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Silvia Meschi, Monica Sané Schepisi, Emanuele Nicastrì, Nazario Bevilacqua, Concetta Castilletti, Maria Rosaria Sciarrone, Maria Grazia Paglia, Robert Fumakule, Jape Mohamed, Andrew Kitua, et al.

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The prevalence of antibodies to human herpesvirus 8 and hepatitis B virus in patients in two Hospitals in Tanzania

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Complete List of Authors:	Meschi, Silvia; INMI L. Spallanzani, Laboratory of virology Sané Schepisi, Monica; INMI L. Spallanzani, Department of epidemiology and pre-clinical research Nicastri, Emanuele; INMI L. Spallanzani, Clinical Department Bevilacqua, Nazario; INMI L. Spallanzani, Clinical Department Castilletti, Concetta; INMI L. Spallanzani, Laboratory of virology Sciarrone, Maria; INMI L. Spallanzani, Laboratory of virology Paglia, Maria; INMI L. Spallanzani, Laboratory of microbiology and biorepository Fumakule, Robert; Tosamaganga Hospital, Laboratory Mohamed, Jape; Ivo De Carneri, Public Health Laboratory Kitua, Andrew; National Institute Medical Research Mangi, Sabina; Tosamaganga Hospital, Laboratory Molteni, Fabrizio; Italian Ministry of Foreign Affairs, Italian Cooperation Di Caro, Antonino; INMI L. Spallanzani, Laboratory of microbiology and biorepository Vairo, Francesco; INMI L. Spallanzani, Clinical Department Capobianchi, Maria; INMI L. Spallanzani, Laboratory of virology Ippolito, Giuseppe; INMI L. Spallanzani, Department of epidemiology and pre-clinical research
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2 1 TITLE PAGE
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4 2 The prevalence of antibodies to human herpesvirus 8 and hepatitis B virus in patients in two
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7 3 Hospitals in Tanzania
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9 4 Meschi S.¹, Sañé Schepisi M.², Nicastrì E.³, Bevilacqua N.³, Castilletti C.¹, Sciarrone M.R.¹, Paglia
10
11 5 M.G.⁴, Fumakule R.⁵, Mohamed J.⁶, Kitwa A.⁷, Mangi S.⁵, Molteni F.⁸, Di Caro A.⁴, Vairo F.³,
12
13
14 6 Capobianchi M.R.¹, Ippolito G.².

15
16 7 ¹ Laboratory of virology, “L. Spallanzani” National Institute for Infectious Diseases, Rome, Italy;

17
18 8 ² Department of epidemiology and pre-clinical research, “L. Spallanzani” National Institute for
19
20
21 9 Infectious Diseases, Rome, Italy;

22
23 10 ³ Clinical Department, “L. Spallanzani” National Institute for Infectious Diseases, Rome, Italy;

24
25 11 ⁴ Laboratory of microbiology and biorepository, “L. Spallanzani” National Institute for Infectious
26
27
28 12 Diseases, Rome, Italy;

29
30 13 ⁵ Tosamaganga Hospital, Iringa P.O. Box 11, Tanzania;

31
32 14 ⁶ “Ivo De Carneri” Public Health Laboratory, Pemba, Tanzania;

33
34 15 ⁷ National Institute for Medical Research, Dar es Salaam, Tanzania;

35
36 16 ⁸ Italian Cooperation, Italian Ministry of Foreign Affairs, Rome, Italy;

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42 18
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44
45 19 Institution where the work was performed:
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47 20 “L. Spallanzani” National Institute for Infectious Diseases, Rome, Italy.
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51
52 22 Corresponding Author

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54 23 Dr. Silvia Meschi, PhD

55
56 24 Laboratory of Virology

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59 25 “L. Spallanzani” National Institute for Infectious Diseases

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26 Via Portuense 292

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1 00149 Rome

2 Italy

3 Phone: +39 0655170668

4 Fax : +39 065594555

5 e-mail: silvia.meschi@inmi.it

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7 Shortened title

8 HHV-8 and HBV infections in Tanzania.

For Peer Review

1
2 1 ABSTRACT
3

4 2 The aim of this study was to determine the seroprevalence of human herpesvirus 8 (HHV-8) and the
5 3 immunization status for hepatitis B virus (HBV) infection in febrile patients in two districts of the
6 4 United Republic of Tanzania. Between February and March 2007, blood samples were collected in
7 5 Pemba Island and Tosamaganga from 336 outpatients and sent to the virology laboratory in Rome,
8 6 Italy for testing. HHV-8 DNA and HBV DNA were amplified by two in-house molecular methods,
9 7 anti-HHV-8 antibody titers were determined by an immunofluorescence assay (IFA), and anti-
10 8 HCV, HBsAg, anti-HBs, and anti-HBc were evaluated by microplate enzyme immunoassay
11 9 (MEIA). The seroprevalence of HHV-8 was 30.7% (96/313). In Pemba Island, the prevalence was
12 10 lower than in Tosamaganga (14.4% vs. 46.3%). A higher prevalence of low titers of HHV-8 IgG
13 11 (<1:80, 81%) was found among those under five years of age. HHV-8 DNA was detected in 6
14 12 seropositive patients (6.7%). The prevalence of HBsAg, anti-HBs, and anti-HBc was 4.3%, 37.6%
15 13 and 29.3% respectively. Out of 277 patients, 70 had had a previous infection (25.3%). One case of
16 14 occult hepatitis was found. The cover of hepatitis B vaccination was higher among children born
17 15 after 2002 (66.7%) than in patients born before 2002. HHV-8 infection is endemic in Tanzania and
18 16 the seroprevalence rate was higher in the mainland than on Pemba Island. The 3.9% percentage of
19 17 HBsAg in children younger than 4 years of age suggests that increased efforts are required in order
20 18 to achieve universal and compulsory immunization of children against HBV.
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1 KEY WORDS

