Epidemiology of Equine Influenza: Risk by Age, Breed and Sex

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DEDICATION

This manuscript is dedicated to my brothers and sisters and further, to the memory of my grandparents.
ACKNOWLEDGEMENTS

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Introduction

The term influenza has been used to describe epidemics of acute respiratory diseases in horses, and as in man, this disease has a history of great pandemics in which nearly every horse in large areas would be involved.

The last of such pandemics occurred in the United States in the winter of 1872-1873. At that time traffic in the cities was nearly stopped because of the lack of horsepower for horsecars, drays and delivery wagons. This disease used to occur every year in milder forms sparing few horses.

Horse populations in the cities dwindled with the introduction of motor transport and the disease concomitantly disappeared. Today, equine influenza occurs in breeding stables, racetracks or wherever equine "social events" are held.

During the peak prevalence of the disease, virology was undeveloped and no virus strains were isolated. It is then apparent that we cannot be sure that the diseases resembling equine influenza before the era of virology are the same as the disease whose etiology we know today as equine influenza virus.

It was only in 1955 and 1956 that a type-A influenza virus was found to be associated with equine respiratory disease. About that time, Heller et al. (17), while investigating the immunological relationship between infectious cough in horses and human influenza-A, found complement fixing antibodies in sera of horses convalescing from respiratory disease occurring in Sweden. Within a short time a virus was isolated from sick horses in
similar episodes occurring in Czechoslovakia. Since then the study of the disease "equine influenza" has become more meaningful. However, other viruses, namely: adenovirus, rhinopneumonitis virus, rhinovirus and rhabdovirus, have been shown to be associated with respiratory diseases of horses (1).

Although much is known regarding the etiology of the disease and its clinical features, there has not been any controlled study designed to investigate the independent effects of age, breed and sex in this disease. Reports in literature and textbooks quote in passing that the disease affects horses of young ages without a reference to any study in which a test of significance had been done. This study was designed to fill such a gap by testing the following three hypotheses:

1. That horses of any age are subject, as a group, to the same relative risk of contracting influenza virus infection as horses of any other age group. *

2. That for any given breed, the risk of contracting equine influenza virus infection relative to that for all remaining breeds is the same as for any other breed.

3. That there are no sex differences with respect to the contraction of influenza virus infection.

With this in mind, we shall look into what has been documented in the literature concerning the clinical disease, its causative virus (when and where it was isolated, and its structural features that play a part in disease causation) and the possible zoonotic aspects of the disease, and later, the various methods that are available for data collection, analysis and interpretation in the study of disease.

*Positive diagnosis was taken as an index of infection by influenza virus.
Literature Review:

A. Disease description:

Synonyms: (Infectious Equine Bronchitis, Laryngotracheobronchitis, New Market Cough, Hoppengarten Cough, Infectious Equine Cough)

Equine influenza is an infectious respiratory disease of horses characterized by a mild fever and a severe, persistent cough. The disease is not fatal, any mortality being a sequel to complications with bacterial pneumonias, but it is notoriously inconvenient in racing facilities where affected horses cease training and any attempts to treat them are poorly rewarded. Equine influenza outbreaks tend to be highly explosive and communicable, following behind transport routes between shows and any such other "equine social events" which have admitted a sick horse.

In the outbreak in Detroit, Michigan, in May, 1964, clinical illness was found only in 2-3 yr olds and older horses newly entered in the meetings. Horses older than three years and those which had performed in 3 or more meetings did not show clinical illness (1,4,5,6).

B. Virus isolation, serology and antigenic variability:

Two serotypes of the equine influenza virus have been identified: the one (A/equi-1) was recovered from horses with a respiratory syndrome in Czechoslovakia in 1955, while the other (A/equi-2) was recovered from an outbreak in Miami, Florida, in 1963. Since then, the occurrence of the two virus subtypes has been reported in many parts of the world, either individually, each subtype causing an outbreak alone, or both subtypes simultaneously present in horses stabled in the same locality (26,27,33,34,37).

The two equine influenza virus subtypes have been designated A/Equi/Prague/1/56 (A/Equi-1), and A/Equi/Miami/1/63 (A/Equi-2) up through 1971, when a
WHO commission on influenza viruses changed the nomenclature (38) and now the subtypes are currently referred to as: Heq1Neq1 and Heq2Neq2, respectively.

Human influenza-A virus is characterized by a multiplicity of subtypes (H0N1, H1N1, H2N2, H3N2), the one superceding and practically never coexisting with its predecessor at any given moment in time. On the other hand, equine influenza virus subtypes are fairly stable and both have been shown to occur simultaneously in the same epidemic in horses from different areas. Pereira et al. (25) found that an equine strain isolated in Brazil in 1969 differed significantly in its antigenic make-up from the A/Equine/Miami/63 (Heq2Neq2) and deviated even more from the Heq1Neq1 subtype.

However, no antigenic variations have been demonstrated at the World Influenza Centre where post-infection sera is routinely used. Failure to detect such changes has been attributed to the use of hyperimmune sera rather than post-infection sera.

Although there are no cross-reactions between the two subtypes when tested with mono-infection sera, infection with one serotype will recall antibodies to the other (20). Antibodies to equine-1 or equine-2 were found in 60% of the sera of horses 3 yrs or older tested in the periods between epidemics (19).

To protect horses, 2 injections of a killed vaccine are given 6-12 weeks apart; annual boosters and vaccination of foals thereafter is the recommended program.

