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Challenges in the diagnosis and prevention of viral infections

Abstract: Diagnostic assays for detection and monitoring of virus infections have become more and more important and are widely used in routine diagnostics. In particular, the detection of newly emerging infectious diseases is challenging. In addition, travel-associated diseases caused by dengue viruses, chikungunya viruses, influenza viruses, or other viruses draw the attention of physicians. Therapeutic treatment regimens and prevention strategies can also influence the need for diagnostic assays, such as, in the case of the human immunodeficiency virus (HIV) and human papillomavirus (HPV). Moreover, well-known virus infections, such as hepatitis E virus infections, can gain new clinical relevance.

Keywords: hepatitis E virus; human immunodeficiency virus (HIV); human papillomavirus (HPV); influenza virus; travel-associated virus infection.

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The pandemic threat posed by influenza viruses

In the past 100 years there have been four pandemics (1918, 1957, 1968, and 2009) that were caused by influenza viruses. A pandemic is defined as the global spread of an infectious disease. The current World Health Organization (WHO) definition of a pandemic takes into account the occurrence of a new, modified virus variant and human-to-human transmissibility, but not the severity of the disease (Table 1). New influenza virus variants that caused pandemics originated from an avian (bird) influenza virus (1918), from a reassortment of a human and avian influenza virus (1957, 1968), or from a reassortment of two pig influenza viruses (2009). A reassortment is the recombination of the eight gene segments of two different influenza viruses. The newly built virus can differ significantly from the two ancestral viruses in terms of pathogenicity, transmissibility, and infectivity for animals and humans. Influenza A viruses are subtyped based on their hemagglutinin (H) and neuraminidase (N). We distinguish 16 hemagglutinins and 9 neuraminidases. However, this subtyping is only an approximate characterization of the respective viruses, which means that very different viruses may have the same A/HxNx designation (e.g., A/H1N1: pathogen of the Spanish flu, swine influenza viruses, the pandemic 2009 virus, and the seasonal influenza virus prior to 2009) [1].

Animal and human influenza viruses use N-acetylneuraminic acid (NANA), bound to galactose (Gal) on the cell surface, as a receptor [2]. Avian influenza viruses bind to N-acetylneuraminic acid, which is present in an α-2,3 linkage with galactose, whereas human influenza viruses have a preference for N-acetylneuraminic acid in an α-2,6 linkage. Even though both types of NANA linkages occur in birds and humans, their distribution in the respiratory tract is different. In humans, NANA-α-2,6-Gal is found in the upper respiratory tract, and NANA-α-2,3-Gal in the lower respiratory tract, whereas NANA-α-2,3-Gal is found in birds only in the upper respiratory tract. Owing to this fact, avian influenza viruses are easily transmitted from bird to bird, but generally less well from bird to human, because replication cannot occur in the upper respiratory tract of humans. In contrast, pigs have no polarity for either receptor in the respiratory tract, thus pigs may be infected by both avian and human influenza viruses. The viral binding to the cellular receptor is realized by the surface protein hemagglutinin, which is also responsible for the fusion between the cellular and viral membranes.

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Table 1 In the case of influenza viruses, six pandemic phases are distinguished[a].

Phase 1: For the transmission of animal influenza viruses to humans, there is only a very low risk.
Phase 2: An animal influenza virus has a certain potential to adapt to humans.
Phase 3: Occurrence of sporadic cases or small clusters of human infections with a new virus variant that cannot be attributed to human-to-human transmission.
Phase 4: Confirmed cases of human-to-human transmission.
Phase 5: Spread in more than one country within one WHO region.
Phase 6: Spread in at least two WHO regions is also called a pandemic.

[a]After the last influenza pandemic in 2009, we are currently in a post-pandemic phase (phase 1 or phase 2). Phases 3–5 reflect an increasing risk for an impending pandemic.

A/H5N1 influenza viruses were first identified in 1996 in geese and are often called "bird flu viruses" [3]. It is important to consider that there is a large number of different avian influenza A viruses, which possess very different properties and may infect humans only in individual cases (e.g., A/H7N7). Avian influenza viruses are divided into highly pathogenic (e.g., A/H5N1) and low pathogenic viruses, which refer to the pathogenicity in animals (highly pathogenic=100% mortality within 48 h) and not the pathogenicity in humans. The first human A/H5N1 infections were observed in 1997, and since the reappearance/increased incidence of A/H5N1 in birds (starting in 2003), human infections have been documented again and again. Overall, however, the number of these documented and mostly severe cases is still below 1000 (Figure 1).

