Isolation of Reovirus Type 3 from Foals

Hiroshi IMAGAWA, Tomio MATSUMURA, Masanobu KAMADA, Yoshio FUKUNAGA, Ayako HASEGAWA¹⁾, Hideo OHISHI²⁾, and Minoru MATUMOTO³⁾

Epizootic Research Station, Equine Research Institute, Japan Racing Association, 1400-4, Shiba, Kokubunji-machi, Shimotsuga-gun, Tochigi, 329-04, ¹⁾Central Virus Diagnostic Laboratory, National Institute of Health, Musashimurayama, Tokyo, 190-12, ²⁾Hidaka Agricultural Aid Association, 200, Higashi Hourai, Mitsuishi-cho, Mitsuishi-gun, Hokkaido, 059-31, and ³⁾Kitasato Institute, 5-9-1, Shirogane, Minato-ku, Tokyo, 108, Japan

(Received 9 September 1988/Accepted 29 January 1989)

Jpn. J. Vet. Sci. 51(3): 652-655, 1989

KEY WORDS: foal, reovirus isolation.

Reovirus has been isolated from many species of vertebrates. The ubiquity of the virus has also been indicated by the frequent presence of antibodies in a variety of vertebrates [14].

Reoviruses have been isolated from horses. Thein and Härtl [16] isolated a reovirus type 1 from the mucous membrane of upper respiratory tract obtained from a riding horse with severe respiratory illness. Erasmus *et al.* [5] reported the isolation of reovirus type 3 from the blood of a horse and reovirus type 2 from the pharyngeal mucosa obtained from a zebra experimentally infected with african horse sickness virus. Conner *et al.* [3] isolated a reovirus type 3 from a foal with diarrhea. Thein and Mayer [15] presented serological evidence to incriminate reoviruses as etiological agents of equine respiratory disease. It was reported that the 3 serotypes of reoviruses were prevalent among equine population in several countries [1, 3, 8, 13]. This paper describes the isolation of reovirus type 3 from the feces of 5 healthy foals and the lymph node of a foal dying of peritonitis.

A total of 66 fecal specimens were collected in breeding farms of light horses in Hokkaido. These were obtained from apparently healthy foals, 1 week to 4 months of age; 28 of them were obtained from 4 farms in June of 1983 and 38 of them from 7 farms in May of 1984. Six specimens of intestinal contents and 2 mesenteric lymph node specimens were collected in June of 1984 from 6 foals, 1 week to 4 months of age, which died and had macroscopic changes of hemorrhagic or necrotizing enteritis, peritonitis, rupture of the caecum or encephalomalacia. Virus isolation was performed in essentially the same manner as described for equine rotavirus [6]. Briefly, the suspension prepared from the feces, intestinal contents and lymph nodes was treated with 10 µg/ml of acetyl trypsin (Bovine origin, type

V-S, Sigma, USA) at 37°C for 30 min, and each 0.1 ml of the suspensions was inoculated into tube cultures of RK-13, MA-104 and Vero cells washed with Earle's solution. Incubation was carried out in the presence of 1 µg/ml of trypsin. If no cytopathic effect (CPE) appeared in the cells, further three blind passages were performed by the same method as described above. Virus titration was carried out by determining the TCID₅₀ with CPE as indicator. As reference virus, the following strains were used: the Lang strain of reovirus type 1, the Jones strain of reovirus type 2 and the Abney strain of reovirus type 3. These strains were propagated in RK-13 cell cultures. Antisera against the lang, Jones and Abney strains and the isolated K-15 strain were prepared in guinea pigs as described previously [6]. Each virus antigen used for immunization was purified from culture fluid of infected RK-13 cells by ultra-centrifugation with a linear sucrose density gradient. Indirect Immunofluorescent technique [10], electron microscopy [6] were carried out essentially by each method described in the previous reports. HA antigen was prepared from cultured fluid of infected RK-13 cells by ultra-centrifugation.

Virus was isolated from 5 fecal and one lymph node specimens. Of the 5 fecal specimens one yielded virus in cultures of all the cell types used, three only in RK-13 cell cultures and the remaining one only in MA-104 cell cultures. The lymph node yielded virus only in RK-13 cell cultures (Table 1). The strain names given to the isolates are shown in the Table. Cytopathic effects were first observed in the 2nd to 4th passages cultures and further passages were readily made. Cytopathic effects developed in one day of incubation and consisted mainly of rounded cells in RK-13 cell cultures and of long and slender cells in MA-104 and Vero cell cultures. Eosinophilic inclusion bodies were observed in the cytoplasm of infected cells stained with hematoxylin and

Table 1. Virus isolation from foals

Foal		Specimen	Cell culture		
Farm	Age (days)		RK-13	MA-104	Vero
B	98	Feces	3 ^{a)} (K-15)	2	2
B	71	Feces	4 (K-18)	— ^{b)}	—
B	60	Feces	4 (K-19)	—	—
C	62	Feces	4 (K-22)	—	—
E	68	Feces	—	2 (Tuka-1)	—
L	70	Lymph node	2 (D-13)	—	—

a) The number of passages when cytopathic effect appeared. The strain name in parenthesis.

b) Negative virus isolation.

eosin (Fig. 1).

