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Persistence of Antibody to Envelope Antigens of Heq2Neq2 Virus in Ponies after Infection and Vaccination (38166)

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Little information is available concerning the primary serum antibody response and the persistence of such antibody in horses following infection with Heq2Neq2 virus. Moreover, the available information was obtained with prototype virus which contains both the hemagglutinin and the neuraminidase envelope antigens. Recognition that antineuraminidase antibody (ANAb) contributes to host resistance against influenza indicates the need for a more complete evaluation of immune responses to the virus (1–3). Recently it has been shown that ANAb is capable of interfering with the serologic detection of antibody specific for the viral hemagglutinin and *vice versa* (4–7). Therefore, in order to gain further information on the humoral immune response to equine influenza virus in horses, sera from animals that received live virus and were subsequently administered inactivated vaccine were assessed for antibodies to the hemagglutinin and neuraminidase in assay systems using monospecific antigens. The results are described in this report.

Materials and Methods. Initial and follow-up serum specimens collected from 8 Chincoteague ponies that had participated in previous (8) experiments designed to evaluate the infectivity of A/equine/Miami/1/63 (Heq2Neq2) virus after a series of passages in man and horses were used in this study. Following challenge with live virus, all animals became infected and 6 experienced a febrile illness. Forty-eight and 62 weeks following initial challenge with live virus, 5 and 3 animals, respectively, were given an intramuscular injection of a commercially available inactivated vaccine containing A/equine/Miami/1/63 (Heq2-Neq2) virus.

Sera were measured for antihemagglutinin antibody (AHAb) in neutralization tests against 3.2 or 16 50% tissue culture infectious doses ($TCID_{50}$) using the antigenic hydrid Heq2Neq1, derived from A/equine/Miami/1/63 (Heq2)–A/equine/Prague/1/56 (Neq1), and for ANAb by the neuraminidase inhibition test using HONeq2, derived from A/nws/33 (HO)–A/equine/Miami/1/63 (Neq2) as previously described (5, 9). The recombinants, kindly provided by Dr. R. G. Webster of St. Jude's Children's Research Hospital, Memphis, Tennessee, allow for the specific assay of antibody to the desired surface antigen. The lowest dilutions tested by neutralization and enzyme inhibition tests were 1:2 and 1:4, respectively. For geometric mean calculations, a titer of $<1:2$ was classified as 1:1 and $<1:4$ as 1:2.

Results. The geometric mean serum AHAb and ANAb titers are presented in Fig. 1 and the proportion of ponies responding by developing antibody to the envelope antigens of Heq2Neq2 virus is shown in Fig. 2. Following inoculation of antibody-negative ponies, 7 of 8 animals responded to the live virus and the AHAb response was at a maximum level by 2 weeks, ranging from 1:4 to 1:64. All animals had antibody by 8 weeks but the mean titer was slightly lower. Thereafter, the mean level continued to decrease, and at the time of vaccination, it was 1:2 and only 4 of the animals exhibited antibody. While an in-

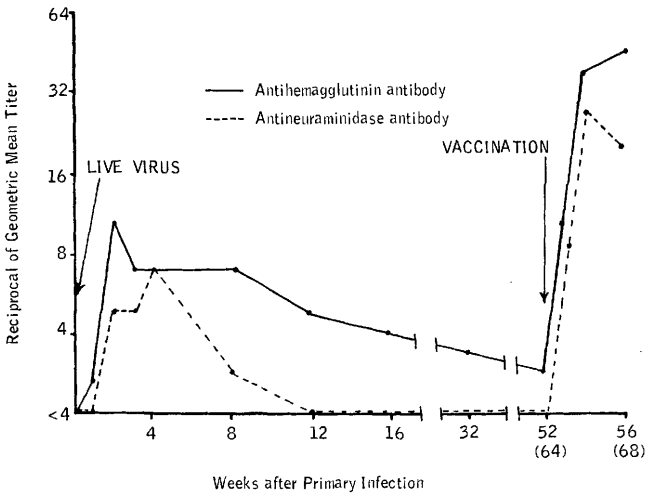

FIG. 1. Induction and persistence of serum antihemagglutinin and antineuraminidase antibodies in ponies following infection and vaccination with Heq2Neq2 virus.

crease in titer occurred in 7 of the 8 animals 1 week after vaccination, a significant rise in AHAb level was evident in all ponies by 2 weeks with a further rise in titer noted by 4 weeks.

The initial exposure of ponies to live virus resulted in a relatively low and transient serum antibody response to the neuraminidase antigen. The peak postinfection antibody titer occurred at four weeks and titers ranged from 1:4 to 1:16. Two of the ponies failed to exhibit an ANAb response and the highest proportion of animals with antibody was observed after 4 weeks. By eight weeks, 2 animals had titers of 1:4 and after 12 weeks none of the ponies were found to possess antibody. Reexposure of animals with Heq2Neq2 virus in the form of an inactivated vaccine was followed by a secondary-type antibody response. ANAb was present in 6 of 8 animals as early as one week and in 7 by 2 weeks. The mean titer was 1:9 by 1 week with further rises evident 2–4 weeks later. At 4 weeks the level of response was approximately 6-fold greater than the maximum level seen among ponies during the primary response to Heq2Neq2 virus.

Discussion. The results presented in this report show that antibodies to hemagglutinin

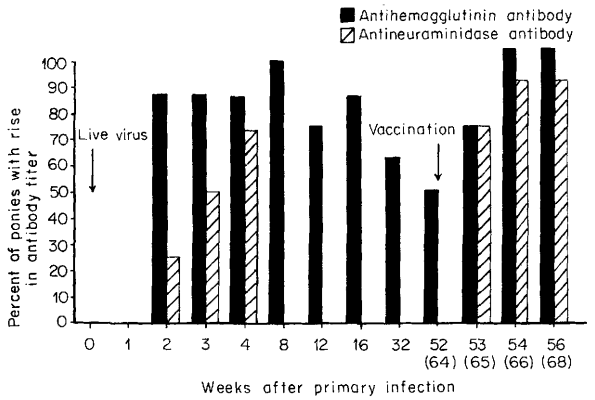

FIG. 2. Relative frequencies of antibody significant rises to envelope antigens of Heq2Neq2 virus after infection and immunization ($\geq$4-fold over baseline).

and neuraminidase antigens are induced in serologically-negative ponies by a primary infection with Heq2Neq2 virus under experimental conditions. However, the titer and frequency of serum responses were of relatively short duration. AHAb was observed to persist at barely detectable levels in only 50% of animals and significant titers of ANAb were not demonstrable in any pony after 4 weeks. Following immunization with an inactivated vaccine, an accelerated antibody response occurred. Although one pony failed to develop an ANAb response at this time, all other animals exhibited marked increases in levels of antibody to each envelope antigen. These findings raise the possibility that equines are subject to reinfection with the same antigenic subtype.

Data from studies in animals and man show that ANAb in addition to AHAb is associated with host immunity to influenza (1–3). Moreover, the degree of resistance to infection and illness appears to vary inversely with the level of antibody present at the time of exposure (3, 10). In the present study, the disappearance of antibodies in horses following primary infection suggests that susceptibility is reestablished. Consequently, immunization needs to be considered for all horses regardless of prior exposure to equine influenza.

Summary. Primary challenge of serologically-negative ponies with Heq2Neq2 virus resulted in the formation of serum antibody to the hemagglutinin and neuraminidase antigens. The titer and frequency of antibody responses were of relatively short duration. Immunization with inactivated vaccine 52–64 weeks after equine infection produced an accelerated and increased antibody response to both envelope antigens.

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