

STUDY REPORTS

1. Title: Influenza A2 Infection in U.S. Military Personnel

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OBJECTIVES

To determine the extent of antigenic shift of 1968 strains of A2 influenza virus in Thailand, to determine extent of protection afforded by military formula polyvalent vaccine, to observe clinical manifestation of disease caused by A2 virus, and to describe epidemiologic aspects of an epidemic in U.S. military personnel.

DESCRIPTION

In August, 1968, an epidemic of influenza occurred in U.S. military personnel stationed at Korat Royal Thai Air Force Base, Korat, Thailand. At the time, approximately 6,200 USAF personnel were stationed there, and the epidemic was monitored in this population during the ensuing three months, by a daily recording of new cases of influenza which were severe enough to require hospitalization or quarters status, and by a recording of the daily dispensary census; that is, of the total number of men excused from duty each day because of illness. 285 clinical records were examined to provide a composite picture of the clinical manifestations of the disease. Chest X-rays and complete blood counts were obtained on all hospitalized patients. A complete influenza immunization history was obtained from 77 men, while the date of the most recent influenza immunization was recorded for all. Throat washings for virus isolation were obtained from 33 men; paired sera for serologic studies were collected from 95. In some instances multiple serum samples were obtained at intervals during convalescence. After the epidemic period, single serum samples were obtained for serologic testing from a random sample of 279 men who had been stationed at KRTAFB throughout the epidemic period. Health records of 159 of these individuals were reviewed to determine whether they had reported symptoms of influenza during the epidemic period.

Virus isolation attempts were performed in tube cultures of primary monkey kidney cells, using the hemadsorption technique. Specific immune sera were prepared in roosters, and serologic testing of these as well as acute and convalescent phase sera from patients consisted of hemagglutination-inhibition, complement fixation, and neutralization (hemadsorption-inhibition) tests.

PROGRESS

During the first two weeks of August a few patients were seen with febrile disease of undetermined origin, which in retrospect were probably cases of influenza. It was not until the third week, however, that cases appeared with increasing frequency, and it became apparent that an epidemic was occurring. Figure 1, a plot of the number of new cases seen each day and the daily hospital census, illustrates the pattern of the epidemic as cases continued to occur during the ensuing weeks. An initial peak of new cases was seen on the 24th of August, followed by a temporary decline for one week. During this early stage of the outbreak, many of the patients were flight personnel or other individuals with a history of

