ized by fever and a cough. The epidemic was explosive. Within 5 months, it had spread to all parts of the country. Horses of all ages were susceptible, and attack rates were 70 to 90 percent in local outbreaks. Few deaths resulted.

This epidemic was apparently one of a series of like events which have afflicted horse populations over many generations. No cases of illness caused by this virus were uncovered in man.

Etiology

Walter R. Dowdle, Ph.D.,

Wilma B. Yarbrough,

Roslyn Q. Robinson, Ph.D.

WHEN equine influenza first appeared in epidemic proportions in Florida in the late winter of 1963, an agent was isolated from nasal washings from an infected horse. This agent was found to be an influenza virus (*4*). Subsequently, a similar virus was isolated from infected horses during an influenza outbreak in Lexington, Ky. (personal communication from Dr. J. T. Bryans and Dr. E. R. Doll).

As the epidemic continued to spread, epidemiologists from the Communicable Disease Center and elsewhere collected specimens for study in the CDC laboratory. Nasal swabs and washings were received from Ohio, New York, and New Mexico. Paired serum specimens were collected from infected horses at racetracks in New York, Ohio, Nebraska, and California. The original virus isolates were sent to us from Florida and Kentucky, and, as the epidemic progressed, we received virus strains isolated from horses at racetracks in Delaware, Maryland, Michigan, New Mexico, New York, Ohio, and Oregon.

Materials and Methods

Clinical material. Nasal swabs or nasal washings or both, were obtained from horses within 24 to 72 hours after onset of illness and frozen in dry ice for shipment to the laboratory. Acute phase serums were collected as soon as possible after onset, and convalescent specimens were collected 2 to 3 weeks later.

Virus isolation. Nasal specimens were treated with an equal volume of tryptose phosphate broth containing 0.5 percent gelatin, 400 units penicillin, 200 μg streptomycin, and 200 units mycostatin per ml. Cell cultures of primary rhesus monkey kidney, human fetal kidney, and diploid human lung fibroblasts were each inoculated with 0.3 ml. of the treated specimens. Ten-day-old embryonated chicken eggs were inoculated with 0.1 ml. of the treated specimens by both the amniotic and allantoic routes. Cell culture tubes were incubated for 7 days for each passage at 33° C. and tested for the presence of virus by hemagglutination and hemadsorption with guinea pig erythrocytes (*5*). Eggs were incubated for 72 hours at 33° C. Amniotic and allantoic fluid harvests were tested for presence of hemagglutinins at dilutions of 1:2 in 0.4 ml. with equal volumes of 0.5 percent chick and 0.4 percent guinea pig erythrocytes at room temperature and at 4° C.

Biochemical and physical properties. Sensitivity of the virus to ethyl ether was determined by the method of Andrews and Horstmann (*6*) and acid lability by the method of Ketler and associates (*7*). A comparison of virus size with a known influenza A virus was made with graded millipore filters. A tissue culture adapted equine virus was mixed with A2/NC/1/63, a current human strain, and portions were passed through filters of 100 mμ and 220 mμ pore diameters. One portion of each filtrate was treated with antiserum specific for one of the viruses in the mixture and the resulting infectivity titer determined in monkey kidney. A second portion of each filtrate was treated in the same manner with antiserum against the second virus in the mixture. The identity of the virus yielding infectivity titers was reconfirmed upon completion of the tests.

Hemagglutinins were titrated in phosphate buffered saline (pH 7.2) with 0.5 ml. of virus dilutions and an equal volume of erythrocytes. All erythrocyte suspensions were adjusted to cell concentrations equivalent to that of a 0.5 percent (by volume) suspension of chick cells, that is, approximately 4×10^7 cells per ml.

Serology. Hemagglutination-inhibition and complement fixation tests were performed as previously reported by this laboratory (*8*).

Table 1. Type-specific complement fixation tests with two representative equine influenza isolates

Serums	A2-equine isolates		Control antigens			
	Ohio/1/63	NY/1/63	A1/FM1/47	Soluble		B/GL/1739/54
				A	B	
Guinea pig:						
Antisoluble type A	16	32	32	64	0	0
Antisoluble type B	0	0	0	0	32	32
Horse (equine influenza):						
BH S_1–S_2	----------	0–128	----------	0–128	0–0	----------
SE S_1–S_2	----------	0–256	----------	0–128	0–0	----------
Human (Asian influenza):						
DJ S_1–S_2	0–256	0–256	0–128	--------	------	0–0
DJT S_1–S_2	0–64	0–64	0–64	--------	------	0–0

NOTE: S_1–S_2=acute–convalescent serums.