Within this group of infected people, approximately 50% of the patients died regardless of the medical intervention, which is why a very high level of mortality has been assumed for A/H5N1 infections in humans. The tropism of A/H5N1 towards the deep respiratory tissue in humans might be responsible for the high pathogenicity observed [2]. The human infections diagnosed and included in the official statistics developed mostly after very intense contact with infected animals or people.

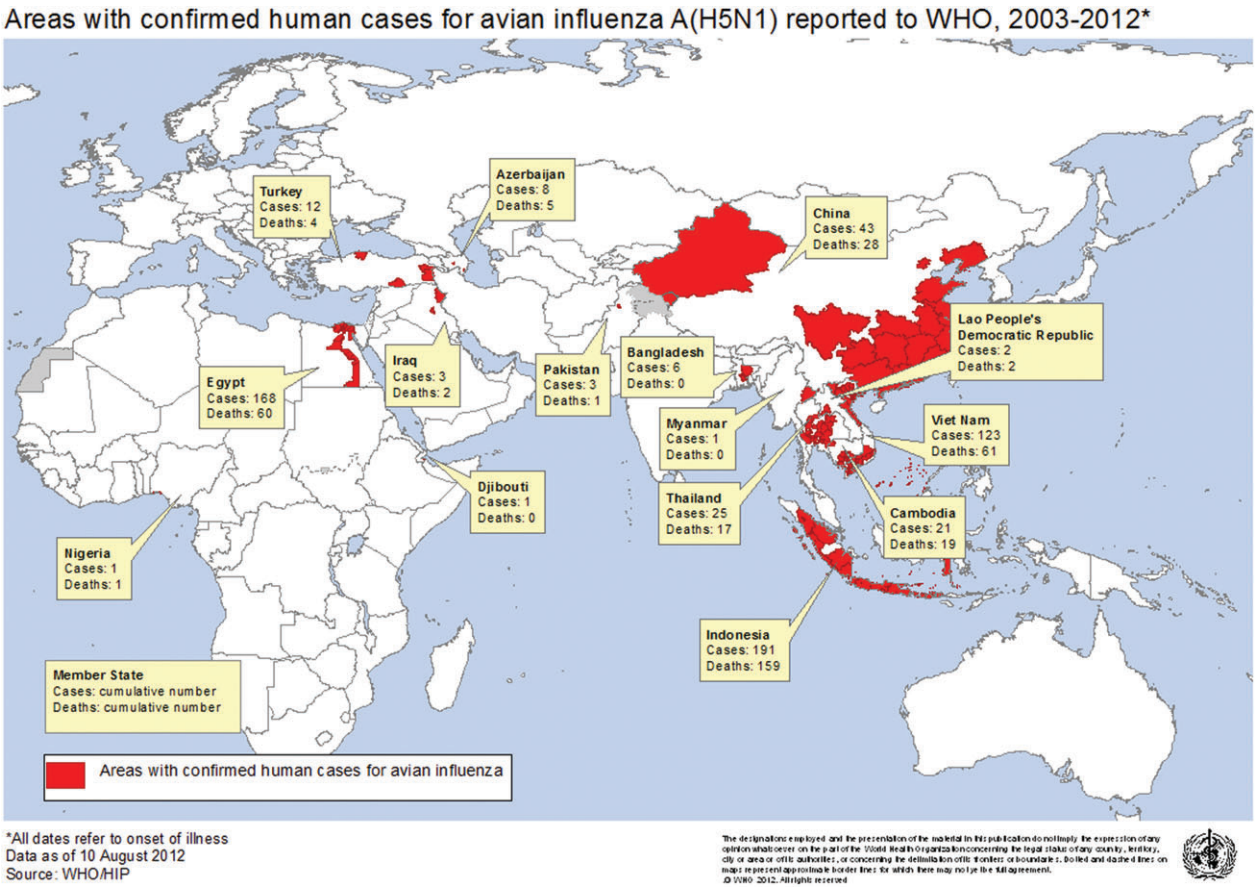

Figure 1 Confirmed human A/H5N1 influenza virus infections.
Source: WHO homepage: http://gamapserver.who.int/mapLibrary/Files/Maps/01_AvianInfluenza_ GlobalMap_2003_10Aug12.png.