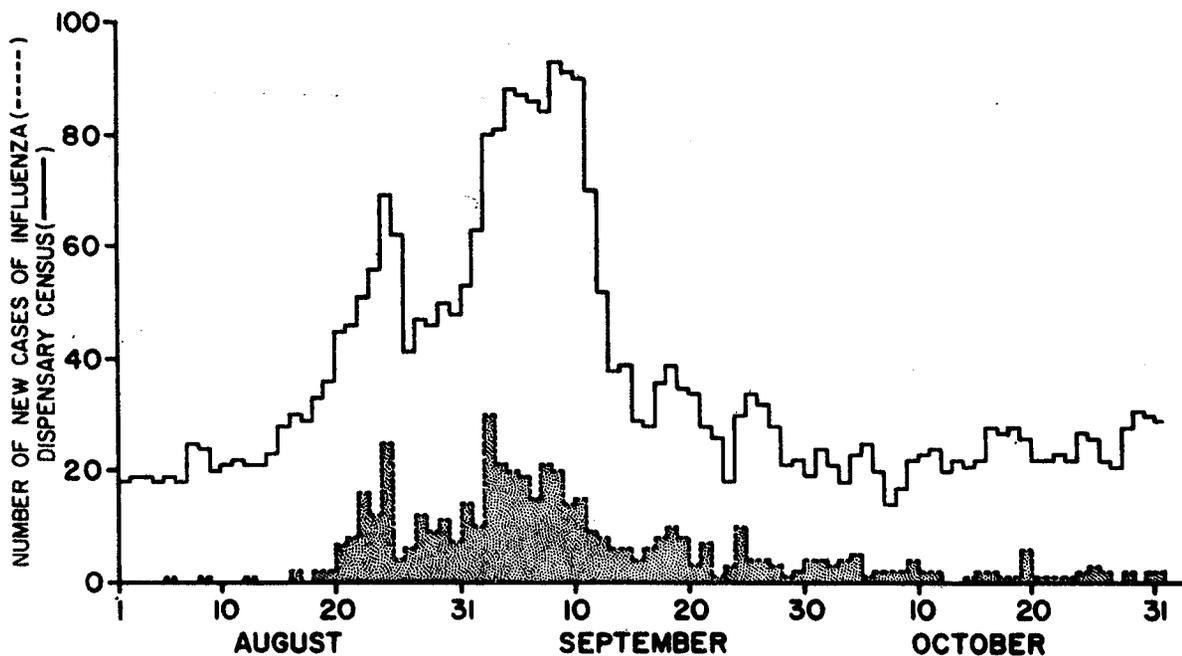


Fig 1. Influenza A2 infection in US Military Personnel

The number of men in whom a clinical diagnosis of influenza was made is plotted according to the day of admission to hospital or quarters status. The number of men on the dispensary census is plotted by day, and represents the total number of individuals in hospital or on quarters status on any given day.

recent travel to other parts of Asia where influenza was occurring. In early September a second larger and more sustained peak of new cases was observed. The dispensary census numbered more than 80 for nine consecutive days during this period, and on 8 Sept. a maximum was reached of 93 men either in hospital or confined to quarters. Between 1 Aug. and 31 Oct., 496 men had influenza of sufficient severity to require relief from duty, representing 8% of the US population at KRTAFB.

Clinical observations

Review of 285 clinical records revealed the study population to consist almost exclusively of young adult men who were in excellent health. None had a clinical history of chronic cardiac or pulmonary disease. The mean age of the group was 28.5 years, with a range of 19 to 55; only 21 were age 40 or older. Table 1 depicts the most common presenting complaints; typically, these were of very sudden onset. Feverishness was reported by 87% of individuals. Other symptoms noted frequently were severe headache, chilliness, myalgias (especially backache) and malaise. Cough, present in a majority of cases, usually was non-productive early in the illness and associated with substernal "tightness". Frequently it later became productive of whitish mucus. Mild sore throat was often present; other presenting complaints included rhinorrhea, a "burning" sensation of the eyes, dizziness, arthralgias, anorexia, and diarrhea. While the patients appeared acutely ill and uncomfortable, there was little in the way of distinctive abnormal physical findings. Fever was the most commonly noted abnormality, usually in the 101–103° range; maximum elevation rarely exceeded 104°. Other abnormal physical findings included conjunctivitis, rhinitis and pharyngitis. Coarse breath sounds and rhonchi were recorded in a few cases.

TABLE 1
Presenting complaints in 285 patients with influenza

Symptoms	Number of men reporting symptom	Percentage of total reporting symptom
Feverishness	248	87
Cough	193	68
Myalgias	192	67
Chills	187	66
Headache	140	49
Malaise	137	48
Sore Throat	61	21
Arthralgia	42	15
Dizziness	21	7
Diarrhea	21	7

Chest X-rays and white blood cell counts were obtained on all patients admitted to hospital. Moderate leucopenia was noted in some cases, and leucocytosis was observed in others; however there was no significant or consistent abnormality. Only three of 100 chest X-rays obtained were abnormal. One man had a left lower lobe consolidation, proven subsequently to be due to D. pneumoniae. Two individuals had patchy peribronchial infiltrates; serologic studies for influenza were negative in the one case in which they were done. This same individual had a cold agglutinin titer of 1:32 early in his illness. Thus two of the three individuals with X-ray evidence of pneumonitis apparently did not have influenza and the third case was not confirmed by laboratory diagnosis.

Therapy during hospitalization consisted of supportive measures; in a few instances broad spectrum antibiotics were employed without apparent effect following the appearance of productive cough. The duration of illness for the entire group ranged from one to 14 days, with a mean of 3.8 days. The average number of days lost from duty was 2.6 days. Convalescence was prolonged in some instances by persistent malaise, cough, and easy fatigability. There were no fatalities or significant sequelae.