Neutralization tests in tissue culture were undertaken with 100 $TCID_{50}$ of virus and equal amounts of twofold dilutions of serum. End points were based on the presence or absence of hemagglutinins.

Results

Virus isolation. Viruses were readily isolated in embryonated eggs in both the amniotic and allantoic cavities, but no isolations were made in tissue culture. Chick cell hemagglutinins were detected in the allantoic fluid from first egg passages ranging in titer from 1:16 to 1:64. Hemadsorbing areas were unmistakably present in monkey kidney and human fetal kidney on first passage of several of the nasal washings, but the virus was not recovered.

All isolated equine viruses studied were easily "adapted" to primary monkey kidney, using virus from the first or second egg passage. After several passages in monkey kidney, infectivity titers of 10^4 to 10^5 were not uncommon, and the cytopathic effect of the virus was pronounced. Highly vacuolated or lacy cells were observed as early as 24 to 48 hours after inocu-

Table 2. Strain-specific hemagglutination-inhibition tests with representative equine influenza isolates

Antigens	Antiserums (chicken)					
	A2–equine/ Ky/1/63	A1–equine/ Prague/56	A2/Jap/ 305/57	A1/FM1/47	A/PR8/34	A/Swine/ 1976/31
A2–equine/:						
Ky/1	80	0	0	0	0	0
NY/1	80	0	0	0	0	0
NY/2	80	0	0	0	0	0
NY/3	80	0	0	0	0	0
Miami/1	80	0	0	0	0	0
N.M./1	40	0	0	0	0	0
Ohio/1	40	0	0	0	0	0
Del/1	40	0	----------	----------	----------	----------
Det/1	40	0	----------	----------	----------	----------
Md/1	80	0	----------	----------	----------	----------
Oregon/1	80	0	----------	----------	----------	----------
Al–equine/Prague/56	0	160	----------	----------	----------	----------
A2/Jap/305/57	0	----------	320	----------	----------	----------
A1/FM1/47	0	----------	----------	640	----------	----------
A/PR8/34	0	----------	----------	----------	320	----------
A/Swine/1976/31	0	----------	----------	----------	----------	160

lation. Infected cells appeared to degenerate very rapidly and fall from the glass surface, a cytopathic effect not unlike that of recent human influenza type A viruses.

By contrast, attempts to "adapt" either the A1-equine/Praque/56 strain or a recent Prague-like isolate to growth in monkey kidney were unsuccessful.

Biochemical and physical properties. Representative equine isolates were found to be ether sensitive, acid labile, and approximately the same size as the recent human influenza A viruses. Tissue culture titrations of both the equine strain and the human strain showed the same percentage of retention after passage through graded millipore filters. Virus isolates from Florida, New York, and Ohio at various egg passage levels from 1 to 4 were titrated in the standard hemagglutination test with erythrocytes from various species. With all erythrocyte suspensions adjusted to the same cell count per ml., the six equine influenza strains tested were observed to agglutinate equally as well the cells from two white leghorn hens, two guinea pigs, two horses, a sheep, and a human "O" donor. At room temperature, variations in titers were no greater than twofold, and a sedimentation overnight at 4° C. did not appreciably change the titers. The spectrum of agglutination was similar to that reported by Sovinova and associates (*3*) for the original Prague/56 equine influenza A isolate. However, chicken erythrocytes were the cells of choice since the agglutination patterns developed rapidly and could be read more easily.

Serologic properties. The results of type-specific complement fixation tests with two representative equine strains against specific antisoluble A and B guinea pig serums, paired horse serums from recent influenza illnesses, and paired human serums from Asian influenza illnesses are shown in table 1. The viruses clearly shared the soluble antigens associated with type A influenza. The results of strain-specific hemagglutination-inhibition tests are shown in table 2. Reciprocal hemagglutination-inhibition tests were undertaken with chicken antiserums against an equine isolate, the prototype strains of the major human influenza A subtypes, swine influenza virus, and the earlier equine influenza virus. Other equine influenza isolates were also included in the test. The A2-equine influenza viruses were serologically unrelated to any of the remaining strains studied.

