

Experimental Infection of the Gibbon with Influenza Virus.

Coordinator : Thomas J. Smith, LTC, MC
Chief, Virus Dept.

Principal Investigators : Chalobon Karnjanaprakorn, M.S.
Dennis Johnsen, MAJ, VC
Lloyd Olson, MAJ, MC
Prayot Tanticharoenyos, D.V.M.
Rapin Snitbhan, M.D.
William Wooding, MAJ, VC

Assistant Investigator : Sumitda Narupiti, B.Sc.

OBJECTIVE: To determine the susceptibility of the gibbon to experimental infection with influenza A₂ viruses as a means of studying antigenic similarities and specificity of humoral and secretory antibodies formed in response to infection:

DESCRIPTION: In 1968 a major A₂ influenza virus variant appeared in the Far East. Although the agent was subsequently shown to be related, albeit distantly, to earlier A₂ strains, biologically it was distinct.

Thus, the host previously infected with earlier strains of A₂ virus, either naturally or via immunization, was not protected.

It is well known that immunity to infection with influenza virus does not correlate with the presence or absence of humoral antibody. It can thus be assumed that susceptibility probably depends on the presence of secretory antibody in the secretions of that organ constituting the portal of entry. This has been true of measles, parainfluenza and polio-viruses.

The gibbon has been found to be strikingly similar to man insofar as his susceptibility and response to infection by a wide variety of human pathogens. Thus the response of the gibbon to intranasally and intravenously administered strains of A₂ influenza virus was studied.

PROGRESS: As a preliminary experiment, two gibbons were inoculated intranasally with A₂/Jap 305/57 virus. Neither possessed HI or neutralizing antibody to this agent at the time they were inoculated. Neither gibbon showed any signs of illness after inoculation, even though one gibbon shed virus for one week thereafter, and both developed significant levels of neutralizing antibody by three weeks post-inoculation. Since this suggested the virus was not unusually virulent in the host, twenty additional gibbons were inoculated according to the following scheme: (five gibbons per group)

- Group A: A₂/Jap 305/57 intranasally
- B: A₂/Jap 305/57 intravenously
- C: A₂/Hong Kong/68 intranasally
- D: A₂/Hong Kong/68 intravenously

None of these gibbons possessed serum neutralizing antibody to either agent prior to inoculation. Following inoculation animals were followed for 28 days. Every fourth day serum was collected from each animal, and tracheal washings collected. No animal showed any signs of clinical illness. Tables 3-6 show results of antibody titers to homologous virus in each animal. All sera were negative to heterologous A₂ virus when tested for neutralizing (hemadsorption-inhibition) antibody at a 1:10 dilution.

An attempt was also made to characterize classes of serum anti-influenza immunoglobulin present in serially collected sera and tracheal washing by use of indirect immunofluorescence using anti-IGA, IGG and IGM antisera. Considerable technical difficulty prevented accurate determination, including antiserum cross-reactivity between monkey renal cells and virus grown in this system and degree of dilution of tracheal washing specimens.

Two to three weeks after inoculation of this experimental group, signs of upper respiratory tract infection began appearing in other members of the gibbon colony. During the subsequent five weeks, approximately 30% of the 120 members of the colony were clinically affected. Nasopharyngeal swabs were collected from twelve animals, and A₂/Hong Kong/68 Influenza virus were recovered from 5.

During the epidemic four animals died. Two of these were in the experimentally inoculated group who died 30 days after inoculation. Autopsy findings were virtually identical to those seen in primary human influenza pneumonitis. The other two animals died during acute respiratory diseases. Autopsy findings in these animals were similar to that seen in humans with influenza complicated by superimposed bacterial infection.

A serological survey suggested that A₂/H.K./68 was widely disseminated throughout the colony during this epizootic. Approximately 80% of animals, where adequate pre- and post epizootic sera pairs were available, showed evidence of infection. Table 7 illustrates antibody titers found in the colony before and after the epizootic and does not include the 22 animals experimentally infected with the virus.

