Upper Respiratory Disease in Thoroughbred Horses: studies of its viral etiology in the Toronto area, 1960 to 1963*

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SUMMARY

From outbreaks of upper respiratory infection of horses in the Toronto area between 1960 and 1963, several viruses have been isolated. The viruses, isolated in tissue cultures or eggs, include an equine strain of Myxovirus parainfluenzae 3; two strains of equine influenza virus, A/equi-1/Prague/56, and A/equi-2/Miami/63; equine rhinopneumonitis virus, and two newly recognized viruses of the horse, equine rhinoviruses. In addition serological evidence suggested a widespread infection with these viruses in the population under study. Because of the identical clinical picture seen and the complex etiology of the disease, immunization against upper respiratory disease of the horse does not appear to be completely feasible at this time.

Upper respiratory disease of horses is world wide in distribution. The condition in Canada is seen in horses of one to three years of age and manifests itself by spasms of coughing, a watery nasal discharge, and congestion of the mucuous membranes of the nose and throat. Slight anorexia is present and a temperature rise is usually noted. If training is continued the cough becomes much more severe, dyspnea results, and secondary bacterial infection of local lymph nodes and pneumonia may appear. If the animal is rested for about two weeks following the onset of signs, recovery is usually uncomplicated.

In 1960 we embarked on a three year programme of investigation of this problem employing the facilities offered by several racetracks and stables in the Toronto area. This paper will deal briefly with a general review of the findings of this study.

Methods

Population studied. The population studied was housed on a large breeding and training farm near Toronto. All ages of horses were housed on this establishment and horses were moved frequently to and from the farm. Specimens for virus isolation were taken from all horses showing signs of acute upper respiratory disease on the first or second day of illness. This was done by inserting a Teigland swab through the nasal passages and, after its removal, cutting off the tip into a vial of Hanks' balanced salt solution containing 0.5% lactalbumin hydrolysate (HLa) and 100 units of penicillin, 100 micrograms of streptomycin, and 100 units of mycostatin per milliliter. The vials of HLa were transported to the laboratory on dry ice. Upon arrival at the laboratory the specimens were transferred to centrifuge tubes and centrifuged at 8000 r.p.m. for 30 minutes in a refrigerated centrifuge to remove bacteria and fungi. The supernatant fluid was used as inoculum for attempted virus isolation in embryonated eggs and tissue cultures.

Acute serum samples were collected from each horse on the day of specimen collec-

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*Supported in part by National Health Grant 605-7-137, from funds allocated by the Province of Ontario.

tion, and convalescent serum three weeks later. In addition all horses on the farm were bled for a serological survey against the myxoviruses at the start of the study, with the exception of suckling foals.

Virus isolation attempts were made in 10 day old embryonated hens' eggs, primary rhesus monkey kidney and HeLa cells, and, during the latter part of the study, rabbit kidney cell cultures.

The eggs were inoculated by the amniotic route and the amniotic fluids harvested in 3 to 5 days and tested for hemagglutinins for human O, fowl, and guinea pig erythrocytes at 4, 22 and 37°C. Two passages of all specimens were made in eggs. The tissue cultures were grown and maintained in stationary tube cultures at 37°C. Each specimen was inoculated, in 0.1 ml. amounts, into four tube cultures of each tissue culture cell type. The tubes were examined daily for cytopathic changes, and hemadsorption tests were performed, using 0.5% guinea pig erythrocytes, seven days after inoculation. Two passages of all specimens were made in tissue cultures.

All viruses isolated were identified serologically by a variety of tests. Viruses which grew in eggs and hemagglutinated were tested in the complement-fixation test against *Myxovirus influenzae types* A, B, and C antisera, and then typed by the use of hemagglutination-inhibiting (HAI) antiserum against influenza A, A1, and A2; influenza B (Great Lakes) influenza B (Lee); and influenza C (1233). Viruses which did not grow in eggs but showed hemadsorption in tissue culture were typed against para-influenza 1, 2, and 3, using the hemadsorption-inhibition test with hyper-immune guinea pig antisera. Isolates which produced cytopathic effects characteristic of equine rhinopneumonitis virus were identified by means of virus neutralization tests using hyperimmune rabbit antiserum prepared against the Ky-D strain (Doll) of the virus. Other virus isolates were tentatively grouped according to the type of cytopathic effect produced, chloroform sensitivity, and acid lability, and tested against antiserum prepared against viruses showing similar properties.

Serological tests on the acute and convalescent sera of infected horses were carrid out against the viruses isolated from the individual animals by employing virus neutralization tests (100 TCD_{50} of virus were tested against serial serum dilutions) and, where appropriate, by means of hemagglutination-inhibition tests using 4 agglutinating doses of virus and serial dilutions of serum. All sera were heat inactivated at 56°C. for 30 minutes, and the sera used in HAI tests were treated overnight with the receptor destroying enzyme of *Vibrio cholerae* followed by heating at 56°C for 30 minutes.