Effect of prior immunization

Every individual in the study group had received at least one influenza immunization at some time prior to the epidemic with the military formula polyvalent vaccine. The vaccine used at KRTAFB in 1968 contained 400 CCA units of A2/Taiwan 1/64 antigen as part of a total 1000 CCA units per dose. The number of immunizations varied with the length of Air Force service, with some older men having received up to 12 annual inoculations. A routine influenza immunization program had been conducted at KRTAFB in May 1968, at which time an effort was made to vaccinate all personnel; in August, when it became apparent that influenza was occurring, another program was instituted to immunize those individuals who did not receive vaccine in May. As a result, 236, or 83%, of the 285 men whose records were examined had been vaccinated in 1968; all but 16 of these after 1 May. The clinical manifestations of infection in the 49 men who had received no influenza vaccine in 1968 were no different from those seen in more recently

vaccinated individuals; the mean duration of symptoms was 3.8 days in the former group, causing an average loss of 2.8 duty days. Analysis of complete influenza immunization histories of 77 men showed that there was no correlation between the number of prior immunizations and severity of illness. The apparent lack of effect of prior vaccination is illustrated by the observation that 18 of the 77 men had received seven or more doses of vaccine during the preceding 10 years; these 18 men lost an average of 3.4 duty days because of illness, compared with an average loss of 2.6 days for the entire study group.

Virus Isolations

Twenty-two virus strains were isolated from samples of pharyngeal secretions collected from 33 suspect cases of influenza. All positive cultures were hemadsorption-positive and showed evidence of gross cellular injury by the third day after inoculation, which progressed to complete destruction of the cell sheet over the next several days. Further passage of the isolates in MK cells or embryonated eggs was accomplished without difficulty, with hemagglutinin detectable in culture fluids at dilutions of 1:80–1:160 and in allantoic fluid at dilutions up to 1:640. Specific rooster antiserum prepared against one of the isolates was tested in the HI test against all isolates for purposes of identification. The antiserum had an HI titer of 1:320 when tested against the homologous antigen, and 1:80–1:160 against all other strains. This result was interpreted to indicate that no significant antigenic difference existed between isolates.

Additional evidence for strain identity and a measure of relationships to A2/HK/68 and earlier A2 strains were obtained by testing rooster sera prepared against several recent strains for HI activity against these strains as well as against older strains (table 2). Homologous HI titer to each of the 1968 strains was 1:320–640 and was the same against other 1968 strains, indicating apparent identity between A2/KT/68 and A2/HK/68 viruses. A2/Puerto Rico/64 antiserum reacted to some extent with the 1968 strains, and low levels of HI activity of A2/KT/68 and A2/HK/68 antisera were observed with A2/Puerto Rico/64 virus. Antisera against A2/KT/68 and A2/HK/68 viruses failed to react with A2/JAP305/57 and A1/FM1/47.

TABLE 2
Comparison of current influenza strains with earlier A2 and A1 viruses
by HI test with specific rooster antisera

Rooster serum	Reciprocal HI titer against indicated virus					
	A2/KT1/68	A2/KT28/68	A2/HK1/68	A2/Puerto Rico/64	A2/JAP305/57	A1/FM1/47
A2/KT1/68	<u>640</u>	320	320	<20	<20	<20
A2/KT28/68	640	<u>320</u>	320	20	<20	<20
A2/HK1/68	640	320	<u>320</u>	20	<20	<20
A2/Puerto Rico/64	80	40	20	<u>160</u>	20	<20

Testing of rooster sera for N antibody against several A2 strains again illustrated the identity of A2/KT/68 and A2/HK/68, and the extent of the difference in antigenic configuration between these viruses and earlier A2 strains (table 3). A2/Puerto Rico/64 and A2/JAP305/57 antisera showed low levels of activity against the 1968 strains in this test. A2/KT/68 and A2/HK/68 antisera reacted slightly with A2/Puerto Rico/64 but not with A2/JAP305/57.

