Serologic and Infectivity Studies of Canine SV-5 Virus (35012)

ESTELLE C. LAZAR, L. J. SWANGO,[1] AND L. N. BINN
(Introduced by A. D. Alexander)
Department of Veterinary Microbiology, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C. 20012

The recovery of parainfluenza SV-5 viruses from laboratory and military dogs with respiratory disease was previously reported (1–3). The virus was first isolated from rhesus monkey kidney cell cultures (4) and subsequently from man (5–8). SV-5 virus appears to infect many mammalian species since specific antibodies are found in man (9) and laboratory and domestic animals (10, 11). The widespread incidence of SV-5 infections in mammals suggests the possible role of this virus as a potential zoonotic agent, although there has been no direct evidence of transmission from animals to man (3, 12).

This study deals with two immediate questions posed by the isolation of SV-5 virus from dogs; its relationship to the simian and human SV-5 isolates and its course of infection in dogs.

Materials and Methods. The techniques used for the collection of specimens, preparation of tissue cultures, isolation of virus, serum neutralization (SN) and hemagglutination-inhibition (HI) tests were described previously (1, 13). In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

Viruses. The origin of the strains of SV-5 studied serologically are summarized in Table I. Dog infectivity studies were done (i) with C958, originally recovered from the lung of a moribund dog and passed 7 times in primary dog kidney cell cultures (DK), and (ii), D008 recovered from a throat swab of an acutely ill dog and passed 3 times in DK (1).

TABLE I. SV-5 Strains Used for Cross Serological Tests.

Strain	Origin	Ref.
D008	Throat of laboratory dog (WRAIR)[a]	(1)
3x84	Throat of military dog (Ft Benning, Ga.)	(2)
Lackland	Throat of military dog (Lackland AFB, Tex.)	(3)
SV-5[b] (21005-2WR)	Rhesus monkey kidney cell culture	(4)
DA[b]	Human blood	(5)
WB-39	Human urine	(6)

[a] WRAIR = Walter Reed Army Institute of Research.

[b] Obtained from American Type Culture Collection.

Antisera. Ferrets were used to prepare specific antisera since SV-5 antibodies are often present in guinea pigs, rabbits, and hamsters (10). Ferrets under diethyl ether anesthesia were given 0.2 ml of virus intranasally. Each antiserum was prepared separately in two ferrets and an uninoculated ferret was held in the same room as a contact control. The ferrets were bled on days 0, 14, 21, and 28.

Experimental infection of dogs. Mixed breed puppies free from detectable antibody to SV-5 were used. The dogs were kept in individual cages in one room. In the first experiment, 4-month-old dogs were inoculated by multiple routes (nasal, oral, tracheal, and peritoneal) with approximately

[1] Present address: School of Medicine, University of Washington, Seattle, Washington 98105.

TABLE II. Cross Serological Reactions Among SV-5 Viruses.

Antiserum vs.	Ferret no.	1/Antibody titer to strain					
		Canine			Simian	Human	
		D008	3x84	Lackland	SV-5	DA	WB-39
Neutralization:							
D008	1	*256*	256	64	64	32	128
	2	*512*	512	256	512	128	512
3x84	1	512	*512*	128	128	32	256
	2	512	*512*	512	128	128	128
Lackland	1	512	1024	*256*	64	64	128
	2	512	512	*512*	32	64	64
SV-5	1	128	64	16	*64*	64	128
	2	256	64	16	*128*	64	128
DA	1	64	16	8	32	*64*	128
	2	128	128	16	64	*128*	128
WB-39	1	16	8	<8	8	8	*32*
	2	128	128	64	64	64	*256*
Hemagglutination-inhibition:							
D008	1	*160*	160	160	80	80	80
	2	*640*	320	320	160	160	160
3x84	1	160	*160*	160	80	80	80
	2	160	*160*	160	80	80	80
Lackland	1	160	160	*160*	40	80	40
	2	160	320	*320*	40	80	80
SV-5	1	80	80	80	*80*	80	40
	2	320	160	160	*160*	160	80
DA	1	40	40	40	<20	*40*	40
	2	80	80	80	20	*160*	80
WB-39	1	20	40	40	<20	40	*40*
	2	80	80	80	40	40	*80*

$10^{5.5}$ tissue culture infective doses ($TCID_{50}$) of virus strain C958. A second group of 7-month-old dogs were inoculated intranasally with approximately 10^6 $TCID_{50}$ of the D008 strain. Two dogs of each group were inoculated with uninfected DK and held in the same room. Body temperature measurements and total white blood cell counts (WBC) were made prior to inoculation to determine normal levels for each dog. Following inoculation, dogs were examined daily for clinical signs of infection. Throat and rectal swab specimens and blood for virus isolation were taken each day for 10 days postinfection (PI) and on alternate days for the next week. Blood for serologic tests was obtained at weekly intervals through day 42. Each virus preparation was also inoculated into ferrets intranasally to detect the possible presence of canine distemper virus in the inoculum. Ferret sera were collected on the day of inoculation and on days 14 and 42 PI.

