Attempts to transmit hepatitis B virus to chimpanzees by arthropods

P. G. JUPP, R. H. PURCELL, J. M. PHILLIPS, M. SHAPIRO, J. L. GERIN

Summary

Bedbugs (*Cimex lectularius* L.) were fed on an infective blood-hepatitis B virus (HBV) mixture. Further bedbugs and tampan ticks (*Ornithodoros moubata* [Murray]) were fed on HBV-carrier chimpanzees. After a 10 - 13 day interval for oviposition, tests done on samples of individual arthropods showed that 53 - 85% of the bugs were HBsAg-positive and none HBeAg-positive, while 100% of the ticks were HBsAg-positive and 88% HBeAg-positive. The remaining arthropods were fed on 3 susceptible chimpanzees, which had failed to develop HBV infection after 11 months, indicating no transmission had occurred. Subsequently the presence of viable virus in the original infective meals was confirmed by inoculation of the relevant donor sera directly into the 3 still susceptible chimpanzees. HBV infections quickly followed in each animal. It is concluded that, while mechanical transmission of HBV is most unlikely after a 10 - 13-day interval between feedings in bedbugs and tampans, it is still possible that mechanical transmission between humans might occur during interrupted feeds.

S Afr Med J 1991; **79:** 320-322.

It has been shown that human populations in the northern Transvaal, the northern territories of Namibia and in West Africa have a high positivity rate for hepatitis B virus (HBV).[1-4] None of the usual transmission methods for HBV seemed adequate to account for these high positivity rates and there was strong epidemiological evidence favouring transmission by bedbugs or argasid ticks.[5-7] Tests on such arthropods collected from huts showed high infection rates, based on the presence of HBsAg and HBeAg in the common bedbug (*Cimex lectularius* L.) in South Africa,[5,8] the tropical bedbug (*Cimex hemipterus* [Fabr]) in West Africa,[6,9] and the tampan tick (*Ornithodoros moubata* [Murray]) in Namibia.[7] Laboratory vector experiments provided further evidence that these arthropods could be capable of transmitting the virus.[8,10-12] Among such *in vitro* experiments HBsAg was transmitted by bite from infected to uninfected blood by *C. lectularius* and *O. moubata*, although other experiments suggested two additional potential routes for mechanical transmission.[8,13] Since it was not possible to know with certainty that viable virus was indeed transferred by bite in these *in vitro* tests, it was decided to determine whether HBV could be transmitted to chimpanzees and so cause hepatitis B infection in recipient animals.

Arbovirus Unit, National Institute for Virology, Department of Virology, University of the Witwatersrand, Johannesburg
P. G. JUPP, M.SC., PH.D., SCI. NAT.
National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA
R. H. PURCELL, M.D.
SEMA Inc., Primate Center, Rockville, Maryland, USA
J. M. PHILLIPS, D.V.M., M.S.
M. SHAPIRO, M.T., R.L.A.T.
Division of Molecular Virology and Immunology, Georgetown University Medical Center, Rockville, Maryland, USA
J. L. GERIN, M.D.

Accepted 26 Mar 1990.

Materials and methods

Three transmission experiments were conducted using 3 HBV-susceptible chimpanzees. In experiment 1, adult bedbugs were given an infective feed in the Johannesburg laboratory on a blood-virus mixture, which contained serum from a human donor, and on the 10th day were allowed to refeed on a non-immune chimpanzee (No. 1296). The 10th day was chosen because 3 *in vitro* transmissions of HBsAg had previously been accomplished with small numbers of bugs after that interval of time.[10] Furthermore, such a period would permit the completion of oviposition by the bugs so that they would then be ready to feed again. In experiment 2, 2 HBV-carrier chimpanzees (No. 1164 and No. 1241) were exposed to adult bugs and on the 10th day the bugs were refed on a non-immune chimpanzee (No. 72). In experiment 3, adult and late nymphal ticks were given their infective feed on the 2 carrier chimpanzees and on the 13th day were refed on a non-immune chimpanzee (No. 73). The 13-day period allowed the nymphal ticks to moult and some of the adults to oviposit.

Arthropods

The arthropods were reared in an insectary at the National Institute for Virology in Johannesburg. Newly moulted *C. lectularius* adults and *O. moubata* adults and late nymphs were taken by the senior author to the National Institutes of Health (NIH) in Washington, DC. They included one group of *C. lectularius*, which had already taken an infective blood meal (experiment 1). The bedbugs originated from a laboratory colony described previously[10] and were maintained in plastic tubes, 6 cm high and 4,5 cm in diameter, closed with organdie mesh. The tampan ticks used were the F_1 generation of an HBV-free and *Borrelia duttoni*-free colony established from ticks collected at Rundu, Kavango region, Namibia. Ticks were maintained in fine sand inside the same kind of plastic tubes as were used for the bugs. Except during their flight from South Africa to the USA and while feeding on the chimpanzees, the arthropods were housed in insectaries where the temperature was maintained at 25 - 27°C, relative humidity at 75 - 80% and the illumination regimen was 12 hours light-12 hours darkness.

