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WOODCHUCK HEPATITIS VIRUS IN NATURAL WOODCHUCK POPULATIONS

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Abstract: Woodchuck hepatitis virus (WHV) was discovered in serum samples from captive woodchucks (*Marmota monax*) at the Penrose Research Laboratory in December, 1977. WHV belongs to the same class of viruses as hepatitis B virus (HBV), the cause of serum hepatitis in man. Both appear to be associated with chronic hepatitis and hepatocellular carcinoma in their respective hosts. Woodchucks were trapped and blood samples collected to determine the prevalence of WHV in natural woodchuck populations. Sera from 217 woodchucks trapped from southeastern Pennsylvania, central New Jersey, and north central Maryland during the spring and summer of 1978 and 1979 were tested for evidence of WHV infection. In 1978, 7 of 51 (13.7%) woodchucks were positive for WHV antigens and in 1979, 28 of 166 (16.9%) tested positive. In addition, 49 of 166 (29.5%) woodchucks trapped in 1979 had antibodies to WHV antigens. The data indicate a high prevalence of WHV in woodchucks from the areas surveyed.

INTRODUCTION

Woodchuck hepatitis virus (WHV) was discovered in December, 1977 in woodchucks (*Marmota monax*) from the Penrose Research Laboratory at the Philadelphia Zoological Gardens.¹ Morphological and immunological studies indicate that WHV is closely related to hepatitis B virus (HBV), the agent of serum hepatitis in man.^{8,11} Prior to the discovery of WHV, HBV was the prototype and the only known member of a new, novel family of DNA viruses. They are distinct from other animal viruses and have the following characteristics; 1) both are double-layered DNA viruses. The inner particle, called the core, contains a circular partially double-stranded DNA genome, approximately 3,000 nucleotides in length, which includes a single-stranded segment of variable length;^{7,8} 2) *In vitro*, an endogenous DNA polymerase uses the single-stranded region of the genome as a template;⁸ 3) Excess viral protein coat

identified as surface antigen is produced during an infection and released into the blood as spherical and tubular particles about 22 nm in diameter;^{1,8} and 4) Both viruses have a tropism for liver tissue and both have been associated with chronic hepatitis and hepatocellular carcinoma in their respective hosts.^{2,8,10}

Hepatitis and hepatocellular carcinoma are the most frequent causes of death of woodchucks in the Penrose Research Laboratory's colony.^{1,5} Although these liver diseases have been found in free-ranging woodchucks, the prevalence is much lower than in their captive counterparts.¹ This paper presents the results of a survey for WHV in wild woodchucks from southeastern Pennsylvania, central New Jersey and north central Maryland.

MATERIALS AND METHODS

Woodchucks were trapped in southeastern Pennsylvania from 23

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March to 8 August 1978. Trapping was discontinued in Pennsylvania due to low woodchuck populations and was initiated in the Assunpink Wildlife Management area in Mercer County, New Jersey from 9 August to 12 September 1978. In 1979, woodchucks were trapped from Assunpink Wildlife Management area during the late spring and throughout the summer, however the majority of woodchucks were trapped from north central Maryland by trappers employed by Cocalico Woodchuck Farm of Denver, Pennsylvania. The precise location of each woodchuck trapped was not available but all woodchucks tested for WHV were trapped within a 220 km radius of the Philadelphia Zoological Garden. The age and sex of each woodchuck was determined using established criteria.⁶ Young woodchucks were less than eight months of age and adult woodchucks were eight months or more of age. Woodchucks were sedated with Innovar Vet (Pitman-Moore, Inc.) and blood was drawn from the femoral vein.

In 1978, serum was tested for particles containing endogenous DNA polymerase activity and some were examined by electron microscope after purification by pelleting twice through a 10-20% sucrose gradient and by equilibrium sedimentation in a CsCl density gradient.⁸ Serum collected after July, 1978 was tested for woodchuck hepatitis virus surface antigen (WHsAg) and its corresponding antibody (anti-

WHs) by passive hemagglutination inhibition and passive hemagglutination tests, respectively.^{9,11}

RESULTS

Seven of 51 (13.7%) woodchucks tested in 1978 had evidence of WHV in their serum. Six of 35 (17.1%) woodchucks tested in 1978 had anti-WHs in their serum. In 1979, a total of 166 woodchucks were tested for circulating WHsAg and anti-WHs. Twenty-eight (16.9%) had WHsAg and 49 (29.5%) had anti-WHs. Eighty-nine of 166 (53.6%) had neither WHsAg nor anti-WHs (Tables 1 and 2).