TABLE 3
Comparison of current influenza strains with earlier A2 viruses by
 neutralization test with specific rooster antisera

Rooster serum	Reciprocal A2/KT1/68	neutralization A2/HK1/68	titer against A2/Puerto Rico/64	indicated virus A2/JAP305/57
A2/KT1/68	<u>320</u>	640	20	<10
A2/HK1/68	640	<u>640</u>	10	<10
A2/Puerto Rico/64	20	20	<u>640</u>	160
A2/JAP305/57	20	40	1280	<u>1280</u>

N and HI antibody responses

Paired sera from 95 patients, collected at two or three week intervals, were tested against A2/KT1/68 and A2/HK1/68 antigens for HI antibody and against A2/KT1/68 for N antibody. Sixty-seven men (70.5%) developed a four-fold or greater increase in N antibody titer in their second serum samples (table 4). The acute phase sera of over half (36 of 67) who showed evidence of sero-conversion contained undetectable levels (<1:5) of N antibody, while 21 were positive at 1:5, nine at 1:10, and one at 1:20. In contrast, most of the men (23 of 28) in whom a four fold rise in N antibody was not demonstrated contained antibody in their acute phase sera. Five of 23 samples titered 1:5, eight 1:10, six 1:20, two 1:40, and two \geq 1:80. These increased levels of antibody in acute-phase samples are highly suggestive of influenza A2/KT/68 infection and indicate that in at least some of these cases, the acute phase serum was obtained too late to detect a four-fold rise in antibody. If a N antibody titer of 1:20 or greater is accepted as indicative of infection, a total of 77 (81%) of the 95 men showed serologic evidence of influenza A2/KT/68 infection.

Fifty men (52.6%) developed a four-fold or greater rise in HI antibody titer to A2/KT1/68 during convalescence (table 4). Forty showed significant rises to A2/HK1/68 as well, while the other 10 showed less than a four-fold rise against the latter antigen. There were two additional men who demonstrated a four-fold rise in antibody against A2/HK1/68 and not against A2/KT1/68. These sera which were positive against only one of the two antigens generally contained low levels of antibody, and the results were interpreted to represent variability inherent in the HI test rather than antigenic differences between the two virus strains. HI antibody titers in the 50 men ranged from 1:40 to 1:320 during convalescence, with geometric mean titers of 1:146 at two weeks and 1:137 at three weeks.

Acute sera frequently inhibited HA at 1:20 or 1:40, and occasionally at higher dilutions. HI tests of 49 sera collected Apr-Jun 1968 yielded the same result; since none contained N antibody against A2/KT1/68 at a 1:20 dilution, the HI activity probably represented a high sensitivity of A2/KT1/68 to normal serum inhibitors persisting after trypsin-periodate treatment.

When HI & N tests for diagnosis of infection were compared, it is clear that the N tests was the more efficient (table 4). Of the 50 infections confirmed by HI test, all but one were also confirmed by the N test. Eighteen additional infections were confirmed by a rise in N antibody alone, with no corresponding rise observed in HI antibody. Thus, almost 19% of the total of 67 confirmed infections were detected only with the N test.

TABLE 4
Results of testing acute and convalescent phase sera from 95 patients
for A2/KT/68 neutralizing and HI antibody

Sample	Number	% of Total
Sera tested for neut. and HI antibody	95	100
Sera with 4X rise in neut. antibody	67	70.5
Sera with 4X rise in HI antibody	50	52.6
Sera with 4X rise in both neut. & HI antibody	49	51.5
Sera with 4X rise in neut. antibody and not HI antibody	18	18.9
Sera with 4X rise in HI antibody and not neut. antibody	1	1.0

CF antibody response

CF antibody responses were measured in 40 men. Thirty-nine were selected because they showed a significant rise in N antibody during convalescence, with or without an accompanying rise in HI antibody. One man had a rise in HI antibody only. Acute sera contained low or undetectable CF antibody titers; 21 were positive at 1:4, and two at 1:8. Twenty-eight of the 40 developed a significant increase in titer during convalescence (2-3 wk). Titers of positive sera ranged from 1:8 to 1:64 with a geometric mean titer of 1:20. Late convalescent sera (8-10 wk) from four men contained two to four-fold less antibody than the 2-3 wk samples. Twenty-five of the 40 men showed four-fold or greater rises in HI antibody; 19 of these also showed CF antibody rises. The one man who had an HI antibody titer rise but no neutralizing antibody was negative by CF test. Thus five men who were positive by both N and HI tests were negative by CF test. On the other hand, nine men with N antibody were also positive by CF test, yet negative by HI test. Six men with significant levels of N antibody were negative by both HI and CF test. These results indicate that while the CF test was slightly more sensitive than the HI test in detecting A2/KT/68 infections significant rises in HI antibody occurred in the absence of rises in CF antibody.

