Oecologia Montana 2003
12, 37 - 38

Is the hepatitis virus absent from Yellow-Bellied Marmots?

K. B. ARMITAGE
Ecology and Evolutionary Biology Department, The University of Kansas, Lawrence, KS 66045-7534 USA, e-mail: marmots@ku.edu

Abstract. Hepatitis virus has been found in some populations of Woodchucks (*Marmota monax*), but not in others, of eastern United States. This patchy distribution raised the question of whether the virus occurs in other species of North American marmots. Therefore, blood samples from 51 yellow-bellied marmots (*Marmota flaviventris*) from the Upper East River Valley, Colorado, USA, were tested for the presence of the woodchuck hepatitis virus (WHV). The marmot sera were tested for woodchuck hepatitis surface antigen, antibody to woodchuck hepatitis virus core antigen, and virus-specific DNA polymerase at the Laboratory of Infectious Diseases of the National Institute of Allergy and Infectious Diseases of the Department of Health & Human Services in Bethesda, Maryland, USA. These tests would have detected a virus identical with or serologically related to WHV and would have detected a serologically distinct member of the hepadna viruses. All tests were negative for all 51 yellow-bellied marmots. These results indicate that yellow-bellied marmots do not harbor a detectable hepadna virus. However, the patchy distribution of this virus in woodchuck populations indicates that additional populations of yellow-bellied marmots and other species of North American marmots should be tested in order to further understand the distribution of animal hepadna viruses.

Key words: hepadna virus, Woodchuck, hepatitis, WHV, Yellow-bellied Marmot

Introduction

A wide variety of parasites and bacterial and viral diseases occur in marmots (Bassano 1996, Bibikow 1996). A virus similar to human hepatitis B virus (HBV) was found in a laboratory population of Woodchucks (*Marmota monax*) and designated woodchuck hepatitis virus (WHV). Hepatocellular carcinoma was always associated with chronic active hepatitis (Summers *et al.* 1978). Subsequently, WHV was found in Woodchucks from southeastern Pennsylvania, central New Jersey, and north central Maryland (Tyler *et al.* 1981). Another virus similar to HBV was found in Beechy Ground Squirrels (*Spermophilus beecheyi*) in northern California and designated ground squirrel hepatitis virus (GSHV) (Marion *et al.* 1980). Woodchucks infected from birth with WHV are almost certain to develop hepatocellular carcinoma (HCC); hepatoma induction by WHV is similar to that by HBV except tumors develop earlier at a much higher frequency (Ganem 1990). WHV and GSHV are similar to HBV, which does not belong to any previously known family of viruses. The genome consists of 3000 base-pair circular double-stranded DNA, which is smaller than any known virus. A large region of the genome is single-stranded and DNA polymerase activity characterizes all three viruses (Summers 1981). These similarities suggested a high degree of phylogenetic and structural relatedness between the respective surface antigen forms for WHV and GSHV (Summers 1981). Furthermore, all mammalian hepadnaviruses can cause both acute and persistent infection and persistent infection is a recognized risk factor in development of primary hepatocellular carcinoma (Dandri *et al.* 1998). Thus hepadnaviruses could be an important factor contributing to marmot mortality.

Recently a hepatitis virus was reported in *M. bobac*, but whether it is related to HBV was not indicated (Pole 2003). Cova *et al.* (2003) reported that Alpine Marmots (*M. marmota*) were not susceptible to WHV infection. Clearly, it would be useful to determine if other species of marmots harbor WHV or a similar virus. This paper reports the results of an effort to determine if WHV occurs in Yellow-bellied Marmots (*M. flaviventris*).

Methods

As part of a long-term study of the behavioral and population biology of the yellow-bellied marmot, blood samples were taken from the femoral vein of a hind leg during routine trapping in 1983. Methods of trapping, handling, and collecting blood samples are described elsewhere (Armitage 1982, 1991). Samples were centrifuged and the sera separated from the cells and frozen. Blood samples were taken from 51 yellow-bellied marmots: eight female young, 11 male young, one female yearling, six two-year-old females, 18 females three-years-old or older, two two-year-old males, and five males three-year-old or older. Samples were taken from marmots at ten sites ranging in elevation from 2,867m to 3,008m over a linear range of about 12km in the Upper East River Valley, Gunnison County, Colorado, USA.

The frozen sera were shipped to R.H. Purcell, Head, Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. The sera were tested for WHV surface antigens (WHs Ag), antibody to WHV core antigen (Wong *et al.* 1982, Cote *et al.* 1984), and virus-specific DNA polymerase.

Results and Discussion

All tests for HV for all Yellow-bellied Marmots were negative (Purcell pers. com.). The marmots did not harbor a detectable hepadna virus. The tests would have detected a virus identical with or serologically related to WHV as well as a serologically distinct member of the hepadnaviruses. For example, WHs Ag shares at least two and possibly three of five cross-reactive domains with ground squirrel surface antigens (GSHs Ag) (Cote and Gerin 1983).

