JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1977, p. 285-289
Vol. 5, No. 3
Printed in U.S.A.
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Infectious Causes of Equine Respiratory Disease on Ontario Standardbred Racetracks

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Received for publication 29 November 1976

Upper respiratory disease has been a serious problem in Standardbred horses on racetracks in Ontario, with outbreaks occurring once or twice annually in late winter and early spring seasons. To determine the causes of these epidemics, a 3-year investigation was carried out in which nasal swabs and serum samples were obtained at intervals from apparently healthy horses and from horses suffering from upper respiratory disease. The nasal swabs were used to isolate bacteria and viruses. The serum samples were examined for the presence and level of antibodies to equine influenza viruses and equine herpesvirus 1. None of the bacteria isolated were associated with the outbreaks of disease. Equine herpesvirus 2 was isolated 72 times from both diseased and apparently healthy horses. Equine herpesvirus 1 was isolated 10 times from horses with respiratory disease, both during and between epidemics. Influenza equine/1 virus was isolated seven times and influenza equine/2 was isolated once during severe outbreaks of upper respiratory disease. Serological evidence confirmed that influenza viruses were the causes of the major epidemics, with the equine/1 strain being involved most often.

Serious outbreaks of upper respiratory disease have occurred once or twice each year on Standardbred racetracks in Ontario and in some cases were severe enough to interfere with the regular racing schedule. Bacteria, fungi, and viruses have been identified as possible causes of respiratory infections of horses, although viruses appear to be of greatest significance.

Two equine influenza viruses, A/equine/Prague/1/56 (equine/1) and A/equine/Miami/1/63 (equine/2), have been reported to play an important role in epidemics of respiratory disease (6, 11, 13). In Canada, the first reported isolation of the equine/1 influenza virus was made in 1960, and the equine/2 virus was first isolated in Canada in 1963 (3). Both of these isolates were from Thoroughbred horses. Several other viruses produce respiratory disease in horses, and these diseases can be differentiated from equine influenza only by laboratory tests. Equine respiratory infections have been reported to be due to adenovirus (2), equine herpesvirus type 1 (rhinopneumonitis or EHV1) (1, 8, 9), equine herpesvirus type 2 (slowly growing equine herpesvirus or EHV2) (10, 12), and reovirus (4). These investigations were undertaken to identify the causes of the epidemics of respiratory disease at Ontario Standardbred racetracks.

MATERIALS AND METHODS

Horses. The horses stabled at the three Standardbred racetracks operated under the authority of the Ontario Jockey Club, Greenwood (Toronto), Mohawk (Campbellville), and Garden City (St. Catharines), comprised the animals for these investigations. On this circuit racing is continuous throughout the year, and each track is used two or three times annually during approximately the same periods. The incidence of equine respiratory disease was monitored on these tracks, and various samples were obtained from January 1973 to December 1975.

Bacterial cultures. For the collection of specimens for bacteriological culture, conventional cotton-tipped swabs were attached to a 20 cm of thin polyethylene tube encased in a 15-cm firmer and larger tube. The equipment was sterilized in paper envelopes. The tubing was inserted into the nostril the distance of the outer tube; then the encased swab was pushed out into the upper nostril, rotated, and pulled back into the outer tube, and then the entire apparatus was withdrawn. Within 2 h after collection, each swab was moistened in a small amount of tryptose phosphate broth and then streaked onto blood agar, mannitol salt agar, and chocolate agar plates. The swab was placed into broth and, after 4 h at 37°C, replated on blood agar.

The plates were examined after 18 and 42 h of incubation. The number of colonies on the initial plates was counted, and the organisms were classified on the basis of cultural and biochemical characteristics (6).

Approximately 10 horses were sampled weekly from January 1973 to February 1974. To assess the bacterial nasal flora of a larger population at one time, 10% of the horse population was sampled, with separate swabs taken from each nostril in March 1974.

Viral cultures. Nasal swabs for viral isolation attempts were obtained by using a sterile cotton gauze sponge (5 by 5 cm) attached to a 50-cm cotton-covered wire. The swab was inserted as far as possible into the nasopharynx and then withdrawn. The gauze was removed from the wire with sterile forceps and placed in a tube of transport medium consisting of Hanks balanced salt solution with 5% lactalbumin and 10% gelatin and containing 500 IU of penicillin, 250 μg of streptomycin, and 100 U of nystatin (Mycostatin, Squibb) per ml. Fluids from the nasal samples were centrifuged at 8,000 $\times g$ for 30 min and inoculated into primary horse kidney cell cultures. The inoculated cultures were incubated at 37°C and examined daily for up to 10 days for cytopathic effect. If no cytopathic effect was apparent by 10 days, subcultures were made onto primary horse kidney cell cultures, and these were observed daily for another 10 days. EHV1 viruses were identified on the basis of cytopathic effect and neutralization by specific antisera. The EHV2 viruses were identified by cytopathic effect, including the demonstration of nuclear inclusions in infected tissue culture cells stained with acridine orange.