2 Seroprevalence, immunization, PCR, hepatitis, antibody titer.

For Peer Review

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2 1 TEXT

3
4 2 Introduction

5
6 3 Human herpesvirus 8 (HHV-8) is the etiologic agent of Kaposi's sarcoma (KS), primary effusion B-
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9 4 cell lymphoma and multicentric Castleman's disease of the plasma-cell type [Cesarman et al., 1995;
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11 5 Chang et al., 1994; Soulier et al., 1995]. In industrialized countries, HHV-8 infection is acquired
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14 6 most frequently in late adolescence or in adult life, i.e., after the beginning of sexual activity. The
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16 7 sexual route of transmission is supported by the presence of HHV-8 DNA in semen and by
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18 8 increased risk of HHV-8 infection among homosexual men infected with the human
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21 9 immunodeficiency virus (HIV), as compared to others at risk of HIV infection [Pauk et al., 2000].
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23 10 Conversely, HHV-8 infection is relatively common in early childhood in developing countries,
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26 11 particularly in Africa where the seroprevalence of HHV-8 in children increases with age [Serraino
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28 12 et al., 2001]. The risk factors for acquiring HHV-8 infection are still uncertain, although saliva
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31 13 exchange is likely to play a major role [Andreoni et al., 2002; Mbulaiteye et al., 2003]. Although
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33 14 few studies have investigated the prevalence of anti-HHV-8 antibody titers in the population of
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35 15 countries where a high rate of infection occurs, one study shows that anti-HHV-8 antibody titers in
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37 16 blood donors from different regions of Italy mirror the incidence of Kaposi's sarcoma in these
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40 17 regions [Whitby et al., 1998]. Among black South African patients with cancer, the highest anti-
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42 18 HHV-8 antibody titers were observed in KS patients [Sitas et al., 1999]. Finally, high anti-HHV-8
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45 19 antibody titers in males aged 40 years or older parallel the increase in the incidence of endemic KS
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47 20 in older African men [Plancoulaine et al., 2002].

48
49 21 Hepatitis B is a serious global public health problem. In sub-Saharan Africa, where the
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51 22 seroprevalence of hepatitis B virus (HBV) is particularly high [Olubuyide et al., 1997], chronic
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54 23 HBV infection affects an estimated 50 million people with a prevalence of $\geq 8\%$ [Burnett et al.,
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56 24 2005]. The general population is at risk of acquiring the virus in areas where it is endemic and the
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59 25 major route of transmission is horizontal, with most infections established during childhood by
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61 26 inter-family contacts [Jacobs et al., 1997]. In East Africa, post puberty transmission also occurs, and

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2 1 although sexual transmission has been suggested, its role in the spread of the infection has not been
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4 2 clearly demonstrated [Bile et al., 1991; Hudson et al., 1988; ter Meulen et al., 1989; Van de Perre et
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6 3 al., 1987]. In this setting, the only logical approach for preventing transmission of hepatitis B virus
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8 4 in early childhood is mass immunization of newborns and infants [Burnett et al., 2005]. The World
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10 5 Health Organization (WHO) recommended that all countries with a prevalence of HBsAg of at least
11
12 6 8% should have the hepatitis B vaccine incorporated into their national Expanded Programme on
13
14 7 Immunization (EPI) by 1995. Tanzania initiated universal hepatitis B vaccinations in March 2002
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16 8 [Mphahlele et al., 2002], but few studies have examined whether this vaccination campaign was
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18 9 effective.
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23 10 In African populations, the overall prevalence of hepatitis C virus (HCV) infection has been
24
25 11 estimated to range from 3 to 5% [Madhava et al., 2002]. The prevalence of HCV infection in a
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27 12 population-based, cross-sectional study carried out in a rural population of Southwest Tanzania in
28
29 13 1996 was 2% [Puato et al., 2007].
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33 14 The seroprevalence of HHV-8, HBV and HCV and the immunization against HBV in patients with
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35 15 febrile illness admitted to two rural hospitals in Tanzania were investigated to determine the
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37 16 prevalence of HHV-8, HBV and HCV infection and the coverage of the immunization program
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39 17 against HBV.
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44 19 Methods

