

## **Hepatitis B and C virus infections in the three Pygmy groups in Cameroon**

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## Abstract

A high level (11.8%) of hepatitis B virus (HBV) infection was found among 524 Pygmies in Cameroon, whereas the extent of hepatitis C virus (HCV) infection in the same population was low (0.6%). Phylogenetic analyses showed co-circulation of two HBV genotypes, A3 and E. Taken together our results suggest different epidemiological scenarios concerning HBV and HCV infections, respectively, in this population.

## Text

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are considered to be highly endemic in sub-Saharan Africa (6, 11). However, to date little information is available pertaining to the prevalence and genetic diversity of these viruses in Central Africa. In Cameroon, HBV and HCV infections have been mostly studied among Bantu populations (4, 13, 15) and very few data are available for the Pygmies in this region. The Pygmies have lived in Cameroon for more than 20,000 years; in a forest-type environment, mostly as hunter-gatherers (19). The characterization of HBV and HCV isolates from ancient populations may help to reveal the origin and evolutionary history of these viruses (7). Three distinct Pygmy groups currently live in Cameroon: the Baka, the Bakola and the Bedzan. Three studies conducted 15 years ago reported a high HCV prevalence in the Pygmy population, ranging from 6 to 11 % (5, 10, 12). However these studies only considered the Baka. Furthermore, the performances of the tests used at that time are questionable. A more recent study demonstrated an HCV prevalence of only 2.3% (8). In these 4 studies, HBV was found to be highly endemic. Even now, no information is available for the Bakola or Bedzan Pygmies. Thus, the objectives of our study were to assess the prevalence of HBV and HCV markers among the three Pygmy groups from Cameroon and also to study the HBV genetic diversity in these populations.

This study formed part of a survey of viral emergence in Pygmies from Cameroon, conducted from 2005 to 2008 (1-3). Informed consent was obtained from adults or parents, in the case of children, before blood sampling. Furthermore, the participants of the study underwent a medical examination and, if necessary, were treated according to local procedures on site or were sent to local medical facilities. The geographic localization, the number of subjects included in each group, their mean age and sex ratio are shown in Fig. 1A.

The presence of antibodies against HCV (anti-HCV) was checked by a third generation enzyme immunoassay (EIA) (Monolisa anti-HCV Plus version 2, Biorad, Marne-La-Coquette, France). A positive result for anti-HCV was defined as a Monolisa ratio of greater than 6 (16). Out of the 346 available samples tested, only 2 (one Baka and one Bedzan; 0.6%, 95% CI 0.9—1.9%) were anti-HCV-positive. These samples were negative when tested for HCV RNA.

HBV surface antigen (HBsAg) was screened by a third generation EIA (Monolisa AgHBs Plus, Biorad). Of the 524 samples tested, 62 (11.8%; 95% CI 9.2—14.9%) were positive. The prevalence of HBsAg was not statistically different between the three ethnic groups over the same age range. However, in the Baka population, there was a significant association between a decrease in HBsAg and an increase in age, dropping from 23.5% in the  $\leq 10$  years age group to 6.6% among the  $> 50$  year old population ( $P < 0.001$ ) (Table 1). Consequent to their use in a larger epidemiological survey in the same population (1-3), not all samples were available for molecular analyses. A 423-bp fragment of the HBV S gene was amplified as described (18) and sequenced, from 22 HBsAg-positive sera (from 15 Baka, 5 Bakola and 2 Bedzan individuals). After sequence alignment, 17 (77.3%) sequences were seen to belong to the HBV-A3 subgenotype and 5 (22.7%) to the HBV-E genotype. Interestingly, the 15 sequences from Baka individuals were all of the HBV-A3 subgenotype, while there was a co-circulation of HBV-A3 and HBV-E sequences in the Bakola and Bedzam groups, with the

HBV-E genotype being predominant among the Bakola population (80%) (Fig. 1B). The 17 HBV-A3 subgenotype sequences clustered with previously reported HBV-A4 and -A5 subgenotypes (17), although this observation was not supported by a high bootstrap value,. This group was designated as the “quasi A3 subgenotype”.