In attempts to isolate influenza viruses during 1973, the fluid obtained from each swab was inoculated into the amniotic cavity of 13-day chicken embryos, which were incubated at 37°C for 4 days. The amniotic fluid was harvested together with the chicken embryo lungs and was inoculated into the allantoic cavity of 10- or 11-day chicken embryos. These embryos were incubated at 37°C for 4 days. Influenza virus isolation was determined by standard hemagglutination techniques.

These techniques were modified in 1974 and 1975 by (i) obtaining nasal secretions at the onset of clinical illness, (ii) storing them at -20°C until the swabs could be transported to the laboratory, (iii) inoculating them into amniotic cavities of 10-day chicken embryos, (iv) incubating the eggs at 33°C, and (v) harvesting the amniotic fluid after 4 days and passaging in 10-day embryos, which were again incubated at 33°C for 4 days.

Serum. Blood samples were collected on a weekly basis from a random selection of horses with respiratory disease and from horses that were apparently healthy. In addition, all horses reported to be affected with respiratory disease by trainers or veterinarians were immediately given a clinical examination, and an acute-phase serum sample was obtained. A convalescent-phase serum sample was obtained 30 days later whenever possible. The serum was stored at -20°C.

Hemagglutination inhibition titrations were performed by the microtiter method described by Dowdle and Coleman (5) in a doubling-dilution series beginning at 1:10. Concentrations of 4 hemagglutinating units of virus in 0.025 ml were used in 0.3-ml microtiter wells.

RESULTS

Bacteria isolated from the equine upper respiratory tract. The most common pathogens isolated were *Staphylococcus aureus* and *Streptococcus zooepidemicus*, and their distributions are shown in Table 1. Other potential pathogens isolated included *Pasteurella multocida* from two swabs and *Actinobacillus equuli* from three. The presence of a nonhemolytic micrococcus on direct culture from 33 swabs yielding 10 colonies or more suggested that this organism could be part of the normal flora. Environmental contaminants such as *Bacillus*, *Neisseria*, *Corynebacterium*, and *Streptomyces* species were found in some cases. The cultures from the enriched broth did not yield any pathogens not found on direct culture.

Bacteria were isolated from the random sampling of 10% of the horse population as follows: Of 152 horses, *Staphylococcus aureus* was isolated from 11, *Streptococcus zooepidemicus* from 1, and nonhemolytic micrococci (present in large numbers) from 81; 14 horses showed no micrococci. Comparison of the results from each nostril of the horses sampled is given in Table 2. The results indicate that one nostril may harbor a bacterial flora different from the other.

Viruses cultured from the equine upper respiratory tract. During 1973, a total of 315 nasal swabs were processed by inoculation into primary horse kidney cell cultures. These samples

TABLE 1. *Staphylococcus aureus and Streptococcus zooepidemicus isolated from nasal swabs*

Month	No. of samples	*Staphylococcus aureus*	*Streptococcus zooepidemicus*
1973			
Jan.	21	7	5
Feb.	41	0	10
March	42	4	4
April	41	8	4
May	30	5	3
June	41	15	5
July	29	5	0
Aug.	0	–	–
Sept.	20	1	0
Oct.	40	4	2
Nov.	17	1	2
Dec.	10	0	0
1974			
Jan. and Feb.	40	0	7

were obtained from 267 horses, some of which were sampled more than once. EHV1 was isolated five times, and EHV2 was isolated 45 times (Table 3). In 1974, EHV1 was isolated four times and EHV2 was isolated 24 times from the 147 swabs processed. A total of 71 swabs were processed in 1975, with the isolation of one EHV1 and three EHV2. All of the EHV1 isolations and most of the EHV2 isolations were made in the period of cold weather: late fall, winter, and early spring (Table 3).

Many of the isolations of EHV1 were from horses with upper respiratory disease, but EHV2 was isolated with approximately equal frequency from horses with upper respiratory disease and from apparently healthy horses.