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47 20 Study areas. The study was conducted in two peripheral hospitals in Tanzania: in the Chake Chake
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49 21 Hospital (on Pemba Island) the study was carried out from February 9, 2007 to March 12, 2007
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51 22 jointly with the "Ivo De Carneri" Public Health Laboratory (PHL-IdC). In the Tosamaganga
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53 23 Missionary Hospital, (Iringa region) the study was conducted from March 3, 2007 to March 29,
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55 24 2007 jointly with CUAMM-Doctors with Africa (University College for Aspiring Missionary
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57 25 Doctors), an Italian non-governmental organization (NGO).
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2 1 Study design. A cross-sectional observational study was conducted in the outpatient departments of
3
4 2 the Chake Chake and Tosamaganga hospitals. Outpatients involved in the present survey were also
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6 3 included in a study which evaluated malaria infection in the same Tanzanian areas [Nicastri et al.,
7
8 4 2009]. Inclusion criteria were age of ≥ 1 year and a body temperature of $\geq 38^\circ$ for a period of less
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10 5 than 10 days. Exclusion criteria were the presence of signs of severe disease. Patients affected by
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12 6 mental illness or with a clinical picture of measles, chickenpox, otitis, infected wounds, chest X-ray
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14 7 documented pneumonia or with a previous antimalarial treatment were excluded.
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19 8 Ethical Committee. Ethical approval was obtained from the "L. Spallanzani" National Institute of
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21 9 Infectious Diseases in Italy, the National Institute for Medical Research in Tanzania and the
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23 10 Zanzibar Ministry of Health and Social Welfare.
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26 11 Protocol procedure. An easy-to-read, friendly questionnaire was provided for the collection of
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28 12 epidemiological and clinical data. A physical examination with a clinical note of any clinical signs
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30 13 or symptoms, including a final diagnosis, was requested from the local medical officer for each
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32 14 enrolled patient. The protocol procedures and the study collaborators did not change the current
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34 15 standard of care of each facility. After receiving complete information in Kiswahili about the
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36 16 collection of clinical samples for research purposes, all consenting adult patients and parents or
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38 17 legal guardians of minors signed an informed consent form.
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42 18 Laboratory Investigations. Two specimens of venous blood (5ml into a serum-separator tube, 5ml
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44 19 into a vial containing EDTA) and five drops of venous blood which were absorbed on filter paper
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46 20 collection cards (S&S 903TM, Schleicher and Schuell, Dassel, Germany) were collected from each
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48 21 enrolled patient. All blood samples were dispatched at the appropriate temperatures to "L.
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50 22 Spallanzani" National Institute of Infectious Diseases.
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53
54 23 Venous blood and serum samples were processed for detection of HHV-8 DNA and HBV DNA,
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56 24 respectively. 125 microliters of blood and 250 microliters of serum were used to extract DNA using
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58 25 BioRobot[®] MDx and QIAamp[®] DNA Blood BioRobot[®] MDx Kit (Qiagen, Hilden, Germany).
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60 26 For 18 patients no blood aliquot were available and nucleic acid extraction was performed from

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2 1 dried blood spots using DNA with QIAamp DNA Mini Kits (Qiagen, Hilden, Germany).
3
4 2 Amplification and quantification of human herpesvirus 8 were carried out with real-time PCR on an
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6 3 ABI Prism® 7700 instrument (Applied Biosystem, Foster City, CA) thermal cycler as previously
7
8 4 described [Lallemand et al., 2000]. All samples were analyzed in triplicate. A qualitative HBV
9
10 5 nested PCR was performed, as described previously [Lindh et al., 1995] in HbsAg positive samples,
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12 6 and in all samples positive for anti-HBc and negative for the other HBV markers. A PCR for β -
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14 7 globin was conducted in parallel on both blood and serum samples to assess suitability for
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16 8 amplification of the extracted DNA [Saiki et al., 1985]. The sizes of the products were estimated by
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18 9 electrophoresis on 2% agarose gel stained with ethidium bromide.
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23 10 To determine HHV-8 antibody titers, sera were tested at 1:40 dilution in a “in house”
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25 11 immunofluorescence assay (IFA) using latently infected body-cavity-based lymphoma 1 (BCBL-1)
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27 12 cell lines untreated and treated chemically with tetradecanoyl phorbol ester acetate (TPA). Punctate
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29 13 nuclear staining in an untreated BCBL-1 cell line was considered a positive result for latency-
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31 14 associated nuclear antigen (LANA), whereas the entire cell fluorescence in approximately 20% of
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33 15 TPA-treated cells was considered as positive for lytic-phase Kaposi’s sarcoma-associated
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35 16 herpesvirus (KSHV) antigens [Kedes et al., 1996].
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40 17 HBsAg, anti-HBs, anti-HBc and antibodies to HCV in sera were detected using a microparticle
41
42 18 enzyme immunoassay (MEIA) (Abbott Diagnostics Division, Weisbaden, Germany) according to
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44 19 the manufacturer’s instructions. Immunoblot Deciscan HCV Plus (Biorad, Marnes-la-Coquette,
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46 20 France) was carried out to confirm anti-HCV results. HBV markers were performed in all available
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48 21 samples. Antibodies to HCV were performed in a selected group of 101 patients from both sites.
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51 22 Data analysis. Data were entered, processed and analyzed using SPSS software package (SPSS 15.0
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53 23 for Windows, Chicago, IL). Frequencies were compared with the Yates corrected χ^2 test. The
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55 24 seroprevalence of HHV-8 was compared with the χ^2 test for trend.
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26 Results

