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Reaction of Squirrel Monkeys to Intratracheal
Inoculation with Influenza/A/New Jersey/76 (Swine) Virus

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Running title: Swine Influenza in Squirrel Monkeys

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In conducting the research described in this report, the investigators
adhered to the "Guide for the Care and Use of Laboratory Animals," as
promulgated by the Committee on Revision of the Guide for Laboratory
Animal Facilities and Care of the Institute of Laboratory Animal
Resources, National Research Council. The facilities are fully
accredited by the American Association of Accreditation of Laboratory
Animal Care.

29 September 1976

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ABSTRACT

To determine whether a model could be established for laboratory investigations, nine squirrel monkeys were inoculated intratracheally with 10^7 EID₅₀ of influenza virus type A/New Jersey/8/76 (Hsw1 N1) (swine influenza virus). They responded with clinically detectable illness including fever, leukopenia, decreased food consumption, increased respiratory rate, occasional coughing, labored breathing, nasal discharge and lethargy. Convalescence was well advanced by the tenth day. All monkeys excreted virus for 7 to 8 days. A scoring procedure (illness score) has been developed for use in studies of vaccine and chemotherapeutic efficacy.

In February 1976, influenza virus bearing swine antigenic determinants (Hsw1 N1) was isolated from several recruits at Fort Dix, New Jersey [1]. Because this virus represented a serotype to which the majority of the American population had no immunity, it was feared that a pandemic might begin during the following fall. Consequently, a decision was made to make vaccine available to all Americans.

Although extensive investigations of the antigenicity and reactogenicity of various swine influenza vaccines have already been carried out [2], no reports of the protection afforded by these vaccines have been published. The principal reason for the lack of protection data is that the virulence and communicability of the recently isolated strain is unknown, and as a consequence, no experimental inoculation of humans with swine influenza has been attempted in the United States.

A desire to limit the dissemination of the virus has also largely precluded experiments with laboratory animals. Because we have recently published the details of a squirrel monkey model for influenza infections [3], and because we have unique containment facilities for the study of potentially hazardous microorganisms, we were requested by the National Institute of Allergy and Infectious Diseases to investigate the protective efficacy of vaccines and chemotherapeutic agents in primates.

In order to carry out investigations of prophylaxis, it was first necessary to determine whether the New Jersey strain of influenza virus caused clinical illness in squirrel monkeys and, if so, whether a means of assessing the degree of clinical illness could be developed to compare

responses among groups of animals given various treatments. A systemic reaction score has been employed by Parkman et al. to evaluate the reaction of human subjects to various vaccine preparations [2], and by Beare and Craig to evaluate the reaction of humans to various test strains of influenza virus (clinical score) [4]. The clinical response (illness score) of squirrel monkeys to intratracheal inoculation of virus is presented in this report.

Materials and Methods

Virus preparation and assay. A sample of the fifth egg passage of influenza A/New Jersey/8/76 (Hsw1 N1) was obtained from the Bureau of Biologics, Food and Drug Administration, diluted 1:1000 in sterile heart infusion broth (HIB) and inoculated into the allantoic cavity of 10 to 12 day embryonated eggs. After 48-hr incubation at 35 C, the allantoic fluid was collected, distributed in 3-ml aliquots, and stored at -70 C. Estimates of the concentrations of virus in terms of egg infectivity were accomplished as previously described [5]. The allantoic fluid preparations had a titer of $10^{7.2}$ median egg infectious doses (EID₅₀) per ml. Antigenic purity of the viral preparation was confirmed with reference standard materials supplied by the Center for Disease Control (CDC). The serological tests required for confirmation were carried out by standard CDC procedures [6].

Monkeys. Nine male squirrel monkeys (Saimiri sciureus), obtained from commercial sources, were used in this study. Average weight was 0.7 kg (range 0.493 - 1.01 kg). Monkeys were housed in wire-bar cages and fed commercial monkey chow supplemented with fruit until the time of the experiment. Water was provided ad libitum. During experiments food was limited to six commercial biscuits daily.

Intratracheal inoculation. The procedure for intratracheal inoculation of virus has been described previously [3]. Briefly, it involves inoculation through a plastic catheter that has been passed over the epiglottis and into the trachea of a lightly anesthetized monkey.