C. Biology of the virus:

The equine influenza virus belongs to the genus Orthomyxovirus, which includes viruses consisting of several pieces of a ribonucleic acid (RNA) genome, a tubular nucleocapsid and a pleomorphic lipoprotein envelope that
carries the properties of hemagglutination and enzymatic elution. Specific structural subunits associated with the latter two functions have been isolated from mature virions and infected cells. Within the envelope is a membrane protein (M).

Inside the lipoprotein envelope lies the ribonucleoprotein (RNP) evidently occurring, as the RNA does, in several separate pieces both in the virion and within infected cells; and possibly a polymerase. Using the complement fixation test, three types of influenza viruses, namely A, B, and C have been identified. Type A influenza viruses have been recovered from animals (horses, birds, swine) and man. Types B and C have so far been recovered from man only.

The difference between strains/or subtypes resides in the envelope subunits, the hemagglutinins (HA) and the neuraminidase (NA) peplomers, and the hemagglutination and neutralization tests are used to elucidate such differences.

The existence of the viral genome in separate pieces has been evoked to explain the plasticity of the influenza virus surface antigens and the antecedent antigenic changes that precede pandemics or epidemics. In theory, two parental influenza viruses which possess different HA and NA subunits are thought to doubly infect a cell and undergo genetic recombination. Among the progeny would be a virus subtype that is different from either parent and which would be virulent to man. Alternately, the theory of genetic mutations is thought to contribute to the antigenic changes. There is accumulating evidence for the recombination theory (12,35,36).

D. Equine influenza, a zoonosis?

It has been postulated that the human influenza viruses showing antigenic shifts and which made possible such pandemics as those of 1918 and 1957
had their origin in swine or some other unidentified animal reservoirs. To
investigate the latter hypothesis, the WHO organized a world survey of animal
influenza, particularly equine influenza at the time of the 1957 pandemic in
man and since then has sponsored co-ordinated efforts geared at clarifying
the salient epidemiological, virological and biological relationships between
human and animal influenza.

A short summary of the results of these efforts is recorded in Dr.
Schwabe's book (32) covering the surveillance through the 1958-1968
period, while some more detailed work is contained in a series of papers
on animal influenza appearing in the 1972 Bulletin of WHO (vol. 47) edited
by Kaplan and Beveridge.

Kasel et al. (18) reported antibody response to A/equi-2 in human
volunteers exposed to the HongKong human influenza strain, thus suggesting
that both the equine and the human strains tested shared common antigen.
When volunteers were exposed to B-propiolactone inactivated A/equi-2 (Heq2
Neq2), antibodies to the homologous strain were elicited in volunteers of all
ages. However, the greatest difference in the paired sera was observed in the
very young and the very old. Subjects showing a response to the equine virus
had no such antibodies in pre-exposure sera. The response to the hetero-
logous human subtype A₂ (H2N2) seemed independent of the response against
the homologous equine subtype (Heq2 Neq2). Two subjects aged 53 and 67
developed antibodies against the equine subtype following vaccination
with the human A-2 subtype, suggesting a recall phenomenon (22,23,24).

In sera of persons aged from two to ninety-nine years sampled in 1956-
1957, hemagglutination inhibition antibodies against the HongKong and the
equi-2 viruses were found in 357/913 and 65/913 sera respectively. The latter
were persons aged 66 to 73 and 80-83 years; this suggests that a virus,
antigenically similar to A-equi-2, was abundant in the human population about the turn of the century, when such persons were still alive (17,18,19).

The current evidence of serological cross-reactions of equine influenza virus (Equi-2/Miami/63) with avian (duck/Ukraine/1/63), human (A/HongKong/68) and swine (swine/Taiwan/69) viruses on hemagglutination-inhibition tests performed using the corresponding reciprocal sera is very suggestive of a zoonotic tendency.

E. Prospective and retrospective studies; methods of data analysis:

Since the days of Hippocrates, physicians and veterinarians have used the case history method as a tool to study the etiology of disease. Cornfield (7) believes that this implies the study of the characteristics of an individual with a disease. This method fails to provide a basis for judging the validity of the observations obtained by its use. A standard of comparison is implicitly necessary for the evaluation of case history data in the study of the etiology of disease.

The case history method has been modified so that a study would only involve the comparison of the characteristics of a group of individuals having a specific disease with those of a group of individuals not having this disease.

The comparison group has to be representative of the population in which the individuals originated. There are several problems inherent in a study such as this, hereafter referred to as a retrospective study:

1. Subjects are selected after the disease has developed.

2. The population of origin (called hereafter, the population at risk) and the events occurring prior to the onset of the disease are difficult to identify or are unknown.

3. There is a lack of the knowledge concerning possible selective
processes that could bias observations.

4. There is consequent uncertainty on the validity and relevance of the acquired conclusions to the population at risk.

5. There is lack of agreement between measures of relative risk and incidence rates as well as variations in the estimates of relative risk, both of which are due to lack of specifying the methods of sampling and the population to be sampled (a phenomenon called probability sampling). However, the data cannot be called unrepresentative, since the population at risk is unknown.

The alternative to probability sampling is to select samples so as to include all individuals in a specified population during a specified time interval and then during statistical analyses to adjust for factors contributing unknown variabilities.