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2 1 During the study period, 1,800 outpatients were seen to at the two sites: 960 patients (53.3%)
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4 2 were screened at the Tosamaganga Hospital and 840 patients (46.7%) were screened at the Chake
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6 3 Chake Hospital; 1,463 patients (81.3%) were excluded because they did not meet the study
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8 4 enrollment criteria; 337 patients (18.3%) were eligible and 336 patients (18.7%) were recruited
9
10 5 (figure 1). One hundred -seventy patients were adults (50.6%), represented equally in the two study
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12 6 sites. 92 out of 336 patients (27.4%) were children under five years of age and had a higher
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14 7 representation in the Chake Chake Hospital (60 patients, 65.2%). In both sites there were slightly
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16 8 more female patients than males. The symptoms reported most frequently were headache (46.7%),
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18 9 cough (41.4%), joint pain (35.4%) and vomiting (22.3%).
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21 10 Anti-lytic antibodies to HHV-8 were tested in 313 out of 336 (93.2%) recruited patients, while
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23 11 HHV-8 DNA was tested in the all 336 patients recruited. Overall, 96 of 313 (30.7%) patients had
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25 12 antibodies for HHV-8 lytic-phase antigens. No differences in the prevalence of HHV-8 seropositive
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27 13 individuals were found between men (39 of 131, 29.8%) and women (57 of 182, 31.3%). The
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29 14 seroprevalence of HHV-8 decreased from 28.4% (21 of 74) among children under age five to 22.5%
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31 15 (16 of 71) among subjects aged 5 to 15 but increased to 35.1% (59 of 168) in adults. The
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33 16 seroprevalence of HHV-8 (22 of 153, 14.4%) was significantly lower ($p<0.001$) among patients
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35 17 screened at the Chake Chake Hospital than those patients screened at the Tosamaganga Hospital (74
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37 18 of 160, 46.3%) (figure 2). In the Pemba Hospital the prevalence of anti-lytic HHV-8 antibodies
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39 19 among children under age five (8 of 45, 17.8%) was higher than that observed in subjects aged 5 to
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41 20 15 years (3 of 36, 8.3%) or in adults (11 of 72, 15.3%). In the Tosamaganga Hospital, the age-
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43 21 specific seroprevalence presented the same trend observed in the entire study population. A
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45 22 significant difference in the seroprevalence of HHV-8 between Pemba Hospital and Tosamaganga
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47 23 Hospital was noted in all age categories: under five ($p=0.024$), 5 to 15 ($p=0.009$) and adults
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49 24 ($p<0.001$). The percentage of patients with anti-lytic HHV-8 antibodies titer $\geq 1:640$ was higher in
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51 25 older patients than in those aged 5-15 and <5 years (χ^2 for trend: $p=0.01$) (figure 3). All samples
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53 26 were tested for HHV-8 DNA independently of the presence of anti-HHV-8 antibodies, considering
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1 all patients in the acute phase of disease. No HHV-8-seronegative patients were positive for HHV-8
2 DNA. Among 96 HHV-8 seropositive patients, 6 (6.3%) patients (all from the Tosamaganga
3 Hospital), were positive for HHV-8 DNA (Table I). Three patients had detectable but not
4 quantifiable HHV-8 viral load (<250 copies/ml) and the remaining three patients had a higher
5 HHV-8 viral load. The only child under the age of five who was positive for HHV-8 DNA (viral
6 load of 2,880 copies/ml) had a high anti-lytic HHV-8 antibody titer. All six patients had febrile
7 syndrome, with prevalent high respiratory tract involvement in 4 cases and gastrointestinal
8 involvement in the remaining two cases.

9 HBV markers were examined in 282 patients. The prevalence of HBsAg, anti-HBc and anti-HBs
10 was 4.3% (12 of 277), 29.3% (82 of 280) and 37.6% (106 of 282), respectively. Among the 12
11 patients with active HBV infection, eight (66.6%) had at least one gastrointestinal symptom. The
12 prevalence of HBsAg, anti-HBc and anti-HBs was lower in Pemba than in Tosamaganga: 1.7% (2
13 of 120) vs. 6.4% (10 of 157), 15.9% (20 of 123) vs. 39.5% (62 of 157) and 32.3% (40 of 124) vs.
14 41.8% (66 of 158 p<0.001) (figure 4).

15 Among the 13 patients with anti-HBc only, one person (7.7%) was positive for HBV DNA in sera,
16 whereas among the 12 patients with positive HBsAg, seven cases (58.3%) were positive for HBV
17 DNA.

18 Among the children born after 2002, the prevalence of persons with anti-HBs seroprotective titer
19 (>10 UI/ml) was higher than in patients born before 2002: 66.7% (34 of 51) vs. 44% (10 of 226),
20 respectively (p<0.001) (figure 5).

21 No patients had active co-infection with HHV-8 and HBV. Conversely, in 33 patients, 94% in
22 Tosamaganga, had antibodies to HHV-8 and to hepatitis B core antigen: seven (21.2%) were under
23 five years of age, two (6.1%) were aged 5 to 15 and 24 (72.7%) were adults. The seroprevalence of
24 HHV-8 among anti-HBc positive subjects (33 of 82, 40.2%) was slightly higher than among
25 patients without previous HBV infection (58 of 198, 29.3%) (p=0.1).