Persistence of N and HI antibody

Additional sera were obtained late in convalescence, between five and ten weeks after onset of illness, from 18 individuals who had shown a rise in HI antibody in their two week samples. Sera were collected from seven men at both five and ten weeks, while single specimens were obtained from the remainder at either five weeks or between the eighth and tenth week of convalescence. The results of testing these sera for HI and neutralizing antibody against A2/KT1/68 are shown in table 5. Antibody levels remained elevated in all samples during this period, and the geometric mean titers of HI and N antibody was not significantly different in two, five or 8-10 week serum samples.

N and HI antibody in post-epidemic sera

Post-epidemic sera, collected between 14 Nov and 10 Dec from a random sample of 279 men who had been present at KRTAFB during Aug.-Oct., were tested for A2/KT/68 HI and N antibody in an attempt to determine the true attack rate and incidence of subclinical infection. The vast majority contained non-detectable or low levels of HI activity (1:20-1:40). Only 22 men had HI antibody at levels of 1:80 or greater, with a maximum titer of 1:320 observed in three individuals. Since the mean titer of N antibody in 12 men with documented influenza was shown to be greater than 1:200 during the 8th to 10th week

TABLE 5
Neutralizing and HI antibody response in sera of patients convalescent
from influenza A2/KT/68 infection

Week serum obtained	No. of sera tested	Reciprocal HI antibody titer		Reciprocal neutralizing antibody titer	
		Range	Mean	Range	Mean
0	18	<20-40	—	<5-10	—
2	18	40-320	143	40-1280	226
5	13	40-640	156	80-1280	236
8-10	12	20-320	146	80-1280	242

TABLE 6
Persistence of influenza neutralizing antibody in post-epidemic sera of a random sample of 279 men

Clinical history of influenza	No. of men	No. with neutralizing antibody	Percent positive
Symptomatic (hospitalized)	10	4	40
Symptomatic (dispensary visit)	18	4	22.2
Asymptomatic (no dispensary visit)	129	12	9.3
Unknown	122	14	11.5
Total	279	34	12.2

after illness (table 5), sera were screened for N antibody at a 1:50 dilution. Thirty-four of the 279 were positive, and 17 were among those who showed HI titer of \geq 1:80. Thus 12.2% of the men tested had evidence of prior A2/KT/68 infection in the form of a significant titer of N antibody.

Review of 157 clinical records revealed that 28 men (17.8%) had experienced influenza-like symptoms severe enough for them to seek medical care during the epidemic, and 10 of these 28 were included in the study group of hospitalized patients. Eight of the 28 men (28.5%) with a history of influenza had an N antibody titer of \geq 1:50. Table 6 illustrates the relationship between history of clinical influenza and the presence of N antibody. While the number of men with a positive history is small, there does appear to be a direct correlation, in that only 9.3% of men with a negative history had a detectable level of serum N antibody, compared with 22% of those who sought medical aid, and 40% of those requiring hospitalization. Included in the post-epidemic sample were four men from among the 95 whose sera had been tested previously and found to contain N antibody during convalescence (range 1:80-320). Only two of the four had detectable N antibody in their sera approximately three months after onset of illness.

SUMMARY

An epidemic of A2 influenza occurred in US airmen at Korat, Thailand, after the infection was introduced by flight crews returning from other parts of Asia. Virus strains were identical with A2/HK/68 virus, and serologic testing with specific antisera emphasized the extent of the antigenic alteration which had occurred; there was slight cross-reactivity with earlier members of the influenza A2 subgroup.

During a three month period, 8% of the population experienced an influenza-like disease severe enough to require relief from duty. All illnesses were brief and uncomplicated. The neutralization test was the most sensitive serologic test for detecting these infections. Most individuals had received polyvalent influenza vaccine within the preceding six months; severity and duration of illness were apparently unmodified by either recent vaccination or by multiple prior vaccinations.

By conservative estimate, 12% of the population became infected during the epidemic period. The relatively low attack rate and low morbidity probably resulted from the fact that the study population consisted exclusively of healthy young adults living in an environmental situation which was not conducive to rapid or wide-spread dissemination of influenza viruses.