The present study confirms the very low rate of infection of the Baka in Cameroon by HCV (8) and extends this conclusion to the Bakola and Bedzan populations. Such a low viral prevalence, especially among elderly pygmies (people aged above 50 years), contrasts with the high rates found among the neighbouring Bantu populations (5, 8, 10, 13, 15) and points to a different route/dynamics of HCV transmission in Cameroon. A hypothesis is that transmission in the past among the Bantu had occurred during mass treatment and vaccination with unsafe material (14).

The prevalence of HBsAg noted in our study was comparable to that reported previously among the Baka (8, 12), was similar to that reported among Bantu populations (4, 8) and indicated a high endemicity of HBV in Cameroon. The age-dependent HBsAg prevalence profile showing that children under 10 years old were highly infected strongly suggested perinatal transmission, or transmission in early childhood. Systematic HBV screening during pregnancy is not yet practiced in Cameroon. Furthermore, the expanded child vaccination program introduced into Cameroon in 2005 is limited to urban areas. To prevent the spread of HBV, it is required that these programs are extended to all remote parts of the country.

From a molecular point of view, our data confirm the co-circulation of HBV-A3 and HBV-E genotypes in Pygmies living in Cameroon (8). Interestingly, Baka Pygmies were only infected by the HBV-A3 subgenotype, whereas the HBV-E genotype was predominant in the Bakola population. Despite the relatively small sample size, the different HBV genotypes present in Baka versus Bakola Pygmies raise questions regarding the origin of these viruses.

The high prevalence of the HBV-A3 subgenotype, and its presence in the three groups of Pygmy, suggests a protracted natural history of this subgenotype in Cameroon, whereas the low prevalence of the HBV-E genotype may indicate its recent introduction into this area. These results are in agreement with previous studies that reported a Central African origin for the HBV A3 subgenotype and a West African origin for HBV-E (9). It is also of note that even though Pygmies still frequently hunt, none of the recent HBV isolates are related to HBV genotypes from chimpanzee or gorilla, suggesting the absence of cross-species transmission of this virus. This situation contrasts with that observed for foamy retroviruses in the same populations (2).

In conclusion, our data demonstrate a low prevalence of HCV infection among Pygmies living in Cameroon, reinforcing the hypothesis of a massive iatrogenic transmission of HCV in neighbouring Bantu populations. Furthermore our results extend high endemicity in the 3 Pygmy groups to include HBV infection. HBV-A3 appears to be a longstanding, indigenous HBV strain in Cameroon. Further studies destined to extensively characterize HBV strains from other indigenous populations in central Africa will reveal new insights into the origin and the evolutionary history of this oncovirus.

**Nucleotide sequence accession numbers.** The GenBank accession numbers of the HBV-A3 subgenotype and HBV-E genotype sequences described in this study are HM355550 to HM355571.