The range of age of the Yellow-bellied Marmots should have been sufficient to detect hepatitis virus (HV). WHV was not found in woodchucks less than three months old; young Yellow-bellied Marmots were three to four months old. The remaining 32 marmots ranged in age from one to ten years old. Because infection rate is similar among woodchucks aged seven months or older, the age distribution of the yellow-bellied marmots was adequate for detecting the presence of HV. About 30% of woodchucks trapped in eastern United States had antibodies to WHV antigen and about 17% tested positive for WHV antigens (Tyler *et al.* 1981). If yellow-bellied marmots had similar rates of infection, we could expect about nine animals to test positive for HV and 15 marmots to test positive for antibodies. It is unlikely that HV was missed in Yellow-bellied Marmots because of early mortality; the average age of death of captive woodchucks was 52 months (Summers 1981) and our sample of Yellow-bellied Marmots ranged from four to 120 months, a range sufficient to detect HV or hepatocellular carcinoma.

The absence of a hepadna virus in the East River population of yellow-bellied marmots should not be construed to mean that HV is absent from the species *M. flaviventris*. WHV is patchily distributed. Woodchucks from southeastern Pennsylvania, central New Jersey, and north central Maryland harbored WHV (Tyler et al. 1981), but none of 82 woodchucks trapped in New York was positive for WHs Ag (Wong et al. 1982) and no captive Woodchucks from New England housed for over nine years evidenced chronic WHV or hepatocellular carcinoma (Young and Sims 1979). Furthermore, GSHV is patchily distributed; one population near Stanford, CA had an infection rate greater than 50% whereas infection could not be demonstrated in populations from Point Lobos and Santa Barbara (Marion *et al.* 1980).

The patchy distribution of WHV and GSHV suggests that additional populations of yellow-bellied marmots should be tested for the presence of HV. Also, the other species of North American marmots should be surveyed for the distribution of HV in order to determine the effect, if any, of HV on marmot survivorship and fitness.

Acknowledgements

The field research was conducted at the Rocky Mountain Biological Laboratory, Colorado, and supported by NSF Grant BSR-8121231. I thank R.H. Purcell for providing the results of the analyses for hepatitis virus and Sharon Lee Green for typing the manuscript.

References

Armitage, K.B. 1982: Yellow-bellied Marmot. In *CRC Handbook of Census Methods for Terrestrial Vertebrates*, (Davis D.E. ed.), pp. 148-149.

Armitage, K.B. 1991: Factors affecting corticosteroid concentrations in Yellow-bellied Marmots. *Comp. Biochem. Physiol.*, **98A**: 47-54.

Bassano, B. 1996: Sanitary problems related to marmot-other animals cohabitation in mountain areas. In: *Biodiversity in Marmots*, (eds. M. LeBerre, R. Ramousse and L. LeGuelte), pp. 75-88.

Bibikow, D.I. 1996: Die Murmeltiere der Welt. Westarp Wissenschaften, Magdeburg.

Cote, P.J. Jr. and Gerin, J.L. 1983: Non overlapping antigenic sites of woodchuck hepatitis virus surface antigens and their cross-reactivity with ground squirrel hepatitis virus and hepatitis virus surface antigens. *J. Virol.*, **47**: 15-23.

Cote, P.J., Engle, R.E., Langer, C.A., Ponzetto, A. and Gerin, J.L. 1984: Antigenic analysis of woodchuck hepatitis virus surface antigen with site-specific radioimmunoassay. *J. Virol.*, **49**: 701-708.

Cova, L., Jamard, C., Hantz, O., Zoulim, F. and Trepo, C. 2003: Woodchuck (*Marmota monax*) model for study of chronic hepatitis B virus infection. In *Adaptive Strategies and Diversity in Marmots*, (eds. R. Ramousse, D. Allainé and M. LeBerre), pp. 11-12.

Dandri, M., Peterson, M.J., Stockert, R.J., Harris, T.M. and Rogler, C.E. 1998: Metabolic labeling of woodchuck hepatitis B virus X protein in naturally infected hepatocytes reveals a bimodal half-life and association with the nuclear framework. *J. Virol.*, **72**: 9359-9364.

Ganem, D. 1990: Of marmots and men. *Nature*, **347**: 230-232.

Marion, P.L., Oshiro, L.S., Regnery, D.C., Scullard, G.H. and Robinson, W.S. 1980: A virus of Beechy ground squirrels that is related to hepatitis B virus of humans. *PNAS, USA*, **77**: 2941-2945.

Pole, S.B. 2003: Marmots and zoonotic infections in CIS. In *Adaptive Strategies and Diversity in Marmots*, (eds. R. Ramousse, D. Allainé and M. LeBerre), pp. 13-18.

Summers, J. 1981: Three recently described animal virus models for human hepatitis B virus. *Hepatology*, **1**: 179-183.

Summers, J., Smolec J.M. and Snyder R. 1978: A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. *PNAS, USA*, **75**: 4533-4537.

Tyler, G.V., Summers, J.W. and Snyder, R.L. 1981: Woodchuck hepatitis virus in natural woodchuck populations. *J. Wildl. Dis.*, **17**: 297-301.

Wong, D.C., Shih, J.W.-K., Purcell, R.H., Gerin, J.L. and London W.T. 1982: Natural and experimental infection of woodchucks with Woodchuck hepatitis virus, as measured by new, specific assays for woodchuck surface antigen and antibody. *J. Clinical. Microbiol.*, **15**: 484-490.

Young, R.A. and Sims, A.H. 1979: The Woodchuck, *Marmota monax*, as a laboratory animal. *Lab. An. Sci.*, **29**: 770-780.