TABLE 2. *Horses with different microorganisms in right and left nasal passage*

Microorganism	Horses with different nasal flora	
	No.	Percent
Micrococci[a]	20	26
Alpha-hemolytic streptococci	13	17
Neisseria sp.	6	8
Diphtheroids	5	7
Gram-negative rods	3	4
Flavobacterium and *Bacillus* sp.	2	3
Fungi	6	8

[a] Micrococci absent in the cultures from one nostril but present in moderate or large numbers in cultures from the other nostril.

Samples from 90 swabs collected in 1973 were inoculated into eggs in attempts to isolate equine influenza viruses, but no equine influenza viruses were demonstrated (Table 4). During 1974, efforts to isolate equine influenza viruses were intensified, and 51 nasal swabs were processed. During the period 23 April to 6 July an outbreak of upper respiratory disease occurred and the equine/1 strain of influenza virus was isolated from seven horses. One swab obtained on 18 October 1974 from a horse with upper respiratory disease yielded the equine/2 strain of influenza virus (Table 4). No influenza viruses were isolated from 41 swabs processed during 1975.

Serological conversions. Between 15 January and 15 March 1973 during the Greenwood winter meet, 149 cases of upper respiratory disease were recorded. During this period 126 paired serum samples were obtained, and the results of the serological tests on these sera are shown in Table 5. Most of the significant serological responses (at least a fourfold rise in titer between the paired samples) were to the

TABLE 4. *Isolation of equine influenza viruses from upper respiratory tract of horses*

Year	No. of samples	No. of isolations	
		Equine/1	Equine/2
1973	90	0	0
1974	51	7	1
1975	41	0	0

TABLE 3. *Isolation of equine herpesviruses from upper respiratory tract of horses*

Race meet	Dates	No. of samples	No. of isolations	
			EHV1	EHV2
Greenwood	1/22 to 3/16/73	79	4	14
Mohawk	3/17 to 4/14/73	39	0	8
Greenwood	4/15 to 5/13/73	29	0	3
Garden City	5/14 to 7/15/73	80	0	4
Greenwood	7/16 to 9/2/73	10	0	0
Garden City	9/3 to 10/13/73	39	0	6
Mohawk	10/14 to 12/2/73	39	1	10
Greenwood	12/3/73 to 3/16/74	55	1	17
Mohawk	3/17 to 4/14/74	20	2	7
Greenwood	4/15 to 5/11/74	31	1	0
Garden City	5/12 to 7/14/74	29	0	0
Greenwood	7/15 to 8/31/74	8	0	0
Garden City	9/1 to 10/12/74	0	–	–
Mohawk	10/13 to 12/2/74	4	0	0
Greenwood	12/3/74 to 3/14/75	15	1	3
Mohawk	3/15 to 4/13/75	5	0	0
Greenwood	4/14 to 5/10/75	11	0	0
Garden City	5/11 to 7/13/75	40	0	0

TABLE 5. *Serological conversions in horses during the Greenwood winter and Garden City spring meets of 1973*

Respiratory disease	Period	No. of paired sera	Serological conversions[a]			No serological conversion[a]
			Influenza		EHV1	
			Equine/2	Equine/1		
	Greenwood					
Yes	1/15-3/15	90	34 (38)		26 (29)	30 (33)
No	1/15-3/15	36	9 (25)		5 (14)	22 (61)
Yes	2/24-3/6	58	18 (31)		18 (31)	22 (38)
	Garden City					
Yes	5/14-7/15	126		85 (67)	4 (3)	37 (30)

[a] Numbers in parentheses are percentages.

equine/2 strain of influenza virus. A total of 25% of apparently healthy horses also showed seroconversion to equine/2 virus. A number of horses showed evidence of infection with EHV1 during this winter meet.

This outbreak of upper respiratory disease was most severe between 24 February and 6 March 1973. During this 10-day period, 68 cases of upper respiratory disease were recorded, and 58 paired serum samples were obtained from these horses. The results of the serological tests on these samples are also shown in Table 5. These data indicate that both equine/2 and EHV1 viruses were active in this outbreak.

The most serious outbreak of upper respiratory disease in 1973 occurred during the Garden City spring meet from 14 May to 15 July. There were 161 cases of upper respiratory disease during this period, and paired serum samples were obtained from 126 of these horses. More than two-thirds of the horses showed seroconversion to equine/1 virus, and only 3% showed serological evidence of EHV1 infection (Table 5).

The peak of the outbreak occurred during the second week of the Garden City meet, when 60 cases were recorded, and in the next 2 weeks, when 33 cases were recorded each week. Thus from 21 May to 10 June, 126 cases of upper respiratory disease were recorded, with 80% of all cases occurring in this 3-week period. Although no influenza or rhinopneumonitis viruses were isolated during this epidemic, serological data showed that this explosive outbreak was due to equine/1 virus.