Prospective studies were developed to overcome some of the problems of the retrospective method. Prospective studies involve specifying a population at risk and the sampling methods and rules well in advance prior to conducting the study (7, 9, 10). Despite the many disadvantages which are apparent, retrospective studies do show the following advantages:

1. They may be the only tenable approach to the study of a disease of low incidence, or a disease in a very expensive species (e.g. man).

2. They provide tentative exploration of hypotheses in the face of scanty information on a disease preparatory to conducting a prospective study.

3. They are cheap to run.

4. They may uncover previously unknown forays or they could represent refinements of current knowledge.

The primary goal of retrospective studies is to reach the same conclusion as would have been obtained from a forward study, if one had been done.
It would be of interest to understand what properties appertain to the carrying out of retrospective studies and the limitations of the conclusions obtainable by such studies:

a. Retrospective studies pick factors associated with becoming a diseased or a disease-free subject, rather than factors associated with the presence or absence of the disease.
b. Contrasts will test significant if a long series is done, even in the absence of true association.
c. To offset the loss due to spurious associations, often extreme probability levels are set, this makes real associations difficult to detect.
d. Multiple comparisons are problematic when inferences are to be drawn from a single set of data.
e. The bias due to unrepresentative sampling decreases as more data sources are used.
f. When estimating a relative risk, two assumptions are made:
   (1) that all new cases of disease (or a sample of them) can be enumerated correctly;
   (2) that the individuals (or a sample of them) not developing the disease provide(s) an unbiased estimate of the prevalence of the characteristic under study among the entire non-diseased population of interest. In most retrospective studies control groups are selected from individuals with some disease other than that being studied, assuming that the prevalence of the characteristic in these groups would be an unbiased estimate of the required proportions. Both assumptions are usually not satisfied in most studies and spurious associations may occur.
g. **Significance of a study:** One has to weigh and balance alternative risks in order to decide which is important; to detect an effect when present or to reject findings when they may not reflect the true association.

The treatise by Cornfield (7) and those by Dorn (9,10) were more concerned with simple, homogenous groups of test and control individuals testable in a 2x2 frequency table. However, investigations may involve comparisons of heterogeneous groups requiring combined summary estimates from multiple classifications. Mantel and Haenszel (21) provide methods for the analysis of such data. For example the combined relative risk is:

\[
(R) = \frac{\sum \frac{ad}{N}}{\sum \frac{bc}{N}}
\]

which [in the case of the modified formula (M·l) derived to test R for any risk factor] would reduce to:

\[
(R'') = \frac{\sum \frac{a_id_i}{T_j}}{\sum \frac{(M_{ij}-a_i)(T_j-a_i-c_i)}{T_j}}
\]

where \(a_i, d_i, M_{ij}, c_i\) and \(T_j\) are cell frequencies in Table (M·l) discussed below.

Use has been made of such analyses for data from individual institutions and also from the data bank available through the National Cancer Institute's Veterinary Medical Data Program (VMDP).

The risk by breed, age and sex of several conditions, e.g. tumors (15,29), diseases presumed to have predisposing factors (13,14,28,30,31) and one infectious disease (16) have been studied using the VMDP source. Equine influenza, an emerging possible zoonosis, seemed a good candidate to be investigated using this data bank.
Materials and Methods

A case series composed of patients with confirmed diagnosis of equine influenza was compiled from abstracts of medical records submitted to the VMDP* of the National Cancer Institute by 12 schools of veterinary medicine in the United States and Canada, March 1965 through December 1974.

Three hundred and sixty such cases were identified. A reference population was assembled to reflect patient years at risk for all horses reported to the VMDP for any reason.

Segments of interest in the case series were related to the reference population with the use of estimated relative risk (R; R')** and comparative and usual Chi-square tests of significance. Data were pooled for all institutions and all years but no adjustments for data sources were made.

The \( \chi^2 \) test for multiple comparisons in contingency tables was carried out at:

\[
\alpha' = 1 - (1 - \alpha)^{1/n}, \quad \text{where}
\]
\[
\alpha = \text{level of significance used for one comparison},
\]
\[
n = \text{number of comparisons to be done at } \alpha'.
\]

Thus, for \( \alpha=0.05, n=7, \alpha'=0.0073. \)

The contingency table hypothesis (\( H_0 \)) states that the row and column classifications are independent.

If we let the probabilities (P) be:

\[
P_{ij} = P \text{ (an observation falls in row i and column j)}
\]

*The abbreviation used is: VMDP, Veterinary Medical Data Program.

**R = Mantel-Haenszel Procedure; R' = procedure developed in this paper.
P_{i} = P \text{ (an observation falls in row } i)\text{)}

P_{j} = P \text{ (an observation falls in column } j)\text{)}

and recall that two events A and B are independent if \( P(A \text{ and } B) = P(A) \cdot P(B) \),
then we can write the contingency table hypothesis (H_0) in probability terms,
using the notation above, as follows:

\( H_0: \quad P_{ij} = P_{i} \cdot P_{j} \) and the alternative hypotheses (H_A) as:

\( H_A \neq P_{i} \cdot P_{j} \).

Then the test statistic is the \( \chi^2 \) test statistic.

\[
\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}}
\]

with \((r-1)(c-1)\) degrees of freedom; levels of significance as discussed above.