Fifty-nine percent (30/51) of the woodchucks trapped in 1978 were young of the year, whereas 31% (51/166) of the woodchucks trapped in 1979 were young of the year. The prevalence of WHV infection did not differ significantly between males and females ($P > .05$); however it did differ significantly between adult and young woodchucks ($P < .05$). Evidence of WHV infection was not found in woodchucks less than 3 months of age.

DISCUSSION

Woodchuck hepatitis virus is a newly discovered virus of woodchucks and little is known about its prevalence in natural populations. Our data indicate a high prevalence of WHV in woodchucks from areas surveyed. Woodchucks infected with WHV either develop protective an-

TABLE 1. The prevalence of WHV in 51 woodchucks trapped in 1978.

Sex	WHV***	Negative
Male	5/30 (16.7%)	25/30 (83.3%)
Female	2/21 (9.5%)	19/21 (90.5%)
Age Class		
Adult*	3/21 (14.3%)	18/21 (85.7%)
Young**	4/30 (13.3%)	26/30 (86.7%)

*Adult \geq 8 months of age

**Young $<$ 8 months of age

***Tested for DNA polymerase activity and WHsAg.

TABLE 2. Prevalence of WHsAg and anti-WHs in 166 woodchucks trapped in 1979.

Sex	WHsAg+*	anti-WHs**	Negative
Male	11/74 (14.9%)	24/74 (32.4%)	39/74 (52.7%)
Female	17/92 (18.5%)	25/92 (27.2%)	50/92 (54.3%)
Age Class			
Adults	26/115 (22.6%)	41/115 (35.6%)	48/115 (41.7%)
Young	2/51 (3.9%)	8/51 (15.7%)	41/51 (80.4%)

*Tested by passive hemagglutination inhibition test.

**Tested by passive hemagglutination test.

tibodies (anti-WHs) or they become carriers and have continuous titers of WHsAg in their serum. Experimental infection of woodchucks with WHV has resulted in transient infections with subsequent immunization.⁹ Currently, woodchucks which are carriers must be obtained from free-ranging woodchuck populations.

The prevalence of hepatitis B infection in humans differs significantly between the sexes, because males more frequently become carriers than females. Our data would indicate that male and female woodchucks were equally susceptible to WHV and equally capable of producing protective antibodies.

Adult woodchucks had a significantly higher prevalence of infection than did young woodchucks ($P < .05$). This could correspond to the increased chances of exposure to WHV with increasing age. Mid-August (4 months of age) was the earliest appearance of WHsAg in sera from young woodchucks. The rather late appearance of WHsAg may indicate a long viral incubation period, especially if the virus is vertically transmitted or it may indicate that young woodchucks acquire WHV when they contact other unrelated woodchucks. Young woodchucks remain with their mothers for the first two months of life. After weaning, from late May through early June, they leave their mother and search for their own dens. During this period many young come into contact with unrelated adult woodchucks for the first time.⁶

Experimental transmission of WHV has shown that the incubation period increases with increasing dilutions of WHV injected. Woodchucks infected with diluted solutions of WHV positive serum did not develop viremia until 60 days post-infection.⁹ This small dose of WHV probably mimics the natural exposure of young woodchucks to WHV. Thus if young woodchucks acquire the virus after they leave their mother, they would not develop circulating WHsAg until August of the year they were born.

Chronic hepatitis and hepatocellular carcinoma are associated with WHV and constitute the major cause of death in the Penrose Research Laboratory's colony of woodchucks. However chronic hepatitis and hepatocellular carcinoma do not appear to be major causes of death among free-ranging woodchucks, despite the high prevalence of WHV in natural populations. The average age of death from hepatitis-associated hepatocellular carcinoma is 56 months in our captive woodchucks.^{3,4} Since an estimated 98% of free-ranging woodchucks die before reaching the age of 45 months, only 2% live long enough to develop hepatitis-associated hepatocellular carcinoma.^{3,4} The effect of WHV on the mortality rate and reproductive success in natural populations of woodchucks is unknown and should be studied.

The geographical distribution of WHV in natural populations of woodchucks as well as the susceptibility of the eight different subspecies of woodchucks to WHV also should be investigated. The

subspecies we have studied both in captivity and in nature is *M. m. monax*. Young^{1,2} has maintained a woodchuck colony in New England and has studied over 300 woodchucks. Woodchucks in her colony were captured in Vermont and Massachusetts and therefore are most likely the subspecies *M. m. rufescens*. She has found "no cases of hepatitis or malignant hepatomas" in animals captured in

Vermont or Massachusetts.^{1,2} The lack of these diseases in woodchucks from these areas indicates that WHV is either absent from the area or that the subspecies *M. m. rufescens* is more resistant to WHV infection than the subspecies *M. m. monax*. The latter possibility seems more probable and suggests that a genetic factor may be involved in the susceptibility of woodchucks to WHV.

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