Bacterial and viral isolation. Established methods were employed to detect bacteremia [3, 5]. Virus was isolated from the oropharynx by swabbing the back of the throat and the tonsil area with a swab moistened with HIB. The contents of the swab were washed into 1.0 ml of HIB containing 50 µg/ml of gentamicin, 100 units/ml of penicillin and 100 µg/ml of streptomycin. The suspensions were incubated at room temperature for 60 min to kill bacteria and then inoculated into embryonated eggs (6 eggs per sample). After 48-hr incubation at 35 C, followed by overnight refrigeration, the presence of virus was detected by routine hemagglutination procedures.

Clinical and laboratory determinations. Rectal temperature, hematocrit, total and differential leukocyte counts, respiratory rate, pharyngeal virus isolation, food and water consumption, body weight, nasal discharge, coughing and sneezing, labored breathing, and activity were recorded daily. Blood for serum HAI antibody determination was obtained prior to virus inoculation and at 7, 14 and 28 days after infection.

Results

Preliminary observations. Nine monkeys were inoculated intratracheally with 10^7 EID₅₀ of virus. Figure 1 presents the deviation from baseline values (mean of three observations) of five of the response parameters. Frequency of occurrence of three more parameters is also illustrated in the same figure. Activity and dyspnea are omitted from the figure because lethargy and labored breathing were seen daily in every monkey. Hematocrit values are not included because of variation due to daily bleeding. Although sick monkeys seemed to drink less water than healthy ones, it was impossible to measure water consumption accurately because these monkeys would only drink from bowls, and lost water by splashing.

Monkeys became ill within 24 hr after virus inoculation; fever was most prominent at this time and then slowly subsided. Most of the other parameters seemed to reach a maximum (minimum for leukocyte count) in 2 to 5 days, and then slowly subsided. All values had returned to normal within 10 days (not shown); virus shedding also ceased by this time. At 10 days the monkeys had also begun to gain weight. The geometric mean of the reciprocal of the serum HAI titers was <10, 1.9, 33.1, and 127.0 at -5, 7, 14, and 28 days after inoculation, respectively.

Pathology. In a separate experiment an untreated monkey receiving influenza virus was killed and necropsied six days after virus inoculation. Pulmonary changes were similar to those previously described

for squirrel monkeys infected with the Aichi/2/68 strain of type A influenza virus [3]. Microscopically, the bronchial and bronchiolar epithelium was moderately altered by proliferative changes accompanied by focal erosions and areas of deciliation. There was focal ulceration, loss of cilia, and moderate proliferative change. In some areas, the proliferative epithelium extended along alveolar septa and partially occluded alveolar spaces. There was mild infiltration of the pulmonary interstitium around affected bronchioles by macrophages and a few neutrophils. Proteinaceous fluid and a few inflammatory cells partially filled alveoli around some of the more severely affected bronchioles.

Illness score. An illness score was devised to score the response of monkeys over the first seven days after infection. After that, variation in rate of recovery from the illness precluded the use of a scoring system. The scoring system was arbitrary and was designed to weight most heavily those parameters that could be objectively measured. In the case of virus shedding, additional weight was given if relatively large amounts of virus were recovered (>4 of 6 eggs contained virus). The score calculated for the nine monkeys described above is given in table 1. The theoretical minimum possible score would be zero, a critically ill monkey would score approximately 77 (assuming a 20% weight loss and maximum values for the other parameters). Hence, the mean score of 47.6 represents only moderate illness although all monkeys were

infected.

Dose response. A single experiment was performed to determine the effect of dose of virus on response. Groups of four monkeys each were inoculated intratracheally with 10^3 , 10^5 and 10^7 EID₅₀ of virus and examined for seven days. The illness indices were 37.8, 22.8 and 39.2 for 10^3 , 10^5 and 10^7 EID₅₀ doses, respectively. Although the high and low doses appeared to produce the same degree of illness, the pattern was different. Monkeys receiving 10^3 EID₅₀ of virus had significantly more fever (score of 9.0 vs 3.3) and less change in respiratory rate (score of 0.75 vs 2.7) than did those receiving 10^7 EID₅₀. The cause of these differences is presently unknown.