Arthropod feeding

For their infective feed in experiment 1, bugs were fed through a membrane on a defibrinated blood-virus mixture as described previously except that stretched parafilm was used for membranes.[10] For the feedings on chimpanzees, either donor or recipient, each animal was anaesthetised and an open PVC ring, 7 cm deep and 10,5 cm in total diameter, was tightly applied and secured to the shaven abdomen; 120 - 200 bugs or ticks were then placed within a ring whose top was closed with Terylene mesh. The age of the chimpanzees was 4 - 5 years.

Virological techiques

Infective donor blood. In experiment 1, the infective meal consisted of a mixture of defibrinated blood and serum in the proportion 2:1. The blood was drawn from an HBV-negative (negative for HBsAg, anti-HBs and anti-HBc) donor less than 2 hours before the bugs were fed, while the serum was obtained 3 days earlier from the blood of a carrier-donor. Until used, the serum was kept at -20°C except for a sample which was tested to confirm the donor was still an HBV carrier. Tests done on this sample were for HBsAg, HBeAg, anti-HBs, anti-HBe and anti-HBc by solid phase radio-immunoassay (RIA) (AUSRIA II, HB_c, AUSAB, HB_e and CORAB; Abbott, Chicago) and HBV DNA was detected by DNA probe as described by Bowyer *et al.*[14] All tests were positive except anti-HBs and anti-HBe. The donor chimpanzees were bled and a liver biopsy taken just before they were exposed to arthropods (experiments 2 and 3) so that tests could be done to confirm that they were still HBV carriers and probably highly infectious. Serological RIA tests already mentioned (except anti-HBe) and tests for the HBV DNA and HBV DNA polymerase were performed. The latter was done as described previously.[15] The serum levels of three enzymes were determined in liver function tests: alanine aminotransferase (ALT), isocitrate dehydrogenase (ICD) and γ-glutamyl-transpeptidase (GGTP). The recipient chimpanzees were assessed just before exposure to the potentially infected arthropods and after exposure weekly for 9 months. The same three liver function tests were done but only tests for HBsAg, anti-HBs and anti-HBc.

Arthropods. Samples of arthropods were killed immediately after their second feed by placing them in a -70°C freezer where they were stored until extracts could be prepared from them for antigen assay. For this, each individual bug or tick was homogenised in 1,0 ml bovine phosphate albumin (0,75% bovine serum albumin in phosphate-buffered saline (BPA)) centrifuged at 3 000 rpm for 30 minutes and the supernatants frozen immediately at -70°C. Subsequently, supernatants were thawed and tested for HBsAg. Those found HBsAg-positive were also tested for HBeAg. Samples of arthropods which had not engorged on an infecting meal and samples of the BPA were also tested individually as controls.

Results

The bedbug feeding rates on chimpanzees were consistently high at both the first and second feeds (81 - 97%) after the insects had been allowed ample time (20 - 80 min). The number of bugs, an equal number of adult males and females, which fed a second time in the transmission attempts were 102 (experiment 1) and 99 (experiment 2). With the ticks, however, although feeding rates were high (88% and 92%) at the first feed (adult females and mature nymphs), at the second feed it fell to 32 out of 149 adult females (21%). This was probably because only a proportion of the female ticks had completed oviposition before the second feed.

The antigen positivity rates for both bedbugs and ticks on the day of transmission attempts are given in Table I. These rates were very high for both HBsAg and HBeAg in the ticks, while HBsAg positivity varied from 53% to 85% in bedbugs and no HBeAg was detected. No arthropods in samples of 6 - 10 individuals that had not taken infective meals were found positive for HBsAg in each experiment and 4 aliquots of BPA were also HBsAg-negative. In experiment 3, a sample of coxal fluid was collected from the ticks at the second feed and diluted in 0,5 ml BPA. This was HBsAg-positive but was not tested for HBeAg.

Although the 3 recipient chimpanzees were monitored for almost a year after the transmission attempts, there was no indication of infection by HBV as measured by serological tests and liver function tests.

