1 showed the rate of distribution of HI antibodies titer to reovirus type 2 by sex. There, we can find in the table that 450 positive cases (73.29%) out of 614 sera were examined; the rates are estimated at 76.47 % in 172 female ones.

However, in this ivestigation, the difference of the rate of HI titer according to the sex distribution

was a little significant. In general, reovirus antibody was distributed in all age groups except under the age 10 but unfortunately we have not got sera from the children under the age in this investigation. In the groups of age 41-50 and 31-40 playing an active role, the positive rates were 85.71% and 78.72% respectively.

In the other hand, the monthly alterations of the rate of HI antibody titers decreased from June to December, and the maximum positive showed the 93.22 per cent in June 1979, but the rates of antibodies showed from 93.22 % to 47.62 % in this investigation.

Also positive rate of the swine was shown 542 out of the 877 cases (61.80 %) from the 22 different areas.

For this reason, we have a conclusion from the this data that the reovirus type 2 is widely distributed in all the Korea and antibodies are frequently found in Koreans and swine.

Summary

The purpose of this paper is to study the incidence of humoral antibody to reovirus type 2 in the sera of the Koreans and animal (swine) at random. All the 614 of human beings and 877 of swine sera were collected during the period from June to December, 1979, from the healthy persons in the National Seoul Hospital and swine blood was collected from 25 different areas of June to 30th of September in 1977. The HI test was put with plastic plates according to the methods by Rosen (1960 a and 1974).

The total 73.29 % of the 614 cases in human and the 61.80 % of the 877 in swine confirmed as a hemagglutination inhibition antibodies. In human the 76.47 % of the 442 male cases and the 65.12 % of the 172 female ones were confirmed to have humoral antibodies.

The positive rate was widely shown in each age group. But the 31 to 50 old age groups showed a little higher than any other age group, which came to the 85.71% in 41-50 and the 78.72% in 31-40 old age groups.

The monthly distribution of HI antibody was shown to reach the 93.22% of the 59 cases in June. This per cent was much higher than of any other distribution.

Accordingly, the auther came to the conclusion that there is reovirus type 2 in all the parts of Korea and most of the Koreans have the higher rates of antibody.

However, the positive rate of antibody was the 542 out of the 877 cases (61.8 %) from the swine and antibodies was confirmed from the 25 different areas in Korea. The seasonal distribution of the antibody showed these high rates. In domestics animals; blood from the swine showed that distribution of HI antibodies to reoviras type 2. These antibody appears from the various areas of the province in Korea. For this reasons, reovirus was widely distributed in this country.

References

Bell, T. M. (1967) : *Virus associatated with Burkitt's tumor. Prog. Med. Virol. 9 : 1-34.*

Bell, T. M., Massie, A., Ross, M. G, R. & Williams, M. C. *(1964) : Isolation of reovirus of from a case of Burkitt's lymphoma. Brit. Med. J. 1 : 1212-1213.*

Bell, T. M., Massie, A., Ross, M·G. R., Simpson, D. H. I. & Griffin, E. (1966) : *Further isolations of reovirus type 3 from case of Burkitt's lymphoma. Brit. Med. J. 1 : 1514-1517.*

Brubaker, M. M., West, B. & Ellis, R. J. (1964) : *Human blood group influence on reoviruses hemagglutnination titer. Proc. Soc. Exp. Biol. Med. 115 : 1118-1120.*

Davidson, G. P., Bishop, R. F., Townley, R. R. W., Holmes, I. H. & Ruck, B. J. (1975) : *Importance of a new virus in acute sporadic enteritis in children. Lancet 1 : 242.*

Flewett, T, H., Bryden, A. R. & Davies, H (1975) : *Epidemic viral enteritis in a long-stay children's ward. Lancet 1 : 4.*

Halomen, P. (1961) : *Growth, stability and hemagglutination of a reovirus. Ann. Med. Exp. Fenn. 39 : 132-142.*

Hull, R. N., Minner, J. R. & Smith, J. W. (1956) : *New viral agents recovered from tissue cultures of monkey kidney cells. Amer. J.*

Hyg. 63 : 204-215.

Jackson, G.G. & Muldoon, R.L. (1973) : *Virus causing common respiratory infection in man, IV. Reoviruses and adenoviruses. J. Infect. Dis. 128 : 811.*

Lee, H.W., Sung, I.H. & Lee, P.W. (1975) : *Reovirus isolated from rodent and incidence of antibodies in Koreans. The 6th Annual meeting of the Korean Society for Virology. Korean. J. Virology 4 : 53-54.*

Leers, W.D. & Rozee, K.R. (1966) : *A survey of reovirus antibodies in sera of urban children. Canad. Med. Ass. J. 94 : 1040-1042.*

Ramosi-Alvarez, M. & Sabin, A.B. (1958) : *Enteropathogenic viruses and bacteria. Role in summer diarrheal diseases of infancy and early childhood. J. Amer. Med. Ass. 167 : 147-156.*

Rosen, L. & Abinanti, F.R. (1960 a) : *Natural and experimental infection of cattle with human types of reoviruses. Amer. J. Hyg. 71 : 250-257.*

Rosen, L. (1960 b) : *Serologic grouping of reoviruses by hemagglutinatton-inhibition. Amer. J. Hyg. 71 : 242-249.*

Rosen, L. (1662) : *Reoviruses in animals other than man. Ann. N.Y. Acad. Sci. 101 : 461-465.*

Rosen, L. (1968) : *Reoviruses pp. 73-107 In virology monograph Vol. 1 Shringerverlag, Wien, New York.*

Rosen, L. (1974) : *Reovirus Manual of clinical microbiology. Second edition, ASM. pp. 735-739.*

Sabin, A.B. (1956) : *The significance of viruses recovered from the intestinal tract of healthy infants and children. Ann. N.Y. Acad. Sci. 66 : 226-230.*

Sabin, A.B. (1959) : *Reoviruses; A new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. Science 130 : 1387-1389.*

Stanley, N.F. (1967) : *Reoviruses. Brit. Med. Bull. 23 : 150-155.*

Whang, Y.W., Lee, P.W., Seong, I.H. & Lee, H.W. (1975) : *The distribution of hemagglutination inhibition antibody for reovirus in Koreans. Korean J. Virology 5 : 21-25.*