26 Antibodies to HCV were undetectable in all analyzed patients.

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4 2 Discussion

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7 3 This study on the differential diagnosis of febrile syndromes, conducted in two hospitals in
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9 4 Tanzania in 2007, found a 30.7%, 4.3% and 0% seroprevalence rate of HHV-8, HBsAg and HCV
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11 5 with no significant differences in age and gender. Among 96 HHV-8 seropositive persons, six
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13 6 patients (6.3%) from Tosamaganga Hospital with respiratory tract infections (four patients) and
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15 7 gastrointestinal symptoms (two cases) were positive for HHV-8 DNA. HHV-8 DNA was not
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17 8 detected among patients without antibodies to HHV-8.

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21 9 The overall prevalence rate of HHV-8 found in this study is consistent with previous data from
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23 10 different African countries. A review of 28 seroepidemiological studies of HHV-8 in adult
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25 11 populations from 16 African countries reported that between 1996 and 2002, most African countries
26
27 12 (namely Botswana, Cameroon, Democratic Republic of Congo, Egypt, Gambia, Ghana, Ivory
28
29 13 Coast, Nigeria, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe) had high seroprevalence
30
31 14 rates of HHV-8 ranging from 26% to 86%, with the exception of Central African Republic, Eritrea
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33 15 and Senegal which had relatively lower seroprevalence rates (up to 25%) [Dedicoat et al., 2003]. A
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35 16 39.8% prevalence rate was reported in 5 to 10 year-old children in northern Cameroon [Rezza et al.,
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37 17 2000], 36% in Ghana [Nuvor et al., 2001] and approximately 50% in Zambia [Olsen et al., 1985]
38
39 18 and Uganda [Mayama et al., 1998]. Among families in rural Tanzania, the seroprevalence of HHV-
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41 19 8 increased from 3.7% in infants to 58.1% in 3 and 4-year-old children [Mbulaiteye et al., 2003].
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43 20 No difference in the seroprevalence of HHV-8 was described between men and women in the
44
45 21 different countries. Similar HHV-8 prevalence rates was reported in children (44%) and adults
46
47 22 (66%) from North Mara District in Tanzania during the period of 1972 through 1975, the period
48
49 23 prior to the onset of the AIDS-related epidemic of Kaposi's sarcoma in East Africa [de-Thé et al.,
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51 24 1999].

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54 25 These seroepidemiological studies suggest that HHV-8 is transmitted mainly among family
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56 26 members and close contacts by a horizontal, non-sexual route. Sexual transmission may be an

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2 1 important route of transmission in endemic population in Africa [Eltom et al., 2002; Volpi et al.,
3
4 2 2004].

5
6 3 A higher seroprevalence of HHV-8 was found in Tosamaganga (46.3%) than in the Pemba Island
7
8 4 (14.4%). The low HHV-8 circulation among the general population of Pemba may be related either
9
10 5 to the intrinsic geographical isolation, Muslim-based religious behavior with extensive social and
11
12 6 ethical control and the low HIV prevalence rate of Pemba Island in the adult population compared
13
14 7 to the Tanzanian mainland (0.3% vs. 5.8%) [National Bureau of Statistics (NBS) Tanzania, 2008].
15
16 8 Recently, in a cross-sectional study conducted in Ghana, the seroprevalence of HHV-8 was
17
18 9 significantly lower in HIV-seronegative healthy blood donors than in asymptomatic persons
19
20 10 infected with HIV (23.7% and 65.6% respectively, $p < 0.005$) [Adjei et al., 2008]. Consistent with
21
22 11 the previous report [Plancoulaine et al., 2002], in this study, an age-related increasing anti-HHV-8
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24 12 antibody titer was found: this seroprevalence trend parallels the increase of the incidence of
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26 13 endemic KS in elderly African men [Taylor et al., 1972].

27
28 14 In this study, the 4.3% prevalence rate of HBsAg was found in the general population receiving
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30 15 hospital care for febrile syndromes is consistent with the 4.2% prevalence rate reported recently in
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32 16 the Moshi urban area in Tanzania among women of childbearing age [Msuya et al., 2006]. Similar
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34 17 seroprevalence rates of HBsAg were found in Tosamaganga (6.4%) and in Pemba Island (1.4%). As
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36 18 mentioned previously for HHV-8, the low prevalence of HBV infection in Pemba Island may be
37
38 19 related to geographical restriction and to social behavior.

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40 20 In 2002, HBV vaccination was included in the Tanzanian Expanded Programme on Immunization
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42 21 [Mphahlele et al., 2002]. Anti-HBs titer (>10 UI/ml) among children born before or after 2002
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44 22 increased significantly from 4.4% to 66.7% with no substantial differences between the two
45
46 23 surveyed areas or between gender. Despite this considerable result obtained in only 5 years, further
47
48 24 public health efforts are needed to protect Tanzanian children (approximately 1/3) and prevent
49
50 25 susceptible children who are likely to acquire HBV in early infancy from becoming chronic carriers
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52 26 and potential transmitters of the virus. In this setting, the only logical approach to preventing the