During 1974, a vaccination trial was conducted and many young horses were vaccinated against influenza. The presence of these young vaccinated horses probably modified the epidemics observed in the second year of this investigation. However, 72 cases of upper respiratory disease were observed in horses on the circuit between 21 April and 13 July. The first peak of the outbreak occurred from 21 April to 4 May at the Greenwood Spring meet, when 31 (43%) of the cases recorded in 1974 occurred. The second peak was observed between 19 and 25 May at the Garden City spring meet, when 14 (19%) of the year's cases were recorded in 1 week. During these two meets the equine/1 strain of influenza virus was isolated on seven occasions, and serological results confirmed that equine/1 was the cause of the epidemic.

During the Mohawk fall meet of 1974 a mild outbreak of upper respiratory disease occurred, and the equine/2 influenza virus was isolated from a horse with the disease.

In 1975, serological evidence of equine/1 virus infection was shown in 97 horses between 11 May and 12 July during the Garden City spring meet. Of these 97 horses, 23 did not show clinical upper respiratory disease. The peak of the outbreak occurred between 1 and 21 June, when 63 (65%) of the cases were recorded in the 3-week period. No viruses were isolated during this outbreak.

DISCUSSION

Significance of bacteria in the upper respiratory disease epidemics. The bacterial culturing of nasal swabs showed that 11% of samples yielded the beta-hemolytic *Streptococcus zooepidemicus*. This bacterium was isolated throughout the year, with the greatest number of horses carrying it during the winter months. On the other hand, *Staphylococcus aureus* was isolated most often in early summer.

Actinobacillus equuli was isolated from three swabs. It is known to be a pathogen in foals, but its significance in the nasal tract of adult horses is not known. The pathogenic significance of *Pasteurella* spp. in horses is not established, but it was isolated from two horses.

In analyzing the bacteria isolated from normal horses and from horses with upper respiratory disease, no species was associated with

upper respiratory illness. It was concluded that the epidemics of upper respiratory disease on this racing circuit was not caused by infection with bacteria.

Significance of viruses in respiratory disease epidemics. A number of viruses were isolated from the respiratory tract of horses during these investigations. The virus most frequently isolated, 72 cultures, was the slowly growing equine herpesvirus, EHV2. This virus was isolated most often in late fall, winter, or early spring and was demonstrated in healthy horses as well as in horses with respiratory disease. The virus was not associated with upper respiratory disease in any obvious manner, and its significance is not clear.

EHV1, equine rhinopneumonitis virus, is a known pathogen of horses. It was isolated from 10 horses with upper respiratory disease during the 3 years of this research. It was isolated only during late fall, winter, and early spring seasons. EHV1 caused clinical upper respiratory disease in horses, particularly during the cold season, but it was not considered to be a primary cause of the annual severe epidemics of upper respiratory disease.

The isolation of equine influenza viruses is difficult and requires stringent methods. From 182 samples processed, 8 isolates of influenza viruses were recovered. The seven isolations of equine/1 were made during one severe outbreak of upper respiratory disease between 21 April and 13 July 1974. These isolations of equine/1 virus during a typical outbreak and the serological evidence of infection with equine/1 during this and other epidemics led to the conclusion that the equine/1 strain of influenza virus was the primary cause of most severe epidemics of upper respiratory disease on this racing circuit.

The isolation of one equine/2 strain of influenza virus and serological evidence of its involvement in some outbreaks of upper respiratory disease on this racing circuit indicate that this virus was responsible for some of the epidemics observed.

Examination of sera from horses on other Standardbred racetracks in Ontario and the tracing of outbreaks from one track to another showed that equine/1 circulated widely among the Standardbred racing population in Ontario and probably in Quebec in 1975. The epidemic among horses stabled on this circuit ceased in July, as had occurred in previous years. But the infection reappeared in August and caused a small outbreak in susceptible horses.

ACKNOWLEDGMENTS

This research was supported in part by the Ontario Ministry of Agriculture and Food and by a grant fund coordinated by the Ontario Harness Horsemen's Association with contributions from: Canada Horse Council, Canadian Standardbred Horse Association, Canadian Thoroughbred Association, Canadian Trotting Association, Horsemen's Benevolent and Protective Association, National Association of Race Tracks, Ontario Harness Horsemen's Association, Ontario Jockey Club, Ontario Quarterhorse Association, Ontario Racing Commission, Orangeville Raceway, Western Fair Association, and Windsor Raceway.

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