showed a prevalence of 14.8 per cent (12/81) in all GT1 infected patients, and were only detected in GT1b, being mainly represented by C316N with 38.5 per cent (10/26) of GT1b infected patients. The combined NS5A RASs (Q30H + Y93H), causing high level resistance to all NS5A inhibitors, were detected at baseline in one HIV/HCV GT1a co-infected patient who later failed a treatment with SOF + LDV for 12 weeks. Finally, an isolated Y93H mutation was also detected at baseline in a GT1b mono0-infected patient experiencing recurrence. Overall 38.3 per cent (31/81) of all GT1 HCV infected patients presented NS5 RASs at baseline, in which 58.1 per cent (18/31) were co-infected with HIV/HCV whereas only 38.7 per cent (12/31) of HCV monoinfected patients showed baseline RASs. Moreover, 27.3 per cent (15/55) of GT1a infected patients presented NS5 RASs at baseline, whereas patients infected with GT1b showed the highest prevalence of natural RASs, namely 61.5 per cent (16/26). These data support the usefulness of resistance testing prior to treatment initiation, thus preventing relapses associated to the presence of baseline RASs, as a statistical significant association was found between treatment failure and the presence of major NS5 RASs, namely Y93C/H ($P = 0.04$). However, this reduced sampling can constitute a limiting factor since it may underestimate the statistical analysis, and lead to relatively higher RASs rates when comparing to other previous studies.

A42 Genetic variability and phylogeography of hepatitis B virus genotype D in Brazil

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Hepatitis B virus (HBV) has been classified into ten genotypes (A–J), some of which are divided into subgenotypes. Genotype D (HBV/D) has a worldwide distribution, and ten subgenotypes (D1–D10) have been described so far. Brazil has received different migratory flows over time. The evolutionary history of HBV/D in Brazil is not well understood and few HBV/D complete genome sequences are available. The aim of this study was (1) to examine the distribution of HBV/D subgenotypes in Brazil, (2) to determine the full-length genomic sequences of HBV/D isolates from different regions, and (3) to investigate the origin and spread of HBV/D subgenotypes in the country. All Brazilian HBV/D sequences with known subgenotype ($n = 215$) were retrieved from GenBank. HBV/D3 was the most prevalent (56 per cent) subgenotype, followed by HBV/D4 (25 per cent), HBV/D2 (17 per cent), and HBV/D1 (2 per cent). Although HBV/D was circulating countrywide, most (57 per cent) isolates were from the South region, which was the only region where all four subgenotypes were found. In addition, forty-five new full-length sequences (one D1, eleven D2, thirty-two D3 and one D4) were determined. To investigate the origin and spread of HBV/D in Brazil, we compiled different datasets of complete genomes for HBV/D1–D4, using Brazilian and worldwide sequences. Phylogeographic analysis, performed using BEAST v.1.8.2, indicated that the most probable origins of HBV/D1 and HBV/D2 were Syria and Eastern Europe, respectively, with times of the most recent common ancestor (tMRCA) in the early nineteenth century for HBV/D1 and the second half of the twentieth for HBV/D2, corroborating historical data on migrations to Brazil. Martinique was found to be the origin of Brazilian HBV/D4, probably reflecting the population of African slaves brought to the Americas. However, the methodology used was not able to determine from where and when HBV/D3 was introduced in Brazil, possibly due to different introduction routes.

A43 Molecular epidemiology of hepatitis B virus in South Kivu, an eastern province of the Democratic Republic of Congo

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Hepatitis B virus (HBV) is characterized by a wide genomic variability that could play a role in different clinical manifestations and response to therapy. The ten HBV genotypes show a distinctive geographical distribution worldwide and genotypes A, D, and E are the most frequently found in Africa. There are only limited studies on HBV genotype distribution in Democratic Republic of Congo (DRC), all performed in the western part and showing a vast majority of genotype E. We performed a study to determine the genotype distribution of HBV in South Kivu (DRC). Blood screening was performed during 2014–2015 at the Hospital Provincial General de Reference of Bukavu where serum samples of newly detected Ag HBs positive subjects were collected. These samples were sent and analysed at the Cliniques Universitaires Saint Luc, Belgium. We undertook HBV DNA load measurement by Abbott RealTime HBV assay on the m2000 system, genome sequencing using an in-house method targeting the S and P overlapping region, phylogenetic analysis using Geneious 4.0 software, and additional mutational analysis focused on the identification of mutations (P region) associated with antiviral resistance using the online HBVseq tool (Stanford University). Genotype determination was performed in forty-one patients. HBV genotype A was detected in 40/41 (97.6 per cent) and HBV genotype E in 1/41 (2.4 per cent). Only two mutations were observed and concerned the I169T nucleotide substitution, both in genotype A samples. The phylogenetic analysis showed that nearly all South Kivu genotypes A (39/40) are closely related to A1 subgenotype strains found in Rwanda, Haiti, and Martinique while only one single strain attached to the A2 subgenotype cluster was isolated. The only remaining genotype E case was linked to the western African E crescent. HBV genotype A seems to be the most predominant genotype in eastern DRC with the majority belonging to the Afro-Asian subgenotype (A1). This contrasts with the western part of RDC where genotype E is the most frequently found genotype. These results support the hypothesis of an East–West genotypic demarcation. Moreover, the low genetic variability of HBV in South-Kivu is suggestive of strong local endemicity.

