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Hepatitis B Infection, Eastern India

To the Editor: The National Institute of Cholera and Enteric Diseases (ICMR), Kolkata, India, conducted a serologic study in July 2003 to determine the rate of hepatitis B virus (HBV) infection of brothel-based commercial sex workers. These study participants worked in the South-24 Parganas district of West Bengal, one of the eastern states of India. Routine immunization to prevent HBV infection is not a practice in India, and chronic HBV infection is endemic (1). The nature of their work makes commercial sex workers more vulnerable to HBV infection, which could accelerate the infection's spread into the general community, particularly in areas with low literacy rates and socioeconomic status.

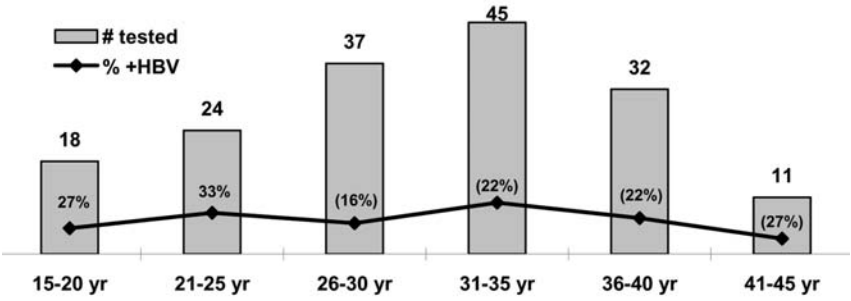

Figure. Distribution of sex workers by age and HBV infection (n = 167)

The study participants were 167 commercial sex workers from three prominent brothels, which were located in small towns and along the national highway of the district. Blood samples from the participants were tested by using the HBsAg unlinked anonymous method. The results showed that 23.3% (unpub. data, National Institute of Cholera and Enteric Diseases) of the commercial sex workers were infected with HBV. The Figure shows the distribution of commercial sex workers and prevalence of HBV infection by age group.

To ascertain a possible route of transmission other than sexual activity, all study participants were questioned about their medical history, including jaundice, injury requiring blood transfusion, surgical events, and tattooing. The evidence indicated that HBV infection in the study participants was not acquired by any route other than sexual activity. Since the carrier rate of chronic HBV in the general population is approximately 5% in eastern India (2), this increase in chronic HBV infection can be attributed to sexual transmission.

The Figure indicates that HBV infection is not correlated to age or duration of commercial sex work; the rate of infection is distributed almost equally in all age groups except the 26- to 30-year-old group in which it was slightly lower. Most of the HBV-infected commercial sex workers likely were infected early in their profession and remained infected throughout their lives, which led to higher rate of chronic infection as compared with the general population.

This higher rate of chronic HBV infection among commercial sex workers (23.3%) is of concern, particularly in a country with an estimated 4.58 million persons infected with HIV. In India, >80% of HIV infection is transmitted sexually (3). HIV infection can affect the natural course of HBV; sexual activity between HIV- and HBV-infected persons could prolong the infected status of those infected with HBV, as was shown in a previous study (4). This sexual activity could facilitate HBV transmission, particularly in areas that have few resources or where the rate of condom use is low or questionable.

After this study concluded, all study participants were notified of their infection status and advised to use condoms when engaging in sexual activity. The commercial sex work-

ers and their family members were advised that they should be tested for HBV infection and receive HBV vaccination if test results were negative. Local health authorities were advised that commercial sex workers and their clients should be vaccinated to prevent HBV infection.

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Ehrlichia Prevalence in *Amblyomma americanum*, Central Texas

To the Editor: *Ehrlichia chaffeensis* and *E. ewingii*, agents of human monocytic ehrlichiosis and ehrlichiosis ewingii, respectively, are transmitted by the lone star tick, *Amblyomma americanum*, which is found from west-central Texas northward to Iowa, and southeastward to the Atlantic Coast (1). In *A. americanum*, *E. chaffeensis* has been found in several states, while *E. ewingii* has only been found in North Carolina, Florida, and Missouri (1,2). *E. ewingii* infection in white-tailed deer (*Odocoileus virginianus*), a potential reservoir, has been found in the states mentioned previously as well as in Kentucky, Georgia, and South Carolina (3,4).

Human ehrlichioses are underdiagnosed in the United States and may be as prevalent as Rocky Mountain spotted fever in some areas (1). Ehrlichioses are prevalent in Texas, and fatal cases have been reported (1,5). This study was conducted to examine ticks from central Texas for *Ehrlichia* and provide information to increase public health awareness of this problem. Adult *A. americanum* ticks were collected from a 38.8-hectare game fenced-pasture (Plot #8) in the Kerr Wildlife Management Area, Kerr County, Texas. Ticks were trapped by using blocks (approximately 85 g) of dry ice centered on smooth, white, nylon cloths measuring approximately 1 m^2. These traps were placed on the ground in the brush or in areas under tree canopies for approximately 1 h.

Trapped adult *A. americanum* were frozen in liquid nitrogen and then bisected with a sterile scalpel. Halves of the bisected ticks were stored at –80ºC. The other halves were pooled in groups of six. DNA was extracted from these pools by using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA), and evaluated by using a nested species-specific 16S rRNA gene polymerase chain reaction (PCR) for *E. chaffeensis* and *E. ewingii*, with *E. canis* as a negative control. The first-round primers were genus-specific for *Ehrlichia* (ECC and ECB). The forward primers of the nested PCR were HE1, EE72, and Ecan, which were specific to *E. chaffeensis*, *E. ewingii*, and *E. canis*, respectively. The reverse primer is a common primer (HE3) for all species (6–8). An aliquot of the negative control reaction containing no DNA template was carried through both rounds of the nested PCR with every reaction set. A dilution series of stock *E. chaffeensis* DNA mixed with tick DNA showed no substantial inhibition of the PCR, even with DNA concentrations as low as 0.2 ng/mL.

Tick pools positive for *E. chaffeensis* or *E. ewingii* by PCR were examined by using DNA from the individual tick halves. DNA was extracted by using the Nucleobond DNA/RNA Isolation Kit (BD Biosciences Clontech, Palo Alto, CA). To confirm positive PCR results for individual ticks, first-round amplicons (primers ECB and ECC) were separated by electrophoresis. The 478-bp band was recovered using the QIAquick Gel Extraction Kit, then cloned into the pCR2.1-TOPO vector with the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). DNA sequences were obtained from both directions of the insert in the recombinant plasmids by using PE Applied Biosystems (Foster City, CA) 373XL automated DNA sequencers in the UTMB Sequencing Core.

Of the 66 adult *A. americanum* ticks examined, 5 were positive for *E. ewingii* (7.6%). The 16S rRNA gene sequences from these five positive samples were most similar to the *E. ewingii* 16S rRNA gene sequence (GenBank accession no.U96436). Sequence variations are summarized in the Table. These mutations may result from polymerase errors prior to cloning. *E. ewingii* has never been cultured or handled by our laboratory, and all negative controls for the nested PCR were negative, minimizing the possibility of false-positive results.

This is the first report of ticks infected with *E. ewingii* in states other than North Carolina, Florida, or Missouri. Ticks are found in damp