The isolated strains multiplied to high titers in RK-13, MA-104 and Vero cell cultures. The K-15 strain at the 6th passage in RK-13 cells had $10^{7.3}$, $10^{7.5}$ and $10^{8.0}$ TCID₅₀/0.2 ml when assayed in RK-13, MA-104 and Vero cell cultures, respectively. The infectious titer of the K-15 strain obtained in RK-13 cell cultures kept in trypsin-containing maintenance medium after inoculation with pretreated virus was 2 to 3 log units higher than that obtained in cultures kept in trypsin-containing maintenance medium after inoculation with non-pretreated virus, and was 4 to 5 log units higher than that obtained in cultures in maintenance medium without trypsin after inoculation with non-pretreated virus. For the propagation of the isolates to high titers the pretreatment of the inoculum with trypsin and the incorporation of a small amount of trypsin in maintenance medium seem to be important. Enhancing effects on virus growth of proteolytic enzymes have been reported for reoviruses [3, 4, 7, 11, 12, 17].

Electron microscopy of the isolated strains revealed spherical virus particles, about 85 nm in diameter, with double-layered capsid and inner core of about 45 nm (Fig. 2).

Specific fluorescence was demonstrated in cell cultures infected with the isolated strains by the indirect immunofluorescent procedure with guinea pig antiserum against the Lang strain of reovirus type 1 (Fig. 3). All the isolated strains gave specific fluorescence with guinea pig antisera against 3 reovirus serotypes and the isolated K-15 strain. No differences were shown between

the homologous and heterologous antibody titers with each of the antisera.

All the isolated strains were found to agglutin-

Fig. 1. Eosinophilic cytoplasmic inclusion bodies in RK-13 cells one day after infection with the K-15 strain (Hematoxylin eosin, $\times 600$). Arrow denotes typical cytoplasmic inclusion body.

Fig. 2. Electron micrograph of negatively stained virus particles of the K-15 strain (Bar=100 nm).

Fig. 3. Immunofluorescence of RK-13 cells one day after infection with the K-15 strain ($\times 389$).

ate swine and human O erythrocytes at 4°C, 24°C, whereas they failed to agglutinate erythrocytes from horse, calf, sheep, guinea pig, mouse, chicken and goose. No difference in HA titer was observed at the different temperature. HA titers of the 6 isolated strains and the prototype strains of reovirus types 1, 2 and 3 with human O and swine erythrocytes are summarized in Table 2. The isolated strains as well as the prototype strains agglutinated human O and swine erythrocytes. Swine erythrocytes gave HA titers higher than human erythrocytes except for reovirus type 2.

HI tests with swine erythrocytes are summarized in Table 3. The isolated strains reacted with antisera against reovirus type 3 and the K-15 strain, but not against type 1 and 2. All the isolated strains were identified as reovirus type 3.

The isolated strains had HI titers essentially the same as the homologous titer with antiserum against reovirus type 3, whereas their HI titers showed differences with antiserum against the K-15 strain ranging from 1: 80 to 1: 1280. The antigenic make-up seems to somewhat differ among our isolates and the prototype strain of reovirus type 3.

Our isolates agglutinated human O and swine erythrocytes. Conner *et al.* [3] reported that their equine isolate of reovirus type 3 agglutinated swine erythrocytes. Erasmus *et al.* [5] showed their equine isolates of reovirus type 2 and 3 to

Table 2. Hemagglutination (HA) titers of reovirus serotypes and the isolated strains with human O and swine erythrocytes

Virus strain	HA titer to erythrocytes of	
	Human O	Swine
Lang (Reo-1)	320 ^a	1280
Jones (Reo-2)	2560	2560
Abney (Reo-3)	40	320
K-15	80	320
K-18	80	640
K-19	40	160
K-22	40	320
Tuka-1	80	640
D-13	40	160

a) Titer was expressed as the reciprocal of the highest antigen dilution which showed hemagglutination.

Table 3. Hemagglutination inhibition (HI) tests with guinea pig antisera against reovirus serotypes and the isolated K-15 strain

Virus strain	HI antibody titer of guinea pig antiserum to			
	Type 1	Type 2	Type 3	K-15
Reovirus type 1	5120 ^a	<20	<20	<20
Reovirus type 2	<20	5120	<20	<20
Reovirus type 3	<20	<20	10240	160
K-15	<20	<20	5120	1280
K-18	<20	<20	5120	640
K-19	<20	<20	10240	1280
K-22	<20	<20	10240	1280
Tyka-1	<20	<20	10240	80
D-13	<20	<20	10240	1280

a) Titer was expressed as the reciprocal of the highest serum dilution which inhibited perfectly hemagglutination.

agglutinate human O and bovine erythrocytes. In our hand one of their isolates (reovirus type 3) was shown to agglutinate swine erythrocytes (unpublished data).