In the light of this, we decided to confirm the presence of viable virus in the original infective blood meals. For this, 0,1 ml of the original human serum used on 4 June 1987 in the infective blood-virus mixture in experiment 1 was inoculated intravenously into chimpanzee No. 1296. Similarly, 0,1 ml of serum from each of the carrier chimpanzees, No. 1164 and No. 1241, bled on 8 and 9 June 1987 was pooled and 0,2 ml inoculated into chimpanzees No. 72 and No. 73, respectively. After 2 - 3 weeks, the 3 previously non-immune chimpanzees became highly HBsAg-positive, HBsAg disappeared by weeks 9 and 8 in chimpanzees No. 72 and No. 73, respectively, followed by conversion to anti-HBs-positive and anti-HBc-positive status. ALT values became elevated 7 and 5 weeks after inoculation in chimpanzees No. 72 and No. 73 and both animals developed anti-HBc. Chimpanzee No. 1296 demonstrated *de novo* synthesis of HBsAg by 2 weeks after inoculation, developed elevated serum ALT values during week 10 and has continued to be strongly HBsAg positive, with markedly elevated results of liver function tests for over 2 years.

Discussion

The direct inoculation of sera corresponding to those of the original infective blood meals into the still susceptible recipient chimpanzees clearly produced HBV infection in all 3 of these animals. Thus it was confirmed that the blood meals were infectious and contained viable HBV. It must therefore be concluded that adult *C. lectularius* and adult *O. moubata* will not transmit HBV mechanically by bite if an interval elapses between the infecting and transmission feeds sufficient to permit digestion of blood, egg development and oviposition. Such an interval would be expected in the normal course of events before bedbugs or ticks would refeed.

TABLE I. TESTS FOR HBsAg AND HBeAg IN ARTHROPODS ON DAY OF TRANSMISSION ATTEMPT

Exp. No.	Infective bloodmeal*	Arthropod	Days after infective meal	No. arthropods tested HBsAg+	No. arthropods tested HBeAg+
1	**Human**	**Bedbugs**	**10**	**16/30 (53%)†**	**0/16**
2	**Chimpanzees**	**Bedbugs**	**10**	**13/20 (65%)**	**0/13**
				17/20 (85%)	**0/17**
3	**Chimpanzees**	**Ticks**	**13**	**40/40 (100%)**	**28/32 (88%)**

*Human and chimpanzee donors were positive for HBsAg, HBeAg, Anti-HBc, HBV-DNA and HBV-DNA polymerase activity and negative for anti-HBs and anti-HBe. Both chimpanzees had elevated levels of serum ALT, ICD and GGTP.
†An equal number of adult male and female bugs but only adult female ticks were tested.

With bedbugs, however, it is still possible that mechanical transmission between humans could occur by interrupted feeding. Unfortunately, a shortage of chimpanzees prevented the inclusion of an experiment to test this in the present study. In such instances there would be an interval lasting only a few minutes between the start of feeding on an HBV carrier and the completion of the blood meal on a susceptible person. Although there is some evidence that interrupted feeding may occur in *C. lectularius* in the laboratory,[16] whether it occurs in natural human bedbug infestations is not known. One previous experiment has been reported in which the mechanical transmission of HBV was attempted by interrupted feeding of mosquitoes on chimpanzees.[17] Two attempts to transmit virus by 100 *Aedes aegypti* from a carrier to 2 different susceptible chimpanzees failed. It would seem unlikely that bedbugs would succeed where mosquitoes failed unless bedbugs differ from mosquitoes in the anticoagulatory enzymes introduced at the time of biting. Perhaps enzymes secreted onto the mouthparts of *C. lectularius* would not inactivate the virus present, whereas this would occur in mosquitoes as suggested by Berquist *et al.*[17] In the case of *O. moubata* it is unlikely that interrupted feeding would occur because once a tick has attached itself to its host it is not easily dislodged.

The two other possible routes for mechanical transmission previously suggested for bedbugs and tampan ticks cannot be ruled out on the basis of the results of the present experiments.[8,13] These are, firstly, the infection of a susceptible person by means of bedbug faeces or by tick coxal or rectal fluid, which could occur by scratching of skin lesions or mucosal surfaces that had been contaminated by these products. Secondly, a person may become infected because he crushes the bug or tick and then scratches the area of the bite or mucosal surfaces causing self-inoculation with infectious material. However, these routes now appear less likely. Serological studies to determine the prevalence of HBV among blacks with viral hepatitis admitted to Johannesburg hospitals,[18] and among residents of Kangwane (South Africa) and Ovamboland (Namibia),[2,19] showed a rise in the HBsAg and HBeAg positivity between the age of 2 years and 4 years accompanied by a fall in anti-HBs positivity. This paucity of perinatal infection but horizontal spread among young children is difficult to explain other than by arthropod transmission. However, the experimental data described here do not support the concept of anthropod transmission as a major factor in the horizontal spread of HBV among young children and other modes of transmission must be considered. These might include contamination from open sores caused by impetigo or by scratches and cuts incurred during rough play.

