elution and after RDE treatment of platelets. Furthermore elution of the virus from platelets is much slower and less complete than that from red blood cells. These phenomena may be due to incorporation of virus particles into the platelets.

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Relationship of Human and Bovine Strains of Myxovirus Para-Influenza 3. (26371)

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The first recognized strain of para-influenza 3 virus was the hemadsorption type 1 (HA-1) virus recovered from children with pneumonia and febrile upper respiratory illness(1). A virus (SF-4) with similar properties of para-influenza 3 virus was recovered from cattle with respiratory disease (shipping fever)(2). The original serological comparison of these human and bovine viruses indicated they were antigenically indistinguishable(3). Subsequent studies by Hamparian and Hilleman, however, showed the viruses could be differentiated by use of serum from intranasally infected guinea pigs (4). The work reported here confirms and extends their findings.

Materials and methods. Virus. The human, HA-1, and bovine, SF-4, strains of the virus used to infect the guinea pigs were grown, respectively, in monkey and bovine kidney tissue cultures. The isolation history and passage of these strains has been reported (1,2). The HA-1 pool had an infectivity of $10^{6.5}$ per ml and the SF-4 pool of 10^{7} per ml. In addition, bovine isolates from different parts of the United States, as well as human isolates from 1955-1959 in Washington, D.C., and from Canada, France, England and Australia, were studied.

Soluble antigens. Preparation of HA-1 and SF-4 soluble antigens is described by Cook, *et al.*(5).

Inoculation of guinea pigs. All guinea pigs were prebled and no hemagglutination inhibition (HI) or complement-fixing (CF) antibodies could be demonstrated with either human or bovine strain antigens. Sera of several animals were also tested for presence of

TABLE I. Antibody Response of Guinea Pigs Infected with Human and Bovine Para-Influenza 3 Viruses.

Days following infection	Infection with HA-1 (human strain)				Infection with SF-4 (bovine strain)			
	HI		Neut.		HI		Neut.	
	HA-1	SF-4	HA-1	SF-4	HA-1	SF-4	HA-1	SF-4
0	<10†	<10	<10	<10	<10	<10	<10	<10
14*	80	10			10	20		
22	640	40	160	40	20	160	<10	20
0	<10	<10	<10	<10	<10	<10	<10	<10
14*	20	<10			<10	10		
22	320	20	160	10	10	80	<10	40
0	<10	<10			10	<10		
14*	40	20			<10	10		
22	320	80			10	160		
0	<10	<10			<10	<10		
14*	<10	<10			10	40		
22	80	<10			160	640		

* Soluble antigen (0.5 ml) inoculated intraper.
† Reciprocal of antibody titers with indicated strain.

neutralizing antibodies and were negative.

The 2 groups of guinea pigs used were housed in separate buildings and were inoculated and tended by separate personnel. They were given 0.2 ml of the respective viruses intranasally. Fourteen days later the guinea pigs were bled and given a 0.5 ml dose, intraperitoneally, of the respective undiluted soluble antigen. Eight days following inoculation of the soluble antigen all animals were bled out.

Human sera. The human sera were from children who had experienced primary infections and some reinfections with para-influenza 3 virus during nursery outbreaks of respiratory disease(6).

Bovine sera were from calves which experienced inapparent infections in nature(7). One calf (#44) was subsequently challenged with the SF-4 strain of the virus.

Serological procedures. Hemagglutination inhibition (HI). All sera were prepared and tested as described by Abinanti, *et al.*(8). Essentially this consisted of adsorption of sera with kaolin followed by adsorption with bovine erythrocytes. Bovine erythrocytes were used as they produced tests which could be easily read. *Neutralization (N).* The neutralization test used has been described by Chanock, *et al.*(1). The test was performed in the usual way and readings were made by means of the "hemadsorption" technic(9).

Results. Guinea pig sera. Table I shows representative responses of 4 guinea pigs in each group to infection. HI antibody to the homologous strain of virus frequently appeared earlier and was 4-fold or greater than antibody for the heterologous strain. Levels of neutralizing antibody were also higher for the homologous than the heterologous strain. There was an increase in antibody titer from the 14th to the 22nd-23rd day.

Human single and multiple infection sera. Results of neutralization tests with the HA-1 and SF-4 strains of virus are shown in Table II. Responses of 4 of the 5 children undergoing first infection were similar to those of the guinea pigs, *i.e.,* moderate to high levels of antibody were observed for the homologous HA-1 strain, while low levels or no antibody for the SF-4 strain was detected. One child (J. Fl.) developed the same level of antibody to both antigens.

Following second infection with para-influenza 3 virus, high levels of HA-1 antibody were produced and one child (D. Pit.) developed a low level of antibody to the bovine strain. The sera of 2 children (S. Tate and D. Pit.) were also tested by HI and no bovine strain antibody was observed following the first infection; however, it was present following the second infection of both children at essentially the same level as HA-1 antibody.

It is of interest that the presence or ab-

TABLE II. Para-Influenza 3 Neutralizing Antibodies in Children during 1st and 2nd Natural Infections.