Table I

Year and virus isolates

Year	Viruses isolated
1960	**Myxovirus influenzae** A/equi-1/Prague/56
1961	**Myxovirus parainfluenzae** 3 (fall) Equine rhinovirus type 1 (fall)
1962	Equine rhinovirus type 2 (spring) Equine rhinopneumonitis (fall)
1963	**Myxovirus influenzae** A/equi-2/Miami/63 **Myxovirus influenzae** A/equi-1/Prague/56 (fall and winter)

The serological survey of *Myxovirus* infection on the farm was performed by the complement-fixation test using two units of antigen and two exact units of complement with fixation overnight at 4°C.

Results

Several viruses were recovered from horses clinically ill with upper respiratory disease, and these isolates are shown in Table 1. Clinically it was impossible to distinguish between illnesses caused by different viruses.

Two strains of *Myxovirus influenza A/equi* were recovered, one in 1960 and one in 1963. The 1960 isolate and several made in 1963, proved to be identical with the Czechoslovakian strain (1); another 1963 isolate was serologically distinct in the HAI test and is identical to A/equi-2/Miami/63 (2). Both of these influenza strains were recovered from horses that were clinically ill with upper respiratory disease and all of these horses showed a rise in HAI antibody to the viruses. In addition, intratracheal inoculation of the isolates into seronegative horses produced a disease that was indistinguishable from that found in naturally infected horses.

A serological survey of the horses on this farm and from other parts of Ontario showed a high incidence of infection with *Myxovirus influenzae* types A, B, and C (3) (Figure 1).

Figure 1: Incidence of Antibodies Against Influenza and Parainfluenza Viruses in Horse Serum, 1960.

Virus	AGE OF HORSES IN MONTHS* 6	12	24	36
Influenza A.....	0+	12	75	6
Influenza B.....	0	2	25	3
Influenza C.....	22	4	52	20
Parainfluenza 1 .	0**	0	25	24
Parainfluenza 2 .	0	6	28	14
Parainfluenza 3 .	12	51	82	100

*25, 6 months old; 49, 12 months old; 125, 24 months old; and 100, 36 months old were tested.

+percentage of horse showing complement-fixation titers greater than 1:4

**percentage of horses showing hemadsorption-inhibition titers in tissue culture at values greater than 1:10

From the outbreaks of racetrack cough in 1961 and 1962 two viruses were isolated, an equine strain of *Myxovirus parainfluenzae* type 3 (RE55) (4), and two new viruses which proved to be equine rhinoviruses (5). The outbreak from which the parainfluenza 3 was recovered was widespread on the particular farm and the virus was isolated from 22 out of 48 specimens from individual horses. Again serological evidence revealed a high incidence of infection with the parainfluenza group (Figure 1). The rhinoviruses were from adult horses with severe upper respiratory disease and in one of the horses the virus was present in the serum as well as the throat swabs.

Equine rhinopneumonitis virus (ERV) was isolated from young horses with a cough, and also from the liver and lung tissue of aborted foals. The year 1962 appeared to be one in which this virus seriously affected the horse population in Canada.

Discussion

It has become apparent that upper respiratory disease of horses is an entity with a complex etiology. Although there has been great demand for vaccines from both veterinarians and owners, our limited studies, which produced five viruses in three years from the same clinical syndrome, cast doubt on the possibilities that the demand for a vaccine can be met. Although all of these viruses cause an economic setback for the horse owner, the disease produced, for the most part, is self-limiting and not, under normal circumstances, life-threatening.

It appears appropriate at this time to discuss each of the viruses involved in equine upper respiratory disease in some detail.

Equine herpesviruses. The *Herpesvirus* group comprises desoxyribonucleic acid containing viruses which possess compound cubic symmetry. All of the members studied so far possess 162 capsomeres on their surface and these appear to be cylindrical and hollow. Development of the virus starts in the nucleus of the infected cell and mature particles, surrounded by a double membrane, appear later in the cytoplasm. A characteristic of infection by herpesviruses is the formation of an intranuclear inclusion. This inclusion, referred to as type A, is seen as a single homogenous eosinophilic body occupying most of the nucleus and clearly separated from the marginated chromatin (6). All members of the group are ether, chloroform, and acid sensitive. Viruses presently included in the *Herpesvirus* group include herpes simplex, virus B of monkeys, pseudorabies, varicella-zoster, virus 111 of rabbits, infectious bovine rhinotracheitis, and the virus of equine rhinopneumonitis.

For many years it appeared as though there was only one serological type of equine rhinopneumonitis virus, but recently Plummer and Waterson (7), and Kawakami and his associates (8), have given proof of a second immunological type. Although there is an antigen shared by both types of virus in serological tests the level of antibody to the heterologous virus is not high (9).