However, to rule out the possibility that these recent equine isolate viruses might in some way be related to the current human type A influenza virus and that monospecific animal serums may not have been sufficiently sensitive to detect a minor relationship, control ferrets and ferrets which had been infected 4 months previously with A2/NC/1/63 were infected with two equine viruses. After 3 weeks the ferret serums were tested for hemagglutinin-inhibiting antibodies to the virus responsible for the first and second infections. While the data in table 3 were obtained with only two previously infected animals, there was clearly no measurable anamnestic response and no evidence to indicate any antigenic relationship between A2-equine/NY/1/63 or A2-equine/Ohio/1/63 and the current human influenza A2/NC/1/63.

Table 3. Hemagglutination-inhibiting antibody response of ferrets infected with equine influenza viruses

Ferrets infected with—	Serums	Antibody titers to—			
		Ohio/1	NY/1	Prague	A2/NC/1
A2-equine/Ohio/1/63 (A2/NC/1/63)[1]	S_1–S_2	0–10	0–10	0–0	320–80
A2-equine/NY/1/63 (A2/NC/1/63)[1]	S_1–S_2	0–40	0–40	0–0	160–160
A2-equine/NY/1/63	S_1–S_2	0–60	0–60	0–0	0–0
A2-equine/Ohio/1/63	S_1–S_2	0–20	0–10	0–0	0–0
A1-equine/Prague/56	S_1–S_2	0–0	0–0	0–640	0–0

[1] Cross-infection of ferrets which had been infected 4 months previously with human strain A2/NC/1/63.

NOTE: S_1–S_2=acute–convalescent serums.

Table 4. Serologic responses of selected horses clinically ill with influenza

Paired serums (acute and convalescent)	Serologic test method and equine influenza antigens			
	Neutralization A2-equine/NY/1/63	Complement fixation A2-equine/NY/1/63	Hemagglutination-inhibition	
			A2-equine/Ohio/1/63	A1-equine/Prague/56
BH [1]	0–320	0–128	0–10	20–20
SE [2]	0–320	0–256	0–20	80–160
17		0–128	0–10	40–40
13–8	0–20	0–64	0–10	10–20
16	40	128	0–10	20–20
13–1	0–320	0–32	0–20	0–0
13–7	0–320	0–8	0–15	40–80
PH	0–80	0–32	0–20	20–1,280
NA	0–160	0–128	0–40	20–320
JP	10–40	128–256	0–20	60–320
TF	0–80	128–256	0–80	80–1,280

[1] Horse from which A2-equine/NY/1/63 was isolated.
[2] Horse from which A2-equine/NY/2/63 was isolated.

Serologic response in horses following natural infection. Horses clinically ill with influenza showed significant serologic rises to the current A2-equine influenza viruses by hemagglutination-inhibition, complement fixation, and neutralization tests (table 4). As has often been observed in the past with new human influenza isolates, the hemagglutination-inhibition response was generally low. Of all serums examined, none showed previously existing hemagglutination-inhibiting antibody to the current A2-equine strains. Antibody to the early A1-equine/Prague/56 strain, on the other hand, was relatively common, with many of these horses also yielding antibody rises to the Prague virus as well as to the current strain (table 4). For example, of 23 paired horse serums showing diagnostic antibody rises to A2-equine/Ohio/1/63 by hemagglutination-inhibition tests, 10 of the 23 demonstrated rises to the Prague/56 strain as well. The pattern of these dual rises was not consistent, and the meaning is not clear at this point, for rises to the Prague/56 strain did not occur in all horses having previously existing antibody.

Discussion

The data presented here confirm the original report of Waddell and associates (*4*). The etiologic agent associated with the 1963 equine influenza epizootic appears to be a heretofore unreported type A influenza virus, which is unrelated to the commonly known swine, horse, and human prototype strains tested.