The Mantel-Haenszel procedure for estimating the relative risk (R) of occurrence was used to evaluate the independent effects of age, breed, and sex for cases compared to the reference clinic-hospital population. In order to be able to calculate a confidence interval and test for a significant R, the 2x2 table was arranged as shown below:

<table>
<thead>
<tr>
<th>State of Nature</th>
<th>+</th>
<th>-</th>
<th>Totals</th>
</tr>
</thead>
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<tr>
<td>Characteristic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>a</td>
<td>c</td>
<td>a+c</td>
</tr>
<tr>
<td>-</td>
<td>b</td>
<td>d</td>
<td>b+d</td>
</tr>
<tr>
<td>Totals</td>
<td>M_1</td>
<td>M_2</td>
<td>T</td>
</tr>
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To use the table we let, for example:

Characteristic: Age

State of Nature: Diagnosis of disease,
where a and c are from test cases and b and d are from reference standard.

Then the estimate of relative risk (R) is:

\[
\frac{\frac{a}{a+c} \cdot \frac{b}{b+d}}{\frac{a}{a+c}} = \frac{a}{b} \frac{(b+d)}{(a+c)},
\]

which is the risk of the special outcome (diagnosis of disease) among individuals having the characteristic of interest (a given age) relative to the risk of the special outcome among individuals not having the characteristic of interest (individuals in a given reference age category, or some other defined reference category, discussed below).

When the reference age category is fixed, e.g. at 1-2 yrs, the risk at other ages relative to the risk at this age is calculated by holding b and (b+d) constant (these being the values at the reference age) and replacing a and (a+c) by the corresponding values at the age of interest whose relative risk is desired.

For example the estimated relative risk (R) at age category 4-7 yrs is:

\[
\frac{105 \times 7175}{38 \times 21860} = 0.9069 = 0.91
\]

where a = 105; (a+c) = 21860; b = 38; (b+d) = 7175.

When the age category (which we shall here call a factor level in the risk factor, "age") is not fixed, then an estimate of relative risk for any factor level relative to the remaining levels (here: other age categories) can be computed from the formula (modified from the general relative risk formula):

\[
R' = (R_{j1}) \frac{a_1 [T_j - (a_1+c_1)]}{(M_j a_1)(a_1+c_1)}
\]
where

\( j \) = risk factor (e.g. age, breed, sex)

\( i \) = any level in the risk factor of interest (e.g. Arabian in risk factor "breeds of horses")

\( R_{ji} \) = the desired estimated relative risk

\( a, b, c, d \) are cell frequencies in a 2x2 frequency table as shown above,

and;

\( T_j \) = grand total

\( M_{1j} \) = total number of individuals with the special outcome

\( M_{2j} \) = total number of individuals without the special outcome in the same 2x2 table.

For example: If the estimated relative risk of diagnosis of influenza virus infection in horses by age is desired, then the factor \( j \) here is age; and the factor levels \( i \) are the different age categories.

Thus the estimated relative risk of diagnosis of influenza virus in horses at a given age, say 4-7 yrs, relative to the risk at all over ages is computed from the formula \( (M_{1} - a_1^i) \) and we obtain:

\[
R' = R_{\text{age}4-7\text{yrs}} = \frac{105 \times 60735}{250 \times 21860} = 1.167 = 1.17.
\]

where

\( a_1 = 105, c_1 = 21,755, a_1+c_1 = 21860; (M_{1j} - a_1) = 250 \) and

\( [T_j - (a_1+c_1)] = 60,735. i = 4-7 \text{ yrs.} \)

Thus, an estimated relative risk can be calculated whether a given reference factor level is fixed or not.

To test whether the relative risks deviated significantly from 1.0, a confidence interval was constructed for each relative risk estimate using
the relationship

\[ 100(1-\alpha)\% \text{ C.I.} = \log_e R \pm Z \left(1-\frac{1}{2}\alpha\right) \sqrt{\text{var} \left(\log_e R\right)} \]

**Graphs and histograms**

The graphs for the relative risks by age (Fig. 5a & b) and the frequency of diagnosis by age (Fig. 4) were drawn such that data points were plotted at the mid point of each age interval on the x-axis, and the corresponding frequency or relative risk plotted on the y-axis. The last open interval for age was assigned the same value as the age interval preceding it.

The moving averages (Figs. 1, 2a & 2b) were centered on the period between the 2nd and 3rd year (for the distribution by year of reporting) and the 2nd and 3rd month (for the month of reporting). The moving average for the distribution by month was closed (i.e. the curve starts and ends at the same height on y-axis) while that by year of reporting was left open.

All histograms were constructed so that the areas of the bars represented the frequencies. However, for the distribution by age, the area of the reference age 1-2 years (one year interval) was fixed on the graph paper and the x and y coordinates calculated proportional to this reference category.

Thus, if \( F = \) frequency for any age interval
\( x = \) distance on paper for any age interval
\( y = \) distance on paper for corresponding frequency,
Then from Fig. (M·1) either of (x) or (y) can be obtained from the relationship:

\[
\frac{(x_{i+1} - x_i) y_{i+1}}{(x_{j+1} - x_j) y_{j+1}} = \frac{F_i}{F_j} \quad \text{--- \quad (M·2)}
\]

where

- \(i\) = the desired factor level
- \(j\) = any chosen factor level (e.g. a fixed reference age in this discussion)

and

\[
y_{i+1} = \frac{F_i}{F_j} \cdot \frac{(x_{j+1} - x_j) y_{j+1}}{(x_{i+1} - x_i)} \quad \text{--- \quad (M·3)}
\]

For example, if 5 units of graph represents one year, and 1 unit represents 2 cases (19 units for 38 cases) then the length on the y-axis for the 105 cases for the age interval 4-7 yrs (15 units for 3 years) is from (M·3)

\[
y_{(4-7 \text{ yrs})} = \frac{105 \times (19)^5}{38 \times (15)} = 17.5 \text{ units}.
\]
Results and Discussion: A. Breeds:

From a total of 84,922 horses reported to the VMDP for any reason, 360 had diagnoses of equine influenza virus infection. The distribution by breeds (Table 9) shows the frequency of diagnosis of equine influenza in the horse breeds commonly reported to the VMDP. Horse breeds having no cases were left out of the study population but appeared in the reference population.