Results. Serologic comparison of canine, simian, and human strains of SV-5. Before inoculation, all of the ferrets were found to be free of HI and SN antibodies to SV-5. Uninoculated ferrets remained seronegative to day 28. All strains provoked HI and SN antibodies in ferrets by day 14 PI. Generally maximum titers were seen on day 21 and

persisted on day 28. Homologous titers ranged from 20 to 640 by HI and from 32 to 512 by SN tests. The variations in cross-reactions were limited to minor nonreciprocal differences with some exceptions. A small significant reciprocal difference in SN tests was observed between the Lackland strain and the SV-5, DA, and WB-39 strains (Table II).

Canine pathogenicity experiments. In both experiments, the temperatures and WBC of dogs following infections were within normal range. A slight nasal discharge and a very mild pharyngitis and tonsillitis were observed in each of the 4-month-old dogs from the 8th through 14th day PI and in one of the contact dogs of this group on days 12 through 14. In the second experiment, only one inoculated dog had a nasal discharge and signs of tracheobronchitis from the 8th through 14th day PI. One contact dog had a serous nasal discharge on days 8 through 13. Virus was recovered from the throat swab specimens of all inoculated and contact dogs (Table III). No virus was recovered from plasma specimens. Virus was recovered from one fecal specimen on day 6 PI. Rises in both SN and HI antibodies were demonstrated in all dogs including uninoculated contact controls. Antibodies were detectable as early as day 7 PI. Peak levels were usually attained by day 21 and persisted at that level through day 42 (Table III). No clinical signs were seen in the ferrets. However, they developed SN and HI antibody titers to C958 by day 14.

Sera from all dogs and ferrets obtained on the day of inoculation and 42 days later were tested for SN antibody to canine distemper virus. Except for 2 dogs in the first experiment which had the same level of antibody in both serum samples, all sera were negative. These sera were also tested for SN and HI antibodies to parainfluenza type 1 (HA-2 and Sendai), type 2 (Greer), type 3 (HA-1 and SF-4), mumps and Newcastle disease viruses. All were negative except for 8 dogs in Exp. 2 which had the same low level of HI anti-

TABLE III. Virus Recovery and Serologic Response of Experimentally Infected and Contact Dogs.

Dog no.	Route of inoculation	Days virus recovered (throat swabs)	Antibody titers on postinoculation day[a]							
			Hemagglutination inhibition				Neutralization			
			7	14	21	42	7	14	21	42
Exp. 1. Strain C958										
2	Multiple	2–7	0[b]	40	40	40	0[c]	16	64	64
3		2–7	0	80	80	80	0	16	64	64
4		2–7	0	80	80	80	0	16	64	64
5		3–7	0	40	40	40	0	16	16	16
1	Contact	9–11	0	20	40	40	0	4	16	64
6		12–16	0	0	80	80	0	0	64	256
Exp. 2. Strain D008										
7	Intransasal	1–6	40	160	320	320	16	64	64	256
8		1–6	20	80	160	160	4	64	64	256
11		1–7	20	320	320	160	4	256	256	256
12		1–3	80	320	320	320	4	64	256	256
14		2–4	40	160	160	160	0	16	64	256
15		2–7	0	160	160	160	0	64	256	256
9	Contact	10–13	0	0	80	80	0	0	64	64
16		6–10	0	80	160	160	0	16	16	64

[a] Preinfection serums from each dog did not contain detectable HI or SN antibody.
[b] Negative at 1:20 dilution.
[c] Negative at 1:4 dilution.

body to mumps virus in both sera.