The reasons for the dual diagnostic rises in hemagglutination-inhibition tests to both the Prague strain and the current equine strains in some infected horses are not clear since the newer viruses have been shown not to be closely related to the earlier A1-equine/Prague/56 virus on the basis of specific antiserums. Interpretation of these results is even further complicated in that the Prague-like virus has been shown to be endemic this past year both by serologic evidence and by isolation of the new virus (personal communication from Dr. J. T. Bryans and Dr. E. R. Doll). It is not evident whether these dual rises are the result of infection with one type of influenza followed closely by a second, a mixed infection, or a true anamnestic response exemplifying the doctrine of "original antigenic sin" (*9*).

In a recent memorandum from the WHO International Influenza Center for the Americas, it was reported that a group of investigators in the respiratory virus field, meeting under the auspices of WHO, proposed a general system of nomenclature for viruses of the myxovirus group which would allow expansion as newer viruses are isolated. The general sys-

tem proposed for influenza viruses is "Type and subtype-animal species (excluding humans)/geographic area/serial number/year of occurrence." The recent equine influenza isolates referred to here, which are antigenically distinct from the A1-equine/Prague/56, are designated according to this scheme; for example, A2-equine/NY/1/63.

Summary

The etiologic agent associated with the explosive equine influenza epizootic of 1963 appears to be a heretofore unreported type A influenza virus, unrelated to commonly known human, swine, and equine prototype strains. The virus has been tentatively designated as A2-equine influenza.

ADDENDUM: It has recently been suggested that the present system of naming animal influenza viruses does not clearly distinguish them from human subtypes. To avoid confusion, it was recommended that the species of animal precede the subtype designation. For example, A2-equine/Miami/1/63 would be changed to A/Equine-2/Miami/1/63.

REFERENCES

(1) Fleming, G.: Animal plagues: their history, nature and prevention. Chapman & Hall, London, 1871.

(2) Doll, E. R.: Influenza of horses. Amer Rev Resp Dis 83: 48–53, February (pt. 2) 1961.

(3) Sovinova, B., et al.: Isolation of a virus causing respiratory disease in horses. Acta Virol [Eng] 2: 52–61 (1958).

(4) Waddell, G. H., et al.: A new influenza virus associated with equine respiratory disease. J Amer Vet Med Assoc 143: 587–590 (1963).

(5) Vogel, J., and Shelokov, A.: Adsorption-hemagglutination test for influenza virus in monkey kidney tissue culture. Science 126: 358 (1957).

(6) Andrews, C. H., and Horstmann, D. M.: The susceptibility of viruses to ethyl ether. J Gen Microbiol 3: 290–297 (1949).

(7) Ketler, A., et al.: Characterization and classification of ECHO 28-rhinovirus-coryzavirus agents. Proc Soc Exp Biol Med 110: 821–831 (1962).

(8) Robinson, R. Q., et al.: A survey of respiratory illnesses in a population. I. Viral studies. Amer J Hyg 75: 18–32 (1962).

(9) Davenport, F. M., and Hennessy, A. V.: A serologic recapitulation of past experiences with influenza A: antibody response to monovalent vaccines. J Exp Med 104: 85–98 (1956).

INVENTION REPORT

Automatic Reader and Converter

Oscar reader and decimal converter model F manufactured by Benson-Lehner Corp., Santa Ana, Calif., developed under a Public Health Service grant.

Data collected on standard dimensional comparators are visually read and hand recorded. In the new model a two-dimensional comparator is connected to an analog reader and decimal converter, and the instrument converts analog information to digital values and automatically reads them out by a modified electrotype or IBM key punch or both. Readout sequence is controlled by an operator format control patchboard.

The mechanical portion of the comparator is modified, and linear action potentiometers are added to the X and Y movements of the comparator. These potentiometers are the exact duplicates of those on the analog reader and decimal converter. A cable connector is installed on the comparator. Necessary circuitry is added so that the cable connector from the comparator can be plugged into the Oscar. This modification permits the encoding and punching of coordinates directly onto IBM cards of any measurements made on the comparator. By eliminating intermediate steps of reading and recording the numerical values from the vernier dials and punching of IBM cards, the instrument reduces labor and sources of error.

This modification is useful wherever a comparator is employed for accurate reading, recording, and computer analysis of a large quantity of data.