Discussion

The New Jersey strain of influenza virus causes a mild disease in squirrel monkeys after intratracheal inoculation. The disease is characterized by fever, leukopenia, anorexia, increased respiratory rate, occasional coughing, slight nasal discharge and proliferation of the virus. The animals were lethargic and demonstrated labored breathing for about seven days. Convalescence was well under way by the tenth day. Signs of disease were very similar to those previously seen in experiments with influenza A/Aichi/2/68 (H3N2) [3]. Virus isolations have not been done routinely in experiments with the Aichi strain, but the illness index for four monkeys inoculated with doses equivalent to those employed with the New Jersey strain was 34.0. The index for the nine monkeys discussed in these experiments (subtracting the score attributed to virus shedding) was 29.3. These differences were not significantly different. Although insufficient monkeys have been necropsied to provide a reliable comparison, the lesions indicate that the disease process in lung tissue is about the same as that seen with the Aichi strain.

Our observations of a mild disease in monkeys is consistent with the observations of Beare and Craig in human volunteers inoculated with the New Jersey strain of virus [4]. These investigators noted mild reactions although all subjects were infected and all excreted virus.

The illness score that we have developed has provided a useful tool for comparing the effect of various treatments. In particular, it has

it has enabled us to determine the identity of the parameters that are most affected. The scoring system has been employed in vaccine experiments that will be published at a later date.

References

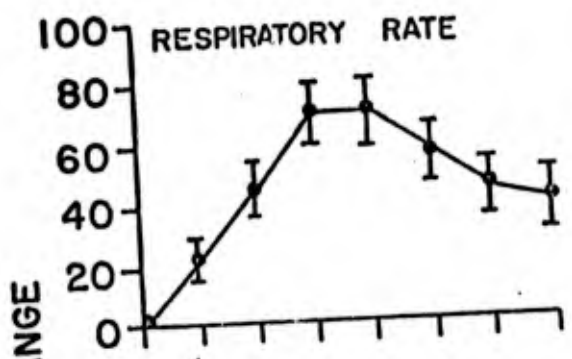
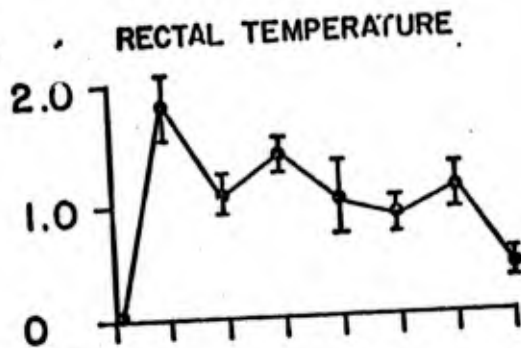
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Table 1. Seven-day illness index for squirrel monkeys infected with swine influenza virus (A/New Jersey/76).