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2 1 transmission of HBV in early childhood is to implement mass immunization of newborns and
3
4 2 infants [Burnett et al., 2005].
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6
7 3 Consistent with previous data on the prevalence of occult chronic hepatitis B in patients from sub-
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9 4 Saharan African countries [Gibney et al., 2008; Hübschen et al., 2009], HBV DNA was detected in
10
11 5 sera in 7.7% of patients with only anti-HBc. The definitive diagnosis of occult chronic hepatitis B
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13
14 6 depends on the detection of HBV DNA in the liver, so the nature of the tested specimen for the
15
16 7 detection of HBV DNA (the serum alone in this study) will underestimate the prevalence of occult
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18 8 chronic hepatitis B by up to 40% [Mariscal et al., 2004]. A possible explanation for this diagnostic
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20
21 9 pattern includes a false positive serological test result, the “window phase” of acute HBV infection
22
23 10 or resolved HBV infection [Knoll et al., 2006]. HBV DNA was also detected in 58.3% of patients
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25
26 11 with HBsAg. These apparently surprising data can be explained by different factors: 1. the
27
28 12 occurrence of false positive results in the HBsAg immunoassay, as reported in African countries
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30 13 [Pirillo et al., 2007]; 2. the occurrence of a false negative PCR, most likely due to the presence of
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32
33 14 inhibitors in the extracted samples or if the sampling, shipping or storing were not performed
34
35 15 accurately. Nevertheless, in the present study, β -globin PCR was used as a positive control to
36
37 16 monitor inhibition, and the extracted amount of DNA from clinical samples was suitable for
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40 17 amplification.
41
42 18 Differently from other epidemiologic studies [Madhava et al., 2002; Puato et al., 2007] which
43
44 19 reported a higher prevalence of HCV among elderly subjects, no positive samples were found in
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46
47 20 this study. It could be related to the lower age of enrolled patients (median, 20 years; IQR 3-32),
48
49 21 and to the low exposure of this rural population to well-known risk factors for hepatitis C.
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51
52 22 Among the 33 patients with previous exposure to HHV-8 and HBV, a higher seroprevalence was
53
54 23 detected in adult patients, suggesting that sexual transmission was an additional factor.
55
56 24 There are a number of limitation to this study. First, this is a cross-sectional study, and neither
57
58
59 25 longitudinal follow-up nor the clinical outcome of febrile patients were included in the study
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1
2 1 protocol. Secondly, a limited number of patients were enrolled at two sites and the conclusions
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4 2 cannot be extended to the entire population.

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6 3 Finally, the age-related increasing seroprevalence of HHV-8 suggests the need for a cancer
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8 4 surveillance system in sub-Saharan African countries for prompt early detection of endemic
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10 5 Kaposi's sarcoma.
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13 14 15 16 7 Acknowledgments

17
18 8 This work has been supported by grants from the Italian Cooperation, Italian Ministry of Foreign
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20 9 Affairs (Project: AID 8282).
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2 1 Figures legend

3
4 2 Figure 1. Number of patients recruited to the study by HHV-8 and HBV test results.

5
6 3 Figure 2. Seroprevalence of HHV-8 in different age groups.

7
8 4 Figure 3. Anti-lytic HHV-8 antibody titers in different age groups.

9
10 5 Among under five year olds, the percentage of subjects with anti-lytic HHV-8 antibody titer <1:80 was higher than for
11 6 subjects aged >15 yrs (17/21 vs. 15/59; $p < 0.001$). Conversely the percentage of subjects with intermediate and low titer
12 7 was lower (3/21 vs. 26/59, $p = 0.003$ and 1/21 vs. 18/59, $p = 0.026$ respectively). All frequencies were compared by the
13 8 Yates corrected χ^2 test.

14
15 9 Figure 4. Prevalence of HBV markers.

16
17 10 Figure 5. Immunization against HBV in patients born before and after 2002.

18
19 11 The graph above does not show the one case of occult chronic hepatitis B among patients born before 2002 (1 of 266,
20 12 0.4%).
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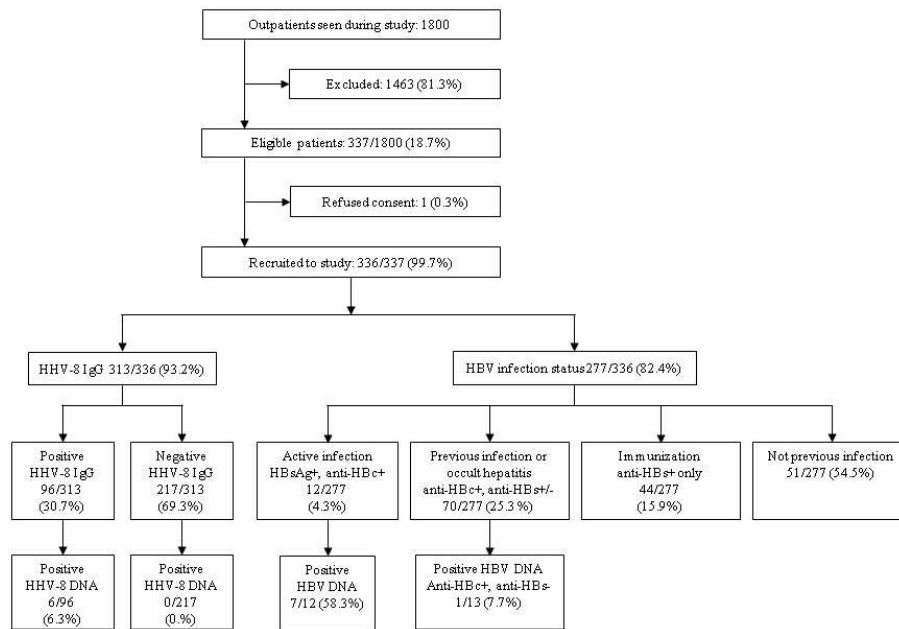