Of the breeds represented, quarter horses were at a significantly high risk, while the data indicated a sparing effect for thoroughbred horses which, as a breed, had a significantly low risk. The other horse breeds were subject to a uniformly low and non-significant risk.

The rate of diagnosis (per 10,000 patients, Table 9) was highest for Palomino horses and least for thoroughbreds.

B. Sex: Sex differences between case series and reference populations were not apparent at the level of significance used (α = 0.05). However no adjustments by breed and data source were performed, the data having been pooled as discussed above.

Thus sex is not a significant contributing factor to the epidemiology of equine influenza as evidenced by this data.

C. Age: A summary of the distribution by age of characteristics of interest in horses is shown in Table 7 and these features are individually illustrated in Figures 4 and 5a and b.

Horses at the age 2-6 months are subject to a significant risk of diagnosis of equine influenza relative to both the reference age category 1-2 yrs and to all age categories other than the 2-6 month group. Middle-aged horses (7-10 yrs) are subject to a significantly sparing (low) relative
risk (Table 7) for diagnosis of equine influenza whereas older horses (10 yrs and over) and very young horses (less than 2 months) tend to have a non-significant low risk. Those horses between the ages of one-half year to 7 yrs have an almost uniform risk which does not significantly deviate from unity.

The age categories were tested for significance by a multiple comparison chi-square test and age 4-7 yrs was found to contribute most to chi-square ($\alpha = 0.0073$).

After removing this age and recalculating the chi-square, the chi-square was not significant at $\alpha'$ (where $0.025 < \alpha' < 0.0073$). Thus all ages except age 4-7 yrs form one homogenous group with respect to diagnosis of equine influenza. However, the relative risk estimates ($R'$) showed that the $R'$ for age 4-7 yrs is not significant, thus the ages are subject to the risks as calculated and discussed elsewhere in the paper.

These relative risk estimates may be cautiously interpreted to have the following medical significance:

a. Very young horses (less than 2 months) and very old horses (>10 yrs) are unpredictably subject to infection by influenza virus;

b. Horses 2 months and ≤ 6 months are at definite risk of infection, whereas;

c. Middle-aged horses would be spared.

D. Length of stay and other parameters:

The largest number of reported horses apparently stayed in the clinic-hospitals for one week (Table 6a & b) and of those within the one week category, the majority seem to have been treated and discharged the same day. The duration of hospitalization of equine influenza cases is shown
Most diseases of the respiratory tract are characterized by repeated attacks, as in the case in the common cold of man. In human influenza infections, affected persons are susceptible to infection by a subsequent human influenza subtype. In epidemics of human influenza, a current virus subtype supercedes its predecessor, thus precluding any coexistence of any 2 subtypes in the same epidemic.

This is not so in equine influenza, where the two equine influenza subtypes have been shown to co-exist in an epidemic (2). The data from this study shows that horses are subject to a high conditional probability of re-infection by influenza virus indicating a tendency of recurrence of the disease in the same patients and probably a short-lived immunity.

Reports of diagnosis of equine influenza were made throughout the year for the years studied with peaks around May and August (Table 2). Taken alone, the period April-June had the most reports of any 3-month pool. However, a 4-month moving average indicates a smooth rise from March with peaks at the June-July and September-October periods and a smooth decline to February. These data may have artifacts due to the pooling of data from the various sources and should be cautiously interpreted.

Although a country-wide epidemic had occurred in 1963, it is remarkable that 2 years later, only 4 cases were reported to VMIDP (Table 1). However, reporting seems to have accelerated and most reports reached the center by 1968 and 1969. This is the period when a concurrent human influenza epidemic was rampant in this country. Another peak in the reporting occurred in 1972, and was yet another coincidental period of concomitant human influenza epidemic occurrence in the United States.
Summary:

When 360 cases of diagnosed equine influenza reported to VMDP were tested for the independent effects of breed, age and sex, relative to a reference clinic-hospital population of 84,562 equine patients, the quarter horse breed was identified to be at excessive risk, while there were no sex differences. Horses of ages 2-6 months were found to be subject to a significant risk above unity while those at age 7-10 yrs had a risk significantly below unity, indicating a sparing effect on the latter group.

A conditional probability of re-infection with equine influenza of 0.05 was identified; and the highest reporting rate was seen to occur in the period April to September. These results should be considered as indicative estimates rather than firm statistics, taking into account the methods used and the type of data available.
Summary:

When 360 cases of diagnosed equine influenza reported to VMDP were tested for the independent effects of breed, age and sex, relative to a reference clinic-hospital population of 84,562 equine patients, the quarter horse breed was identified to be at excessive risk, while there were no sex differences. Horses of ages 2-6 months were found to be subject to a significant risk above unity while those at age 7-10 yrs had a risk significantly below unity, indicating a sparing effect on the latter group.