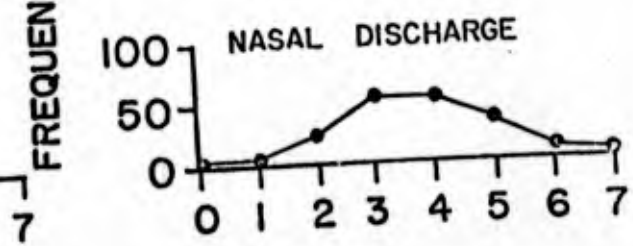
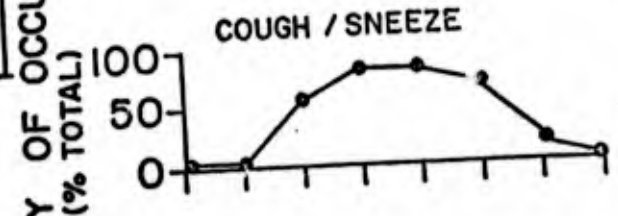
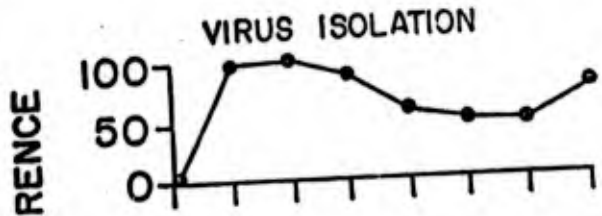
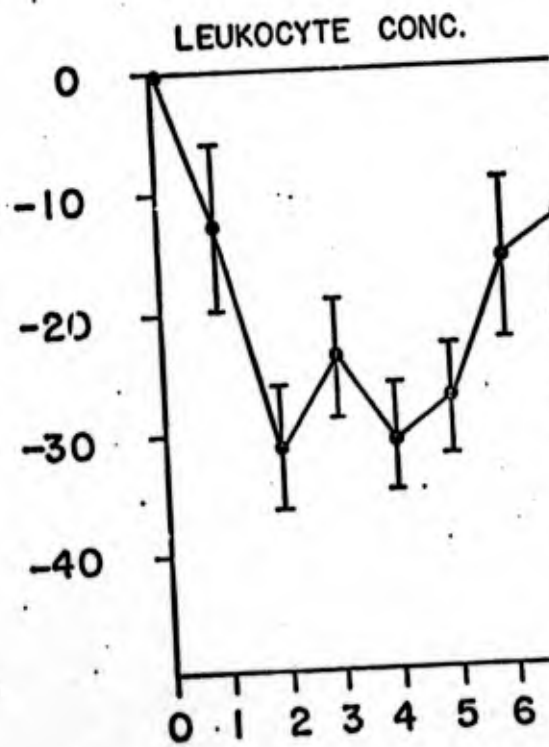
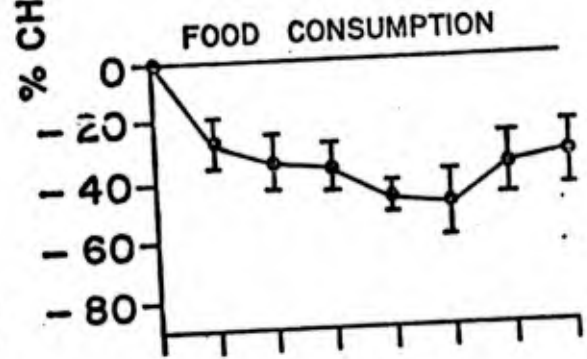
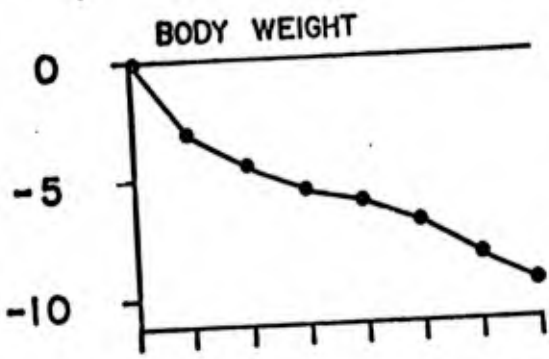
Parameter and scoring procedure	Mean score (SEM) for 9 infected monkeys
1. <u>Virus Shedding</u> - 1 for each day that virus was detected, plus 1 additional for each day that 4 of 6 eggs or more were positive	8.75 (1.25)
2. <u>Temperature</u> - 2 for each day that rectal temperature was ≥ 1 F above baseline value	8.0 (1.31)
3. <u>Body Weight</u> - 1 for each percent loss of body weight at 7 days	9.9 (1.94)
4. <u>Leukocyte Concentration</u> - 1 for each day that total leukocyte count was ≤ 1500 below baseline value	4.4 (0.84)
5. <u>Respiratory Rate</u> - 1 for each day that rate was $\geq 40\%$ above baseline value	3.5 (0.78)
6. <u>Appetite</u> - 1 for each day that biscuit consumption was ≤ 2 less than baseline value	5.9 (0.58)
7. <u>Nasal Discharge</u> - 2 if nasal discharge was observed for 2 or more days	2.0 (0.0)
8. <u>Coughing/Sneezing</u> - 2 if coughing was observed for 2 or more days	1.25 (0.37)
9. <u>Dyspnea</u> - 2 if labored breathing was observed for 2 or more days	2.0 (0.0)
10. <u>Activity</u> - 2 if activity was reduced for 2 or more days	2.0 (0.0)
Overall Mean Illness Index	47.6 (2.89)

FIGURE 1. Change in selected clinical parameters after intratracheal inoculation of the New Jersey strain of influenza virus. Vertical bars represent the standard error of the mean.

CHANGE (°F)



% CHANGE



DAYS AFTER INFECTION