Figure 1. Number of patients recruited to the study by HHV-8 and HBV test results. 254x190mm (96 x 96 DPI)

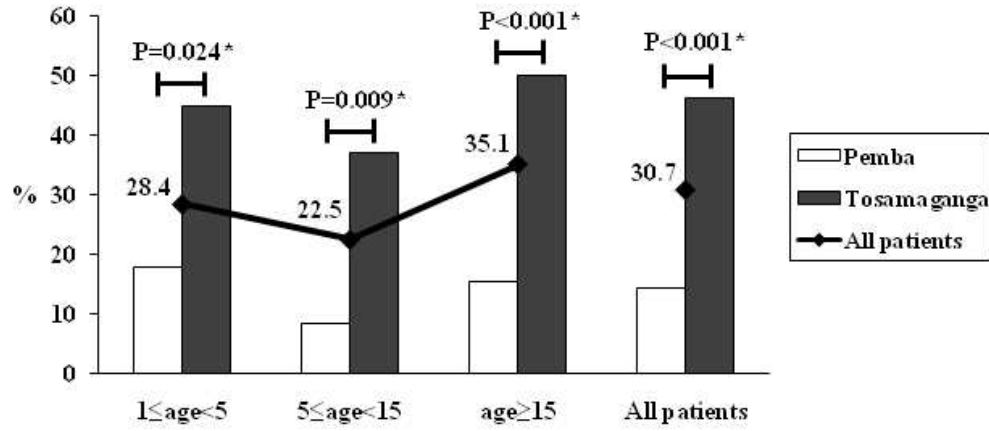


Figure 2. Seroprevalence of HHV-8 in different age groups.
164x75mm (96 x 96 DPI)

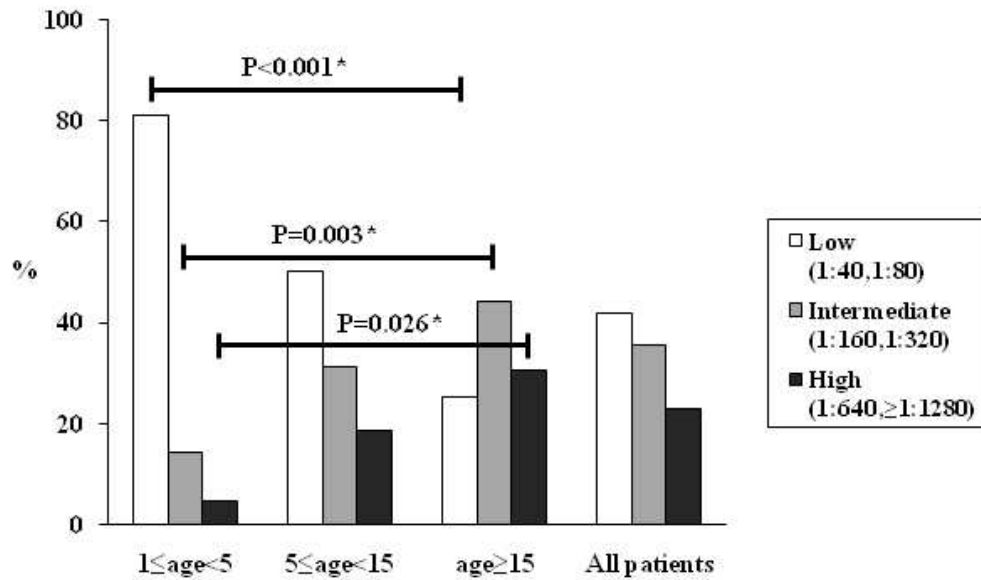


Figure 3. Anti-lytic HHV-8 antibody titers in different age groups.

Among under five year olds, the percentage of subjects with anti-lytic HHV-8 antibody titer <1:80 was higher than for subjects aged >15 yrs (17/21 vs. 15/59; $p < 0.001$). Conversely the percentage of subjects with intermediate and low titer was lower (3/21 vs. 26/59, $p = 0.003$ and 1/21 vs. 18/59, $p = 0.026$ respectively). All frequencies were compared by the Yates corrected χ^2 test.

157x94mm (96 x 96 DPI)

1 Table I. Patients with HHV-8 reactivation or with HBV infection.

2 § All patients had fever $\geq 38^{\circ}$ C.