A conditional probability of re-infection with equine influenza of 0.05 was identified; and the highest reporting rate was seen to occur in the period April to September. These results should be considered as indicative estimates rather than firm statistics, taking into account the methods used and the type of data available.
Cited References


Tables

1. Distribution by year of reporting of equine influenza diagnoses reported to VMDP, March 1965-1974.*

2. Frequency of diagnosis by month for reported cases of equine influenza, VMDP, March 1965-1974.*

3. Distribution of diagnosed equine influenza cases by reporting institution, VMDP, March 1965-1974.*

4. Distribution by status of diagnosis.*

5. Distribution by period of diagnosis.*

*The reference population was not available by the time of writing of this report.

6a. Distribution of equine influenza diagnoses by duration within clinic-hospitals. Weekly intervals.

b. Distribution of equine influenza during the first week of hospitalization.

7. Distribution of equine influenza by frequency and rate (per 10,000 patients) of diagnosis and the estimated relative risks (R and R'), by age for cases reported to VMDP, March 1965-1974.

8. Distribution by age of equine influenza cases reported to VMDP, March 1965-1974. With age classes pooled conveniently.

9. Frequency and rate (per 10,000 patients) of diagnosis and the estimated relative risk (R') for breeds of horses commonly reported to VMDP, March 1965-1974.


11a. Distribution by sex of reported cases of equine influenza diagnoses and estimated relative risk (R'), VMDP, March 1965-1974.
11b. Distribution by sex (data pooled within sex) of reported cases of equine influenza, VMDP, March 1965-1974.
Table 1. Distribution by year of equine influenza diagnoses reported to VMDP, March 1965 through 1974.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnoses</td>
<td>4</td>
<td>11</td>
<td>24</td>
<td>52</td>
<td>53</td>
<td>42</td>
<td>59</td>
<td>61</td>
<td>36</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 2. Frequency of diagnosis by month for reported cases of equine influenza, VMDP, March 1965-1974 June.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>14</td>
<td>12</td>
<td>20</td>
<td>36</td>
<td>46</td>
<td>38</td>
<td>27</td>
<td>58</td>
<td>30</td>
<td>30</td>
<td>37</td>
<td>15</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Institution:</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnoses:</td>
<td>11</td>
<td>20</td>
<td>2</td>
<td>74</td>
<td>0</td>
<td>70</td>
<td>71</td>
<td>0</td>
<td>55</td>
<td>0</td>
<td>1</td>
<td>45</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 4. Distribution by status of diagnosis:

<table>
<thead>
<tr>
<th>Status</th>
<th>Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial call</td>
<td>344</td>
</tr>
<tr>
<td>Re-check call</td>
<td>16</td>
</tr>
<tr>
<td>Total diagnoses</td>
<td>360</td>
</tr>
</tbody>
</table>

Conditional probability of re-infection = \( \frac{16}{344} \) = 0.05

Table 5. Distribution by period of diagnosis:

<table>
<thead>
<tr>
<th>Period</th>
<th>Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Diagnosis</td>
<td>280</td>
</tr>
<tr>
<td>2nd Diagnosis</td>
<td>55</td>
</tr>
<tr>
<td>3rd Diagnosis</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 6(a). Distribution of equine influenza diagnoses by duration within the Clinic-hospital: Weekly intervals

<table>
<thead>
<tr>
<th>Period (days)</th>
<th>0-7</th>
<th>8-15</th>
<th>16-23</th>
<th>24-31</th>
<th>32-39</th>
<th>40-47</th>
<th>48-55</th>
<th>56-63</th>
<th>64-71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnoses</td>
<td>289</td>
<td>41</td>
<td>15</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 6(b). Distribution of diagnosis of equine influenza during the first week in the Clinic-hospitals.

<table>
<thead>
<tr>
<th>Period (days)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnoses</td>
<td>228</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>9</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 7. Distribution of equine influenza by frequency and rate (per 10,000 patients) of diagnosis and the estimated relative risk by age for cases reported to VMDP, March 1965 through 1974.

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency of diagnosis</th>
<th>Rate of diagnosis per 10,000 patients</th>
<th>Estimated relative risk$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Non Cases</td>
<td>Total</td>
</tr>
<tr>
<td>0-2 weeks</td>
<td>0</td>
<td>943</td>
<td>943</td>
</tr>
<tr>
<td>2 weeks-2 months</td>
<td>4</td>
<td>1,016</td>
<td>1,020</td>
</tr>
<tr>
<td>2 months-6 months</td>
<td>24</td>
<td>2,663</td>
<td>2,687</td>
</tr>
<tr>
<td>6 months-12 months</td>
<td>15</td>
<td>2,841</td>
<td>2,856</td>
</tr>
<tr>
<td>1-2 years</td>
<td>38</td>
<td>7,137</td>
<td>7,175</td>
</tr>
<tr>
<td>2-4 years</td>
<td>92</td>
<td>20,398</td>
<td>20,490</td>
</tr>
<tr>
<td>4-7 years</td>
<td>105</td>
<td>21,755</td>
<td>21,860</td>
</tr>
<tr>
<td>7-10 years</td>
<td>35</td>
<td>13,031</td>
<td>13,066</td>
</tr>
<tr>
<td>10-15 years</td>
<td>32</td>
<td>8,695</td>
<td>8,727</td>
</tr>
<tr>
<td>&gt;15 years</td>
<td>10</td>
<td>3,761</td>
<td>3,771</td>
</tr>
<tr>
<td>Unknown$^b$</td>
<td>5</td>
<td>2,322</td>
<td>2,327</td>
</tr>
<tr>
<td>Totals</td>
<td>360</td>
<td>84,562</td>
<td>84,922</td>
</tr>
</tbody>
</table>

$^a$ R = estimated relative risk, relative to age category 1-2 years.
$^b$ R' = estimated relative risk, relative to ages other than the age of interest.