We wish to thank Mrs Doris Wong for technical assistance and Dr Bob Gwadz for allowing the use of his insectary, both of the National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland, USA. We are also indebted to the animal attendants at SEMA for much help. Dr Geoff Dusheiko, recently of the University of the Witwatersrand Hepatology Unit, provided the human donor and certain technical assistance and Professor Jennifer Alexander of the Department of Microbiology at the same university kindly reviewed our manuscript, for which we are most grateful. Our thanks are due to Dr Ron Eagle of Georgetown University Medical Center for assistance with serological tests. One of us (P.G.J.) gratefully acknowledges grants towards the study from the Poliomyelitis Research Foundation, Johannesburg, and the South African Medical Research Council.

This work was performed in part under NIAID contracts No. 1-A1-52567 and No. 1-A1-72623.

REFERENCES

1. Bersohn I, Macnab GM, Pyzikowska J, Kew MC. The prevalence of hepatitis B (Australia) antigen in South Africa. *S Afr Med J* 1974; **48:** 941-944.
2. Botha JF, Dusheiko GM, Ritchie MJJ, Mouton HWK, Kew MC. Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal infection. *Lancet* 1984; **1:** 1210-1212.
3. Joubert JJ, Prozesky OW, Lourens JGH *et al.* Prevalence of hepatitis virus and some arbovirus infections in Kavango. *S Afr Med J* 1985; **67:** 500-502.
4. Szmuness W, Prince AM, Diebolt G *et al.* The epidemiology of hepatitis B infections in Africa: results of a plot survey in the Republic of Senegal. *Am J Epidemiol* 1973; **98:** 104-110.
5. Jupp PG, Prozesky OW, McElligot SE, Van Wyk LAS. Infection of the common bedbug (*Cimex lectularius* L) with hepatitis B virus in South Africa. *S Afr Med J* 1978; **53:** 598-600.
6. Wills W, Larouze B, London WT *et al.* Hepatitis B virus in bedbugs (*Cimex hemipterus*) from Senegal. *Lancet* 1977; **2:** 217-220.
7. Joubert JJ, Jupp PG, Prozesky OW, Lourens JGH. Infection of wild populations of the tampan *Ornithodoros moubata* Murray, with hepatitis B virus in the Kavango, Northern Namibia. *S Afr J Sci* 1985; **81:** 167-168.
8. Jupp PG, McElligott SE, Lecatsas G. The mechanical transmission of hepatitis B virus by the common bedbug (*Cimex lectularius* L) in South Africa. *S Afr Med J* 1983; **63:** 77-81.
9. Brotman B, Prince AM, Godfrey HR. Role of arthropods in transmission of hepatitis B virus in the tropics. *Lancet* 1973; **1:** 1305-1308.
10. Jupp PG, McElligott SE. Transmission experiments with hepatitis B surface antigen and the common bedbug (*Cimex lectularius* L). *S Afr Med J* 1979; **56:** 54-57.
11. Ogston CW, Wittenstein FS, London WT, Millman I. Persistence of hepatitis B surface antigen in the bedbug *Cimex hemipterus* (Fabr.) *J Infect Dis* 1979; **140:** 411-414.
12. Ogston CW, London WT. Excretion of hepatitis B surface antigen by the bedbug *Cimex hemipterus* Fabr. *Trans R Soc Trop Med Hyg* 1980; **74:** 823-825.
13. Jupp PG, Joubert JJ, Cornel AJ, Swanevelder C, Prozesky OW. An experimental assessment of the tampan tick *Ornithodoros moubata* as vector of hepatitis B virus. *Med Vet Entomol* 1987; **1:** 361-368.
14. Bowyer SM, Dusheiko GM, Schoub BD, Kew MC. Expression of the hepatitis B virus genome in chronic hepatitis B carriers and patients with hepatocellular carcinoma. *Proc Natl Acad Sci USA* 1987; **84:** 847-850.
15. Kaplan PM, Greenman RI, Gerin JL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. *J Virol* 1973; **12:** 995-1005.
16. Dickerson G, Lavoipierre MMJ. Studies on the methods of blood-sucking arthropods: II. The method of feeding adopted by the bedbug (*Cimex lectularius*) when obtaining a blood-meal from the mammalian host. *Ann Trop Med Parasitol* 1959; **53:** 347-357.
17. Berquist KR, Maynard JE, Francy DB. Sheller MJ, Schable CA. Experimental studies on the transmission of hepatitis B by mosquitoes. *Am J Trop Med Hyg* 1976; **25:** 730-732.
18. Kew MC, Miller EB, Stevenson CM, Macnab GM, Bersohn I. The prevalence of virus B hepatitis in South African blacks. *S Afr Med J* 1974; **48:** 1837-1838.
19. Prozesky OW, Szmuness W, Stevens CE *et al.* Baseline epidemiological studies for a hepatitis B vaccine trial in Kangwane. *S Afr Med J* 1983; **64:** 891-893.