Code	Age (years)	Sex	Clinical symptoms [§]	HHV-8 DNA (copies/ml)	HHV-8 antibodies titers	HBV infection status
T056	23	F	Cough, joint pain	40,800	1:320	Previous infection
T113	30	F	Difficulty eating/ drinking, headache, chest pain, joint pain	15,440	1:320	Previous infection
T126	1	M	Difficulty eating/drinking, cough, crepitation+++	2,880	$\geq 1:1280$	Previous infection
T158	28	M	Difficulty eating/drinking, vomiting, joint pain	positive, not quantifiable	1:640	Previous infection
T074	19	F	Headache, cough, joint pain	positive, not quantifiable	1:160	Previous infection
T131	44	F	Headache, vomiting, joint pain	positive, not quantifiable	1:80	Not previous infection
T133	46	M	Headache, diarrhoea, joint pain	negative	1:640	Active infection, HBV DNA+
T130	17	M	Headache, joint pain	negative	1:320	Active infection, HBV DNA+
T146	2.2	F	Cough, vomiting, abdominal pain	negative	1:40	Active infection, HBV DNA+
P073	13	M	Chills, headache, vomiting	negative	<1:40	Active infection, HBV DNA+
T101	6	F	Difficulty eating/drinking, headache, vomiting	negative	<1:40	Active infection, HBV DNA+
T105	50	F	Headache, joint pain	negative	<1:40	Active infection, HBV DNA+
T143	21	F	Chest pain, vomiting, abdominal pain, joint pain	negative	<1:40	Active infection, HBV DNA+
T136	9	M	Headache, vomiting	negative	<1:40	Occult hepatitis, HBV DNA+
T093	1.7	F	Cough, vomiting	negative	1:40	Active infection
P085	40	M	Difficulty eating/drinking, chills, fits or convulsions, vomiting, abdominal pain	negative	<1:40	Active infection
T031	33	F	Headache, joint pain	negative	<1:40	Active infection
T147	53	F	Headache, cough	negative	<1:40	Active infection
T156	25	M	Chest pain, abdominal pain, joint pain	negative	<1:40	Active infection

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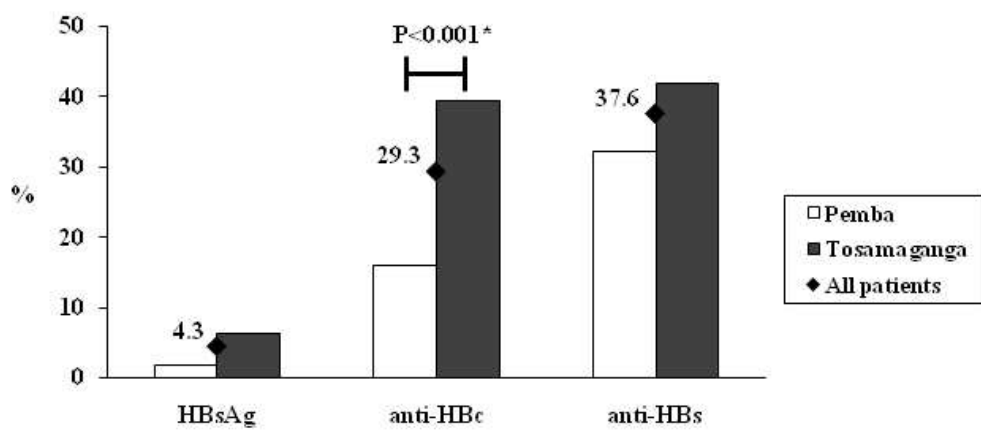


Figure 4. Prevalence of HBV markers.
167x74mm (96 x 96 DPI)

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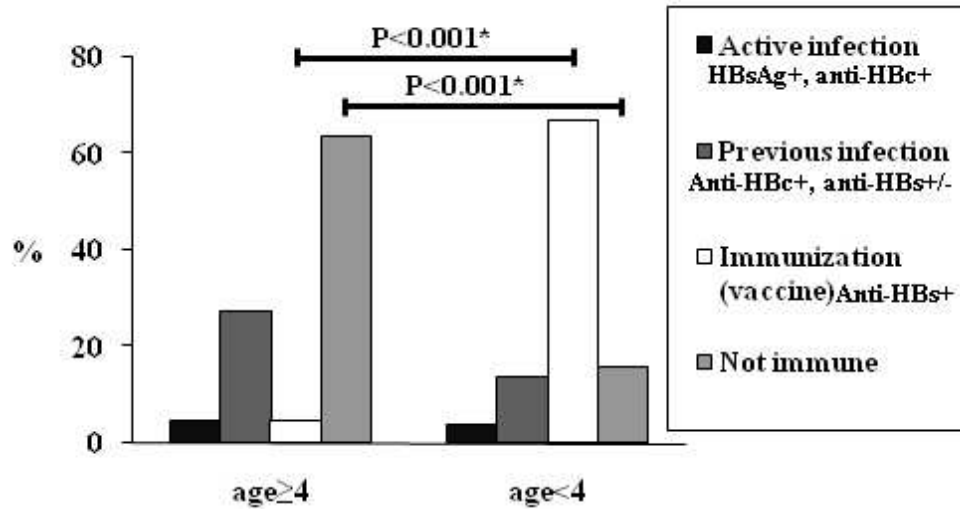


Figure 5. Immunization against HBV in patients born before and after 2002. The graph above does not show the one case of occult chronic hepatitis B among patients born before 2002 (1 of 266, 0.4%).
132x71mm (96 x 96 DPI)