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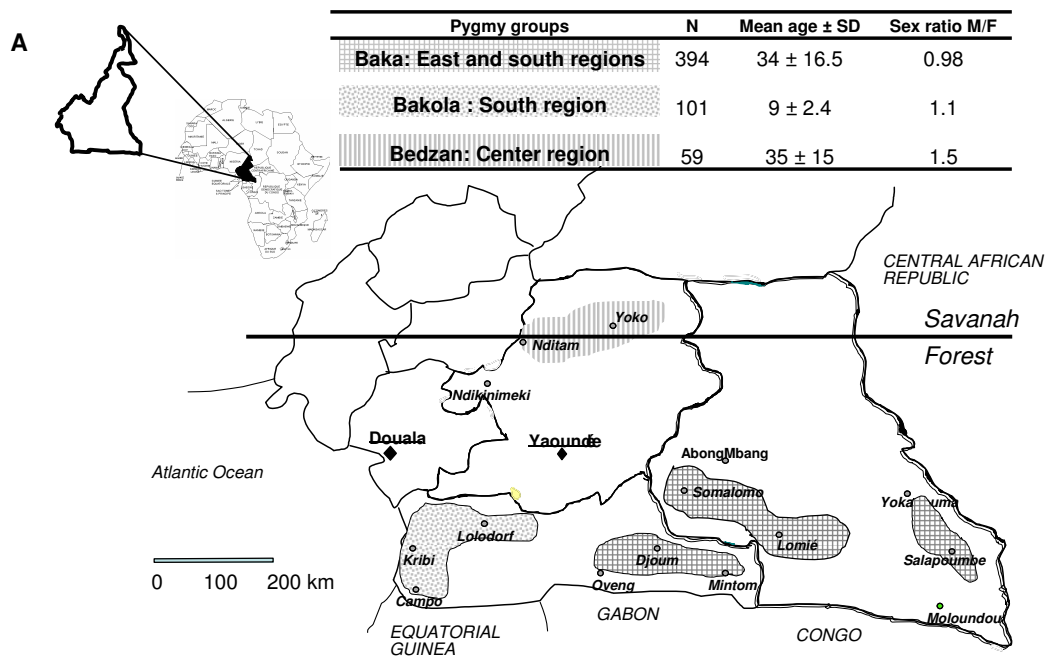
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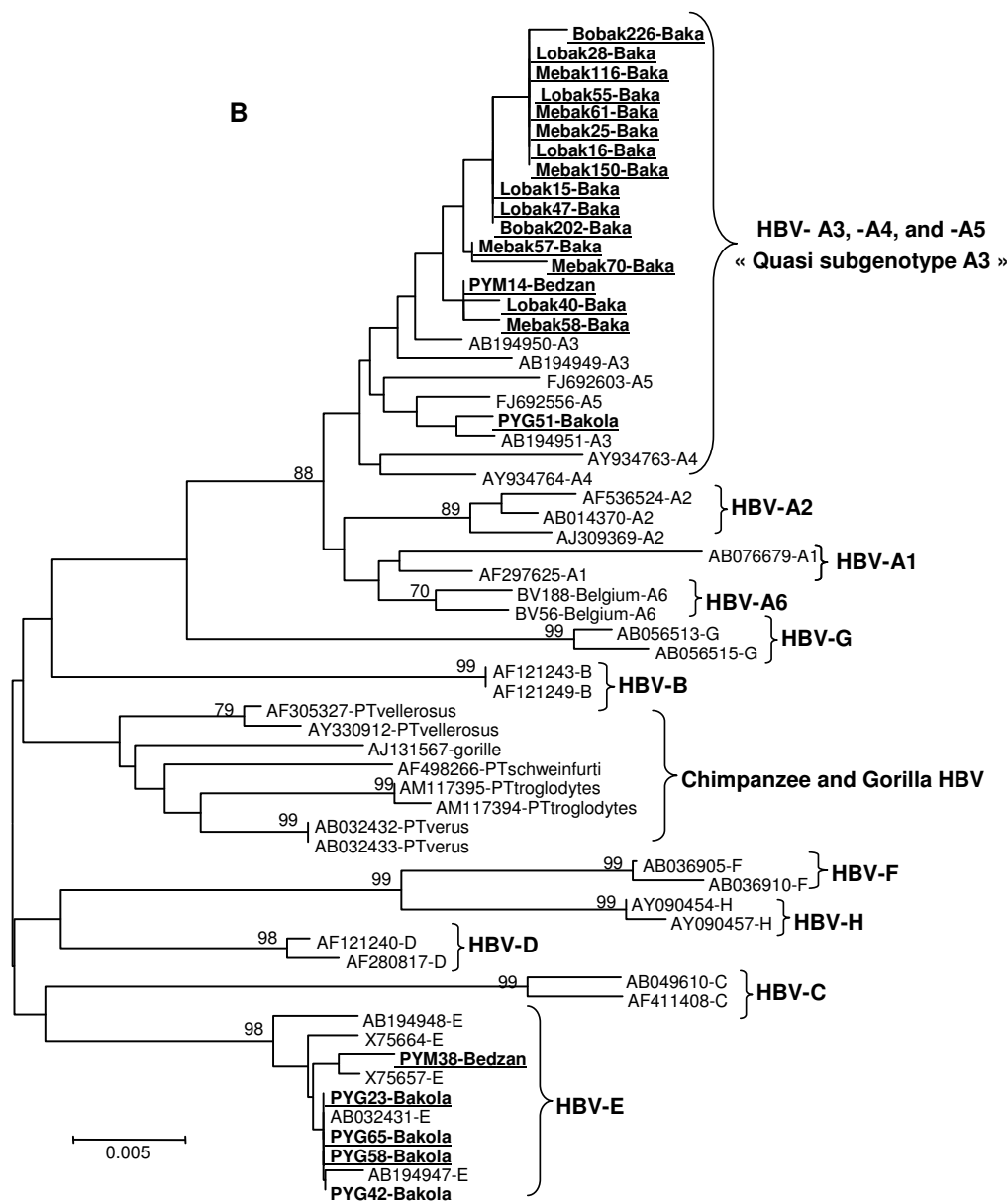