A44 Complete HPV genomes from cervical samples using next-generation sequencing in Luxembourg

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While next-generation sequencing using rolling circle amplification (NGS-RCA) of human papillomavirus (HPV) has been conducted in HIV-HPV co-infected women, we performed a pilot

study for detecting and assembling complete HPV genomes in cervical samples from a cohort of young women attending cervical screening with access to HPV vaccination in Luxembourg. DNA extracts of eighty-one cervical swabs from women (mean age 23 years) positive for HPV by AnyplexIIHPV28® (Seegene) were enriched by rolling circle amplification and sequenced on Illumina Miseq. Reads were mapped to 182 PaVE reference sequences of known HPV types using BBMap and assembled using VELVET. Complete HPV genomes obtained were aligned with genomes published in Genbank using MEGA6. Overall, an average of 1 per cent of reads mapped to HPV. Among the eighty-one positive samples, NGS-RCA detected 186 different HPV types spanning thirty-six of the fifty-one known mucosal types. HPV types 42, 53, 51, 56, 90, and 31 were most frequently detected in twenty-two, fifteen, ten, ten, nine, and seven samples, respectively. Detection of HPV types by NGS-RCA was highly correlated with viral load of Anyplex. About sixty-seven consensus sequences of complete HPV genomes were assembled including two novel lineages of HPV66 and HPV90 and two novel sublineage of HPV67 and HPV73, respectively. NGS-RCA is a powerful method for obtaining complete HPV genomes from cervical samples with a high viral load ($Ct < 30$). After eight years of the vaccination programme in Luxembourg, vaccine-related types 6, 11, 16, and 18 were infrequently detected in the targeted age group.

A45 Merkel-cell polyomavirus and human polyomavirus 6 in Argentina, Uruguay, and Spain: Deep characterization of the South American types

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New human polyomaviruses have been discovered in the last years, including the Merkel-cell (MCPyV) and the human polyomavirus 6 (HPyV6). Although their infection is usually asymptomatic, in immunocompromised hosts they can cause life-threatening pathologies. In particular, MCPyV has been associated with Merkel cell carcinoma, an aggressive skin cancer arising in the elderly and in chronically immunosuppressed individuals. Despite being prevalent viruses, epidemiological data from South America are scarce, as too are the viral types circulating and their origin. The aims of this work were to characterize MCPyV and HPyV6 from environmental samples with different geographical origins and to analyze the phylogenetic and phylogeographic profiles to study their spatio-temporal dispersion patterns, particularly for MCPyV. Partial and complete genome sequences were obtained from sewage samples from Argentina, Uruguay, and Spain. Phylogenetic analysis showed that MCPyV sequences distributed according to their geographic origin in Europe/North America, Africa, Asia, South America, and Oceania groups, suggesting that viral diversification might have followed human migrations across the globe. In fact, the analysis of the viruses reported here emphasized this behavior, given that they reflected the origin of the current population in each country. The South American group presented a high level of clustering, showing subgroups exclusively formed by sequences from southern South America, possibly associated with local diversification events related to early migratory movements in the region. Regarding HPyV6, sequences from South America grouped with high support and were separated from all other sequences available, from USA, France, Australia, and China. The analysis of viruses from the environment allowed us to characterize prevalent infections in different geographic regions, revealing that viruses circulating in each population reflected its origin and that there are specific lineages associated with South America.

A46 Partial genetic characterization of a Brazilian strain of yellow fever virus from an epizootic outbreak in 2009

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Yellow fever is the prototype virus of the *Flavivirus* genus in the Flaviviridae family. Its genome consists of a single-stranded positive-sense RNA molecule of ~10 kb presenting a single open reading frame. It is translated into a polyprotein which is processed by viral and host proteases into three structural (C, prM, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B e NS5). As an arbovirus it is maintained in nature through a cycle in which the virus alternates between vertebrate (human/non-human primates) and invertebrate hosts (*Haemagogus* sp., *Sabethes* sp., and *Aedes* sp.). The species involved in the sylvatic and urban transmission cycles differ and since 1942 no cases related to urban transmission have been reported in Brazil. During a series of epizootics caused by Yellow fever virus in Brazil between 2007 and 2009, a monkey (*Alouatta* sp.) was found dead (May 2009) in a sylvatic area in the State of Paraná (southern Brazil). Brain samples from this animal were used for virus isolation. RNA was extracted from the cell culture supernatant and used to amplify an 861-bp segment, comprising the coding sequence of the C and prM proteins (nucleotides 119–979, according to GenBank accession number U17066). Sequence analyses demonstrated that it is closely related with two isolates from Venezuela (99.5 per cent identity), one isolated in 2005 from a human case and the other isolated in 2007 from *Alouatta seniculus*. Phylogenetic analysis groups them all within South American genotype I. This sequence was also compared to other sequences available in GenBank that presented only partial coverage but 100 per cent nucleotide identity (nucleotides 641 to 981, according to GenBank accession number U17066) and were not included in the phylogenetic analysis. This data indicates that this new Brazilian isolate may be part of the same epizootic that affected monkeys in the state of São Paulo (2008) and the Missiones province (2008 and 2009) and stresses the importance of yellow fever surveillance through sentinel monkeys.

A47 Whole-genome analysis of rotaviruses isolated from humans and domestic animals in Uganda, 2012–2014 reveals possible anthroponosis and multiple rotavirus reasortment events between species

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