The most serious problem caused by these viruses is abortion in pregnant mares. For this reason the virus of equine rhinopneumonitis was previously called equine abortion virus. Doll and Bryans, however, have recently shown that repeated immunization against the virus will reduce the incidence of abortion in vaccinated mares to a remarkable degree (10), although consecutive intranasal infections with live virus were necessary to establish a serviceable immunity. One of the epidemiological problems of equine herpes infections is whether repeated attacks of disease are due to reexposure to the virus or whether they are reactivation of a latent and continuing infection. Doll and his co-workers feel that

pregnant mares become infected with equine rhinopneumonitis virus as a result of contact with young horses suffering from upper respiratory disease (11). On the other hand, is the experience with *Herpes simplex* infection of man where primary infections may be severe but recurrent infections are manifest by local lesions (cold sores) due to reactivation of latent virus (12). Apparently antibody found against *Herpes simplex* in man indicates a carrier state rather than resistance, and this is explained by cell to cell transfer of virus which enables disease to occur, despite circulating antibody. In this case however, only localized lesions are seen. The two theories are not mutually exclusive however. It may be that primary infections in young horses lead to local disease, manifest by upper respiratory signs, plus systemic dissemination of the virus. Subsequent infections of the upper respiratory tract may be repeated but systemic invasion by the virus does not occur due to the presence of circulating antibody. Seeding of the uterus of the young mare probably occurs during the first infection by the virus. In this tissue the virus becomes latent, only to be reinactivated during the period of pregnancy, resulting in local infection of the uterus and the susceptible embryo with subsequent abortion. In support of this theory may be the fact that by far the greatest number of abortions occur in first foal mares and abortion "storms" due to rhinopneumonitis virus appear to coincide with the number of first foal mares on the particular farm (13).

Rhinoviruses. From cases of the common cold, viruses were recovered which shared several properties in common with the enterovirus subgroup of the *Picornavirus* group (14). However these viruses differed from the true enteroviruses in being acid labile and being found in the throat rather than feces. Properties which they share in common with the enteroviruses are those of small size, simple cubic symmetry, chloroform and ether stability. Many serological strains have been recovered from man and one from a calf (15).

The first rhinovirus of the horse was described by Plummer as an equine respiratory virus with enterovirus-like properties (16), and it was also isolated from horse serum at about the same time (17). Equine rhinoviruses are responsible for upper respiratory disease in horses in conjunction with viremia in infected horses. In addition such viruses are capable of infecting humans in close contact with the infected horses or the virus (18), (13).

Following infection with the virus, a high degree of immunity is achieved and serological surveys have indicated a high incidence of infection among selected horse populations (13, 19). There seems little doubt that equine rhinoviruses contribute significantly towards equine respiratory disease.

Myxoviruses. Myxoviruses of the horse have been studied by a large number of investigators. Members of two subgroups are represented in equine infections, true influenzas and parainfluenza type 3. The first influenza virus of equine origin was isolated by Czechoslovakian workers in 1956 (1). This virus, A/equi-1/Prague/56 caused epidemic disease in horses in Sweden and Eastern Europe in 1955 and 1956. Doll (20) provided serological evidence of infection in horses in the United States as early as 1957 but the virus, apparently, had not been recovered from horses on this continent prior to our isolation in 1960. Subsequently the virus was isolated from horses in the United States (Doll, personal communication, 1963).

The recent widespread pandemic on the North American continent among racehorses was due to a new antigenic type of influenza, A/Equi-2/Miami/63. (2). Serological differentiation between the two viruses is made upon the hemagglutination-inhibition test; both viruses share a common complement fixing antigen.

Serological examination of the population under study has shown a widespread infection among horses with not only influenza A type viruses but also influenza B and C. (Figure 1). Of particular interest is the high percentage of horses showing antibody against influenza C, and a recent isolate of *Myxovirus influenza* C from clinically ill horses, with a concomitant rise in antibody between acute and convalescent serum samples, lends more credence to the importance of this virus in upper respiratory disease of horses.

Infection of young horses with parainfluenza 3 was first reported in 1963 (4). All of the horses were clinically ill with upper respiratory disease and a rise in antibody against the infecting virus was shown between the acute and convalescent serum samples collected from sick horses.

Furthermore, recent experiments have shown that low tissue culture passaged virus, when inoculated intranasally into sero-negative horses, produced upper respiratory disease. Again a serological survey on the farm showed a widespread infection with the parainfluenza viruses among the study group (Figure 1). The parainfluenza subgroup of the *Myxovirus* group is differentiated from the influenza subgroup mainly on the basis of its ultrastructure. All of the members of the *Myxovirus* group have proved to have compound helical symmetry, with the nucleocapsid of the virus, in the shape of a coiled spring, bundled within a lipoprotein coat. The diameter of this internal component in the influenza subgroup is 90Å while in the parainfluenza subgroup it is 180Å in diameter.

Parainfluenza infections among children are a particular problem in human infections and it would appear that such a situation also occurs in equine populations. Adult horses show a high degree of immunity to the parainfluenza viruses as estimated from the result of serological tests.

It is apparent from these limited studies that upper respiratory disease in horses is due to a number of causative agents which, for all intents and purposes, cause the same clinical syndrome. Because of this it is apparent that a great deal of work remains to be done before vaccination against such a syndrome is feasible.

ACKNOWLEDGEMENTS

We wish to acknowledge the advice and encouragement of Dr. A. J. Rhodes, Director of the School of Hygiene, University of Toronto, and the excellent technical assistance of Mrs. C. M. Macgregor.

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