**Table 1:** Prevalence of HBsAg in Pygmy populations in Cameroon, divided according to ethnic group and age range.

| Age range<br>(yr) | No. of positive samples/no. tested<br>(%; 95% CI) |                             |                          |                            |
|-------------------|---|-----------------------------|--------------------------|----------------------------|
|                   | Baka  | Bakola                      | Bedzan                   | Total                      |
| ≤ 10              | 4/17<br>(23.5; 6.8-49.9)                          | 13/66<br>(19.7; 10.9-31.2)  | 0                        | 17/83<br>(20.5; 12.4-30.8) |
| 11-20             | 9/69<br>(13.0; 6.1-23.3)                          | 6/35<br>(17.1; 6.6-33.6)    | 1/9<br>(11.1; 0.2-48.2)  | 16/113<br>(14.2; 8.3-22.0) |
| 21-30             | 10/89<br>(11.2; 5.5-19.7)                         | 0                           | 2/20<br>(10.0; 1.2-31.7) | 12/109<br>(11.0; 5.8-18.4) |
| 31-40             | 6/80<br>(7.5; 2.8-15.6)                           | 0                           | 3/13<br>(23.1; 5.0-53.8) | 9/93<br>(9.7; 4.5-17.6)    |
| 41-50             | 4/49<br>(8.2; 2.3-19.6)                           | 0                           | 0/8                      | 4/57<br>(7.0; 1.9-17.0)    |
| >50               | 4/60<br>(6.6; 1.8-16.2)                           | 0                           | 0/9                      | 4/69<br>(5.8; 1.6-14.2)    |
| <b>Total</b>      | 37/364<br>(10.2; 7.3-13.7)                        | 19/101<br>(18.8; 11.7-27.8) | 6/59<br>(10.2; 3.8-20.8) | 62/524<br>(11.8; 9.2-14.9) |



**Fig. 1A:** Map of Cameroon showing the localisation of the three ethnic groups of pygmies. The number of subjects included in each group (N), their mean ages and sex ratio are indicated in the table. The Baka group is the largest (40000/45000 individuals) and its distribution overlaps the two administrative regions of the South and the East. The Bakola pygmies come next (4000/5000 individuals) and these are mostly located in the western part of the South region in the Ocean division. The Bedzan group is the smallest (700/1000 individuals) and is located in the northern part of the Central region



**Fig. 1B:** Phylogenetic neighbour-joining tree constructed using the sequences of a 423-bp fragment of the HBV-S gene from 22 HBV isolates (GenBank accession Numbers: HM355550 to HM355571) found in Baka, Bakola and Bedzan pygmies (highlighted in boldface and underlined) and 49 GenBank sequences of HBV A to -H genotypes and chimpanzee and Gorilla HBV genotypes (GenBank accession numbers and genotypes are indicated). Numbers next to the nodes of the tree represent bootstrap values (100 replicates). Only bootstrap values above 70 are presented.