Thus there is a significant distribution by age of the diagnosis of equine influenza infection.

After removing the highest contributor to Chi-square and recalculating Chi-square the following obtains

\[ \chi^2_{\text{calc.}} = 33.715 > \chi^2_{\text{crit.}} = \chi^2_{\text{cr.}} (9, 0.9927) \]

\[ (21.666 < \chi^2_{\text{cr.}} < 23.589) \]

Thus there is a significant distribution by age of the diagnosis of equine influenza infection.

After removing the highest contributor to Chi-square and recalculating Chi-square the following obtains

\[ \chi^2_{\text{calc.}} = 15.722 < \chi^2_{\text{crit.}} (8, 0.975 < \chi^2_{\text{cr.}} < 8, 0.9927). \]

* Significantly different from R = 1.0.

a  R = estimated relative risk, relative to age category 1-2 years.

b The unknowns were excluded from Chi-square and relative risk calculations.
Table 8. Distribution by age of equine influenza cases reported to VMDP, March 1965-1974 with age classes pooled.\(^a\)

<table>
<thead>
<tr>
<th>Age (x)</th>
<th>Cases</th>
<th>Non Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 &lt; x &lt; 1 yr.</td>
<td>43</td>
<td>7,463</td>
<td>7,506</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 &lt; x &lt; 4 yrs.</td>
<td>130</td>
<td>27,535</td>
<td>27,665</td>
</tr>
<tr>
<td>Middle aged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 &lt; x &lt; 10 yrs.</td>
<td>140</td>
<td>34,786</td>
<td>34,926</td>
</tr>
<tr>
<td>10 &lt; x yrs.</td>
<td>42</td>
<td>12,456</td>
<td>12,498</td>
</tr>
<tr>
<td>Totals</td>
<td>355</td>
<td>82,240</td>
<td>82,595</td>
</tr>
</tbody>
</table>

\(\chi^2_{\text{calc.}} = 7.829\),

\(\chi^2_{\text{crit.}} = 7.815 < \chi^2_{\text{calc.}} = 7.829 < \chi^2_{\text{crit.}} = 9.348\)

\((3, 0.95)\)

\((3, 0.975)\)

Thus when the age categories are pooled in this manner, they present homogeneous groups.

\(a\): The diagnoses with unknown ages were not included in the calculations.
Table 9. Frequency and rate (per 10,000 patients) of diagnosis and the estimated relative risk \( R' \) for breeds of horse reported to VMDP, March 1965-1974.†

<table>
<thead>
<tr>
<th>Breed</th>
<th>Reported Cases</th>
<th>Non Cases</th>
<th>Total Population</th>
<th>Rate per 10,000 patients</th>
<th>Estimated relative risk (( R' ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Paint Horse</td>
<td>1</td>
<td>150</td>
<td>151</td>
<td>66.23</td>
<td>1.65</td>
</tr>
<tr>
<td>American Saddle Horse</td>
<td>3</td>
<td>484</td>
<td>487</td>
<td>61.60</td>
<td>1.54</td>
</tr>
<tr>
<td>Appaloosa</td>
<td>7</td>
<td>1,331</td>
<td>1,338</td>
<td>52.52</td>
<td>1.31</td>
</tr>
<tr>
<td>Arabian</td>
<td>21</td>
<td>4,883</td>
<td>4,904</td>
<td>42.82</td>
<td>1.07</td>
</tr>
<tr>
<td>Morgan</td>
<td>1</td>
<td>225</td>
<td>226</td>
<td>44.25</td>
<td>1.10</td>
</tr>
<tr>
<td>Palomino</td>
<td>1</td>
<td>136</td>
<td>137</td>
<td>72.99</td>
<td>1.82</td>
</tr>
<tr>
<td>Ponies</td>
<td>13</td>
<td>3,566</td>
<td>3,579</td>
<td>36.32</td>
<td>0.90</td>
</tr>
<tr>
<td>Quarter Horse</td>
<td>154</td>
<td>27,380</td>
<td>27,534</td>
<td>55.93</td>
<td>1.93**</td>
</tr>
<tr>
<td>Standard bred</td>
<td>37</td>
<td>11,839</td>
<td>11,876</td>
<td>31.16</td>
<td>0.74</td>
</tr>
<tr>
<td>Thoroughbred</td>
<td>20</td>
<td>13,336</td>
<td>13,356</td>
<td>14.97</td>
<td>0.32**</td>
</tr>
<tr>
<td>Mixed breed</td>
<td>7</td>
<td>2,221</td>
<td>2,228</td>
<td>31.42</td>
<td>0.77</td>
</tr>
</tbody>
</table>

\[
\chi^2_{\text{calc.}} = 43.879 > \chi^2_{\text{crit.}} \quad (23.209 < \chi^2_{\text{crit.}} < 25.188). \quad \text{After removing the breed} \quad \text{(quarter horse) that contributes most to Chi-square, the recalculated Chi-square is:}
\]
\[
\chi^2_{\text{calc}} = 19.379 < \chi^2_{\text{crit.}} \quad (21.666 < \chi^2_{\text{crit.}} < 23.589).
\]

† \( R' \) = the risk of diagnosis in one breed relative to the risk of diagnosis among the remainder of the breeds.