Thein and Mayer [15] reported human isolates of reovirus type 1, 2 and 3 produced mild respiratory illness by artificial infection of horses. Conner *et al.* [2] reported that equine reovirus type 3 isolated in U.S.A. and equine reoviruses, types 1 and 3 isolated in Germany produced mild signs of disease by artificial infection of horses. Erasmus *et al.* [5] reported that no clinical disease was observed in horses inoculated with equine reovirus types 2 and 3 isolated in South Africa, although infection was established serologically.

In our study the D-13 strain was isolated from a mesenteric lymph node obtained from a foal dying of peritonitis. Bacteriological examinations indicated the presence of septicemia of *Streptococcus zooepidemicus* in this foal. The septicemia was probably the cause of death, but the relationship of the isolated reovirus and the disease is unknown. The etiological role, if any, of the isolated strains in equine disease awaits elucidation.

ACKNOWLEDGEMENTS. We wish to thank Mr. T. Yamakawa, Mrs. K. Ito and Miss A. Sakata of Equine Research Institute for their technical assistance.

REFERENCES

1. Boer De G. F., Osterhaus, A. D., and Wemmenhove, R. 1978. pp. 415-418. In: Equine Infect. Dis. 1V. Proc. 4th Int. Conf. Equine Infect. Dis. (Bryans, J. T., and Gerber, H. eds.), Vet. Publ., Inc., New Jersey.
2. Conner, M., Gillespie, J., Schiff, E., Holmes, D., Frey, M., and Quick, S. 1984. *Zentralbl. Veterinaermed.*, B 31: 707-717.
3. Conner, M., Kalica, A., Kita, J., Quic, S., Schiff, E., Joubert, J., and Gillespie, J. 1984. *Vet. Microbiol.* 9: 15-25.
4. Engler, R. and Chopin, M. 1968. *Arch. Gesamte Virusforsch.* 25: 237-240.
5. Erasmus, B. J., Pieterse, L. M., and Boshoff, S. T. 1978. pp. 415-418. In: Equine Infect. Dis. IV. Proc. 4th Int. Conf. Equine Infect. Dis. (Bryans, J. T., and Gerber, H. eds.), Vet. Publ., Inc., New Jersey.
6. Imagawa, H., Wada, R., Hirasawa, K., Akiyama, Y., and Oda, T. 1984. *Jpn. J. Vet. Sci.* 46: 1-9.
7. Lerner, A. M., Cherry, J. D., and Finland, M. 1963. *Virology* 19: 58-65.
8. Reinhardt, G., Poletty, M., and Yevenes, J. R. 1983. *Zentralbl. Veterinaermed.*, B 30: 195-202.
9. Sato, K., Inaba, Y., Kurogi, H., Takahashi, E., Satoda, K., Omori, T., and Matumoto, M. 1977. *Vet. Microbiol.* 2: 83-87.
10. Sato, K., Inaba, Y., Shinozaki, T., Fujii, R., and Matumoto, M. 1981. *Arch. Virol.* 69: 155-160.
11. Spendlove, R. S., Lennette, E. H., Knight, C. O., and Chin, J. N. 1966. *J. Bacteriol.* 92: 1036-1040.
12. Spendlove, R. S. and Schaffer, F. L. 1965. *J. Bacteriol.* 89: 579-602.
13. Sturm, R. T., Lang, G. H., and Mitchell, W. R. 1980. *Can. Vet. J.* 21: 206-209.
14. Tayler, K. L. and Fields, B. N. 1985. pp. 823-862. In: Virology (Fields B. N. *et al.* eds.), Raven Press, New York.
15. Thein, P. and Mayr, A. 1974. *Zentralbl. Veterinaermed.*, B 21: 219-233.
16. Thein, P. and Härtl, G. 1976. *Zentralbl. Veterinaermed.*, B 23: 693-701.
17. Wallis, C., Melnick, J. L., and Rapp, A. F. 1966. *J. Bacteriol.* 92: 155-160.

要 約

子馬からのレオウィルスの分離（短報）：今川 浩・松村富夫・鎌田正信・福永昌夫・長谷川斐子¹⁾・大石秀夫²⁾・松本 稔³⁾（日本中央競馬会競走馬総合研究所栃木支所，¹⁾国立予防衛生研究所，²⁾日高地区農業共済組合家畜診療所，³⁾北里研究所）——北海道の軽種馬繁殖牧場において，6株のレオウィルスが健康な子馬の糞便と溶連菌によると思われる腹膜炎で死亡した子馬のリンパ節から，RK-13細胞，MA-104細胞あるいは Vero 細胞で分離された。分離株は培養細胞において好酸性の細胞質内封入体をつくり，そのウィルス粒子の直径は約 85nm で，二重殻構造を有しており，内部に直径約 45nm のコアを持っていた。分離株は間接蛍光抗体法によってレオウィルスと同定され，さらに赤血球凝集抑制反応によりレオウィルス 3 型と同定された。