Table 3. Antibody responses after intranasal inoculation of A₂/Jap 305/57

Gibbon No.	Hemagglutination - Inhibiting antibody titre ^a at indicated time										
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos.	4 mos.		
P-14	<20	<20	<20	<20	<20	<20	<20	<20	<20		
S-12	<20	<20	<20	20	<20	<20	<20	<20	<20		
S-18	<20	<20	<20	40	40	40	40	<20	<20		
S-25	<20	<20	<20	<20	<20	<20	<20	<20	<20		
S-36	<20	<20	<20	<20	<20	<20	<20	<20	<20		
Gibbon No.	Neutralizing antibody titre ^a at indicated time										
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos.	4 mos.		
P-14	<10	<10	<10	<10	<10	<10	<10	<10	<10		
S-12	<10	16	100	100	80	80	80	<10	<10		
S-18	<10	<10	50	160	200	200	200	32	32		
S-25	<10	<10	16	25	50	50	100	<10	<10		
S-36	<10	<10	50	63	32	32	32	16	<10		

^aExpressed as reciprocal of serum dilution.

Table 4. Antibody responses after intravenous inoculation of A₂/Jap 305/57

Gibbon No.	Hemagglutination—inhibiting antibody titre ^a at indicated time									
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos	4 mos	
S-70	<20	<20	<20	<20	<20	<20	<20	<20	<20	
VM-5	<20	<20	<20	<20	<20	<20	20	<20	<20	
VM-6	<20	<20	<20	20	<20	<20	<20	<20	<20	
VM-8	<20	<20	40	40	40	40	40	<20	<20	
VM-9	<20	<20	20	20	<20	<20	<20	<20	<20	
Gibbon No.	Neutralizing antibody titre ^a at indicated time									
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos	4 mos	
S-70	<10	<10	<10	<10	<10	10	16	<10	<10	
VM-5	<10	16	16	16	16	16	32	63	63	
VM-6	<10	<10	16	16	32	32	32	16	<10	
VM-8	<10	16	63	100	126	126	126	63	63	
VM-9	<10	16	16	16	16	16	16	33	<10	

^aExpress as reciprocal of serum dilution.

Table 5. Antibody responses after intranasal inoculation of A₂/KT1/68

Gibbon No.	Hemagglutination—inhibiting antibody titer ^a at indicated time										
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos	4 mos		
P-9	<20	80	320	320	320	320	640	320	80		
S-5	<20	20	80	160	160	320	320	80	80		
S-20	<20	<20	160	160	320	320	320	160	40		
S-21	<20	80	160	320	640	640	640	160	40		
S-39	<20	40	160	640	640	640	640	80	40		

Gibbon No.	Neutralizing antibody titre ^a at indicated time										
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos	4 mos		
P-9	<10	10	250	250	400	640	1000	250	80		
S-5	<10	<10	32	63	80	100	100	63	63		
S-20	<10	<10	160	250	500	500	500	63	40		
S-21	<10	<10	80	250	500	500	500	250	80		
S-39	<10	<10	200	250	250	250	400	80	63		

^aExpressed as reciprocal of serum dilution

Table 6. Antibody responses after intravenous inoculation of A₂/KT1/68

Gibbon No.	Hemagglutination--inhibiting antibody titre ^a at indicated time										
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos	4 mos		
S-65	<20	<20	20	80	80	80	80	80	20		
S-80	<20	<20	80	320	320	320	320	*			
S-87	<20	<20	160	320	320	320	640	**			
B-18-S	<20	<20	<20	160	320	640	640	320	320		
B-16-S	<20	20	80	640	640	640	1280	640	160		

Gibbon No.	Neutralizing antibody titre ^a at indicated time										
	day 0-4	day 8	day 12	day 16	day 20	day 24	day 28	2 1/2 mos	4 mos		
S-65	<10	<10	<10	40	40	50	50	40	20		
S-80	<10	<10	20	126	126	126	320	*			
S-87	<10	<10	50	80	126	160	160	**			
B-18-S	<10	<10	<10	32	80	200	200	640	640		
B-16-S	<10	<10	50	400	500	500	795	640	160		

^aExpressed as reciprocal of serum dilution.

* Died day 29 post-inoculation.

** Died day 33 post-inoculation.

Table 7. Gibbon Colony HAI antibody to A₂/Hong Kong/68 influenza virus.

Serum Titer	Number of Gibbons	
	Pre — epizootic	Post — epizootic
<1 : 20	18	14
1 : 40	6	10
1 : 80	13	17
1 : 160	none	26
1 : 320	"	22
1 : 640	"	1
1 : 1280	"	2