*+/- = significantly above and below one, respectively.
Therefore the remaining breeds of horses form a homogeneous group (say B; and let us call the previous group of quarter horses - say A.). Then the Chi-square test and relative risk estimates are as calculated below.

Table 10. Homogeneous groups of horse breeds by diagnoses for reported cases of equine influenza, VMDP. March 1965-1974.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Non Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>154</td>
<td>27,380</td>
<td>27,534</td>
</tr>
<tr>
<td>B</td>
<td>111</td>
<td>38,171</td>
<td>28,282</td>
</tr>
<tr>
<td>Totals</td>
<td>265</td>
<td>65,551</td>
<td>65,816</td>
</tr>
</tbody>
</table>

\[
\chi^2_{\text{calc.}} = \frac{(0.11022 - 0.12021)^2 \times 65816}{0.1 \times 0.2 \times 0.1 \times 0.2} = \frac{(154 \times 38171 - 27380 \times 111)^2 \times 65816}{265 \times 65551 \times 27534 \times 38282} = 28.97
\]

\[
\chi^2_{\text{calc.}} = 28.97 > \chi^2_{\text{crit.}} = 3.84.
\]

Therefore groups A and B are significantly different.

The relative risk of diagnosis of equine influenza in quarter horses (group A) relative to other breeds (group B) is:

\[
R = \frac{154}{111} \times \frac{38,282}{27,534} = 1.92876 = 1.93^*
\]

\[
\text{Var } \log_e R = \frac{1}{154} + \frac{1}{111} - \frac{1}{38,282} - \frac{1}{27,534} = 0.0155025 - 0.0000624 = 0.0154401
\]

\[
\text{S.E. } \log_e R = 0.124258
\]

95% C.I. \( R_{A:B} = 0.65698 \pm 0.243546 \)

\([0.413434, 0.900526]\)
Table 11b. Distribution by sex (data pooled within sex) of reported cases of equine influenza. VMDP, 1965-1974.

<table>
<thead>
<tr>
<th>Sex Category</th>
<th>Cases</th>
<th>Non Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>171</td>
<td>37,530</td>
<td>37,701</td>
</tr>
<tr>
<td>Female</td>
<td>186</td>
<td>46,615</td>
<td>46,801</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>417</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>84,562</td>
<td>84,922</td>
</tr>
</tbody>
</table>

Chi-square = 2.854 < $\chi^2_{crit.} = 3.84$

$(1, 0.95)$
Figures

1. Reported cases of equine influenza by year of reporting, VMDP, 1965-1974 (constructed on a 4th order moving average of data pooled for all institutions).

2a. Reported cases of equine influenza by month of reporting, VMDP, 1965-1974 (data pooled for all years and all institutions); a 4th order moving average is super-imposed on the histogram.

b. A 4-month moving average of data in fig 2a.

3. Duration of hospitalization of reported cases of equine influenza:
   a: weekly reports;
   b: reports for the first week.

4. Frequency of diagnosis of equine influenza by age, VMDP, March 1965-1974; (data points were plotted at class boundaries).

5a. Estimated relative risk of equine influenza for any age relative to age categories other than the age of interest. (VMDP data, March 1965-1974; *data points plotted at the midpoint of each corresponding age interval).

b. Estimated relative risk of equine influenza for any age relative to age category 1-2 yrs, VMDP data (March 1965-1974; data points were plotted at the mid point of each corresponding age-interval).
Figure 1. Reported cases of equine influenza, by year of reporting, 1965-1974
Figure 2a. Reported cases of equine influenza by month of reporting. (The data are pooled for all reporting institutions), 1965-1974. VMDP*

* The line superimposed on the histogram is a 4-month moving average.
Figure 2b. Frequency of diagnosis of equine influenza by month of reporting, a 4-month moving average; VMDP, March 1965-1974. (Data pooled for all institutions).
Figure 3. Duration of hospitalization of reported cases [A: weekly intervals, B: reports for the first week]; (all data pooled for all years and all institutions reporting, VMDP, 1965-1974).
Figure 4. Frequency of diagnosis of equine influenza by age; VMDP, March 1965-1974*.

* Data plotted at class boundaries.

A: 0-2 weeks
B: 2 weeks-2 months
C: 2-6 months
D: 6-12 months
Figure 5a. Estimated relative risk of equine influenza for any age relative to age categories other than the age of interest, VMDP, March 1965 through 1974.*

* Data points were plotted at the midpoint of each corresponding age interval.

A: 0-2 wks
B: 2 wks-2 months
C: 2-6 months
D: 6-12 months
E: 1-2 yrs (1 year interval)
Figure 5b. Estimated relative risk of equine influenza for any age relative to age category 1-2 yrs, VMDD, March 1965-1974.

1. Results were plotted at the mid points of the intervals.
2. The intervals assigned relative (proportionately) to the interval of the reference period at age 1-2 yrs.

A: 0-2 weeks
B: 2 wks-2 months
C: 2-6 months
D: 6-12 months
E: 1-2 yrs