Individual	Test virus, para-influenza 3	Reciprocal of neut. antibody titer: 1st infection Pre	1st infection Post	2nd infection Pre	2nd infection Post
S. Tate*	Human‡	<8	64	64	256
	Bovine§	<8	<8	<8	<8
D. Pit†	Human	<8	32	64	1024
	Bovine	<8	<8	<8	16
M. St.	Human	<8	512	—	—
	Bovine	<8	<8	—	—
J. Br.	Human	<8	1024	—	—
	Bovine	<8	64	—	—
J. Fl.	Human	<8	1024	—	—
	Bovine	<8	1024	—	—

* 6 mo interval between infections.
† 3 " " " " .
‡ 100 to 320 TCD_{50} in test.
§ 32 TCD_{50} in test.

sence of bovine strain antibody did not appear to be related to the level of HA-1 antibody. (Table II (M. St., J. Br., and J. Fl.).

Bovine infection sera. It was not possible to secure sera from cattle which had been under as close medical scrutiny as the children just described. However, we did have several pre- and post-infection sera from dairy calves that were infected in nature. One of them was subsequently challenged with an aerosol of SF-4 strain of the virus. These sera were tested only for the presence of HI antibodies. While antibody response to both antigens was poor, homologous levels were higher. The one calf which was challenged with the SF-4 strain of the virus produced high levels of antibody to both antigens.

Identity of human and bovine isolates. It was of interest to determine whether other human and bovine isolates had the same antigenic properties as the prototype strains. The human strains of para-influenza 3 virus were recovered from individuals in Washington, D.C. (from 1955 through 1959), Cincinnati(10), California(11), Canada(12), France(13), England(14), and Australia (15). The bovine strains of the virus were isolated from cattle in Maryland, Pennsylvania(8), Kansas(16), California(17), and New York(18). Each of the strains of virus was used as antigen (4-8 units) in the HI test against dilutions of pooled HA-1 and SF-4 strain guinea pig serums, aliquots of the same sera reported earlier in this paper. Each of the human and bovine isolates resembled its respective prototype (Table III). The human isolates were inhibited by HA-1 serum at a 4-fold or higher titer than with SF-4 serum. A similar specificity was observed for the bovine strains with the SF-4 serum. While there is evidence that the human and bovine strains are related, it would appear that 2 antigenically distinct species specific groups exist.

Discussion. Abinanti and Huebner(3) observed no differences in HI, CF or N antibody responses to the HA-1 or SF-4 strain antigens with human, bovine, rabbit or rooster serums. The work of Hamparian and Hilleman(4), has explained these differences. They observed that guinea pigs developed a specific response following infection but that this specificity was lost following repeated exposure to the same virus. A review of the earlier report(3) suggests that the human and bovine serums came from individuals that had prior experience with the virus (as evidenced by HI titers of 1 in 10 or greater in the acute or preinoculation serums), while the rabbits and roosters were given multiple doses of virus.

From these data it is not possible to determine whether this rise in antibody from the 14th to 22nd-23rd day was due to inocu-

TABLE III. Hemagglutination Inhibition Antibody Titers of Strain Specific Guinea Pig Serum Pools when Tested with Human and Bovine Strains of Para-Influenza 3 Virus from Various Parts of U. S., Canada, and Foreign Countries.

	No. of strains showing indicated pattern	Reciprocal of HI titer: HA-1 guinea pig serum pool	SF-4 guinea pig serum pool
Human	5	80	20
	6	160	20
	7	160	40
	5	320	80
Bovine	1	40	320
	4	20	160
	1	<20	80
	1	<20	40

lation of soluble antigen or reflects the temporal course of antibody development. The first possibility seems unlikely, since the ether-treated soluble antigen preparation which was absorbed twice with erythrocytes was free of demonstrable hemagglutinin. Such a soluble antigen would not be expected to stimulate antibody for the viral hemagglutinin.

Pools of sera from each of these groups of guinea pigs were also tested for presence of complement-fixing antibody with HA-1 and SF-4 tissue culture grown viruses. In each instance serum from HA-1 or SF-4 guinea pigs fixed complement with both antigens, but there was at least a 4-fold greater antibody response to the homologous virus.

An interesting observation was the diverse neutralizing antibody response shown by the 3 children (M. St., J. Br., and J. Fl.) following their first infection with para-influenza 3 virus. The factors which influenced the heterologous response in these circumstances are not known. It is possible that prior experience with other myxoviruses may be a factor.

Based on the available evidence it would appear that para-influenza 3 strains do not cross species boundaries. Additional evidence that human and bovine strains differ was provided when the human virus failed to infect a calf when given by aerosol(3). Subsequent challenge of the calf with a bovine strain resulted in infection. It is clear, however, that additional isolates from both man and cattle must be examined before concluding that infection may not occasionally cross species boundaries.

Summary. Guinea pigs infected intranasally with human (HA-1) and bovine (SF-4) strains of para-influenza 3 virus produced 4-fold or greater HI, N and CF antibodies to the homologous strain than to the heterologous strain. Children and cattle showed similar species-linked responses following infection with para-influenza 3 viruses. Human and bovine strains of the virus originating from various states and foreign countries and isolated in different years were shown to react in the same manner as the HA-1 and SF-4 prototype strains.

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