Discussion. Previous studies of parainfluenza viruses have revealed antigenic differences within a given type between isolates from man and other different species. For example, the mouse strains of parainfluenza type 1 (Sendai) are distinguishable from the human (HA-2) strains (14), and the bovine (SF-4) strains of parainfluenza type 3 are distinguishable from the human (HA-1) viruses (15). Following the recovery of SV-5 viruses from dogs, it was desirable to more carefully compare the canine isolates with the simian and human strains. Previously, small nonreciprocal antigenic differences have been described for the simian SV-5 viruses (16). The large majority of the cross-reactions observed in the present study were within the range observed with the simian SV-5 viruses. However, a small but significant antigenic difference was found between the Lackland canine SV-5 isolate and the simian and human SV-5 viruses. Crandell *et al.* (3) had previously noted antigenic differences with this isolate and simian SV-5. The human WB-39 virus which was previously reported related to the simian SV-5 (6) also appears to belong to this group. In contrast to types 1 and 3 parainfluenza viruses, there does not appear to be consistent antigenic differences between the simian, human and canine SV-5 viruses studied. Further experiments and field observations are required to determine whether SV-5 virus can be communicated among different species.

Dogs given SV-5 develop a respiratory infection with extensive shedding of virus from the throat. The virus was readily communicable to contact dogs. The signs of disease were minimal, *i.e.*, serous nasal discharge with only one dog developing a mild tracheobronchitis. Even under field conditions, only one-third of the dogs with serologic evidence of infection became ill (2). Military dogs and dogs in a laboratory conditioning colony are subject to much greater stresses than the experimental dogs. The SV-5 virus may contribute to the respiratory disease prevalent in the former dogs in much the same manner as the SF-4 strain of parainfluenza virus 3 has been associated with shipping fever in cattle (17). The parainfluenza infection in dogs has many similarities to parainfluenza infections in man and cattle and can serve as a model for studies of this group of viruses.

Summary. The canine, simian, and human strains of parainfluenza SV-5 virus were compared in SN and HI tests. All of the strains studied are closely related viruses.

Dogs given canine strains of SV-5 virus developed very mild respiratory infections with extensive shedding of the virus from the throat and the development of specific antibody.

The skilled technical assistance of David Green and John Rabbege is gratefully acknowledged.

1. Binn, L. N., Eddy, G., Lazar, E. C., Helms, J., and Murnane, T., Proc. Soc. Exp. Biol. Med. **126**, 140 (1967).

2. Binn, L. N., Lazar, E. C., Rogul, M., Shepler, M. S., Swango, L. J., Claypoole, T., Hubbard, D. W., Asbill, S. G., and Alexander, A. D., Amer. J. Vet. Res. **29**, 1809 (1968).

3. Crandell, R. H., Brumlow, W. B., and Davidson, V. E., Amer. J. Vet. Res. **29**, 2141 (1968).

4. Hull, R. N., Minner, J. R., and Smith, I. W., Amer. J. Hyg **68**, 204 (1956).

5. Hsiung, G. D., Virology **9**, 717 (1959).

6. Liebhaber, H., Krugman, S., McGregor, D., and Giles, J. P., J. Exp. Med. **122**, 1135 (1965).

7. Schultz, E. W., and Habel, K., J. Immunol. **82**, 274 (1959).

8. Behbehani, A. M., Melnick, J. L., and DeBakey, M. D., Exp. Mol. Pathol. **4**, 606 (1965).

9. Aulisio, C. G., Wong, D. C., and Morris, J. A., Proc. Soc. Exp. Biol. Med. **117**, 6 (1964).

10. Hsiung, G. D., Chang, P. W., Cuadrado, R. R., and Isacson, P., J. Immunol. **94**, 67 (1965).

11. Cuadrado, R. R., Bull. W. H. O. **33**, 803 (1965).

12. Hsiung, G. D., Bacteriol. Rev. **32**, 185 (1968).

13. Binn, L. N., Lazar, E. C., Helms, J., and Cross, R. D., Amer. J. Vet Res. **31**, 697 (1970).

14. Cook, M. K., Andrews, B. E., Fox, H. H., Turner, H. C., James, W. D., and Chanock, R. M., Amer. J. Hyg. **69**, 250 (1959).

15. Abinanti, F. R., Chanock, R. M., Cook, M. D., Wong, D., and Warfield, M. Proc. Soc. Exp. Biol. Med. **106**, 466 (1961).

16. Hull, R. N., *in* "Virology Monographs" (S. Gard, C. Hallaner, and K. F. Meyer, eds.), Vol. 2, p. 32. Springer-Verlag, New York/Berlin (1968).

17. McKercher, D. G., J. A. V. M. A. **152**, 729 (1968).

Received May 18, 1970. P.S.E.B.M., 1970, Vol. 135.