However, a study of the seroprevalence of specific A/H5N1 antibodies in workers on poultry farms in China pointed to a significant underreporting of infections in people who do not experience any clinical symptoms [4]. The serum of 306 workers on poultry farms in the province of Jiansu, China, was tested by means of a hemagglutination inhibition assay for the presence of specific A/H5N1 antibodies. In total, specific H5 antibodies were detected in eight samples (2.61%). The probability of being seropositive correlated with the quantity of birds managed. There had been no severe respiratory disease in the medical histories of any of those people.

Much attention was also focused on two publications in 2012, which experimentally succeeded to modify A/H5N1 in such a way that airborne transmission between ferrets became possible [5, 6]. The starting point for the experiments of the first study was not a naturally occurring influenza A/H5N1 virus, but a genetically engineered influenza A/H5N1 virus [5]. Into the influenza virus genome of the A/H5N1 isolate, obtained from a patient in Indonesia, mutations were introduced into the hemagglutinin gene (Q222L, G224S) and the PB2 gene (E627K), which are known to influence receptor binding and replication capacity in mammalian cells. Ferrets were nasally inoculated with this altered virus, and then the virus was passaged from ferret to ferret several times. In the process, homogenized material was taken from the nasal concha of the previously infected animal, or material obtained via nasal rinsing/swabbing/respiratory secretions, to infect the next animal nasally. After the tenth passage in ferrets, it was possible to demonstrate airborne transmissibility of the virus (now adapted even further; additional mutations) between ferrets. The infected ferrets did not show any evidence of the emergence of a highly pathogenic A/H5N1 variant for ferrets.

In the experimental approach of the other group, a specific influenza virus variant was engineered in the laboratory, consisting of seven gene segments of the influenza virus A/H1N1 (2009) and the gene segment of the hemagglutinin of A/H5N1 [6]. Random mutations were introduced into the hemagglutinin gene, serving as the starting point for further analyses. As few as four mutations (N158D, N224K, Q226L, T318I) in the hemagglutinin led to the respiratory transmissibility of these altered influenza viruses between ferrets. However, these changes did not result in a more pathogenic course of the disease or a specific mortality.

Influenza pandemics have occurred previously, and they will also occur in the future. Various factors are needed to facilitate the pandemic spread of a new virus. A well-established surveillance system and in-depth knowledge of the functional significance of the detected changes can help in revealing the risk of an impending pandemic and allowing for appropriate measures to be taken in time, such as quarantine and development of a vaccine. The 2009 pandemic showed that it could take up to 6 months before an effective and adapted influenza virus vaccine was available. Whether and to what extent antiviral drugs might be effective in the next influenza pandemic cannot be currently predicted with certainty.

New pandemic threats

In recent months, two different viruses became the center of attention regarding their potential of triggering a new pandemic. One is a new coronavirus MERS-CoV (Middle East respiratory syndrome coronavirus) and a hitherto-unknown influenza virus A/H7N9.

MERS-CoV was first diagnosed in a patient in June 2012 [7]. The 60-year-old patient without any other underlying disease was admitted to hospital in Saudi Arabia with respiratory symptoms and died 11 days later due to progressive renal and respiratory failure. Subsequently, MERS-CoV infections were diagnosed in Europe again and again, but most of those cases had become infected in Middle East countries (Figure 2) [8]. According to a recent report by the WHO, dated 20 September, 2013, there have been 130 laboratory-confirmed MERS infections since September 2012, which led to 58 deaths. The highest diagnostic sensitivity was achieved in samples taken from the lower respiratory tract. The differential diagnosis of MERS infection should also be taken into account in Germany in patients with acute respiratory syndrome and a positive travel history (the Middle East) over the past 14 days. This is particularly true now that already the fifth case of a human MERS-CoV infection was confirmed in a patient who had been transferred from Qatar to Germany for further treatment due to respiratory symptoms [9, 10].

The first human infections with the new influenza virus variant A/N7N9 were observed in Shanghai in March 2013 [11, 12]. The most recent WHO report, dated 12 August, 2013, reveals 135 confirmed infections and 44 deaths, which mainly occurred in March/April 2013 (Figure 3) [13]. The clinical symptoms ranged from mild respiratory symptoms to severe pneumonia [14]. In most cases, close contact with poultry was identified as a risk factor for infection [15]. Unlike A/H5N1 infections in poultry, A/H7N9 infections in animals are often asymptomatic and can thus serve as an important reservoir for human infections.

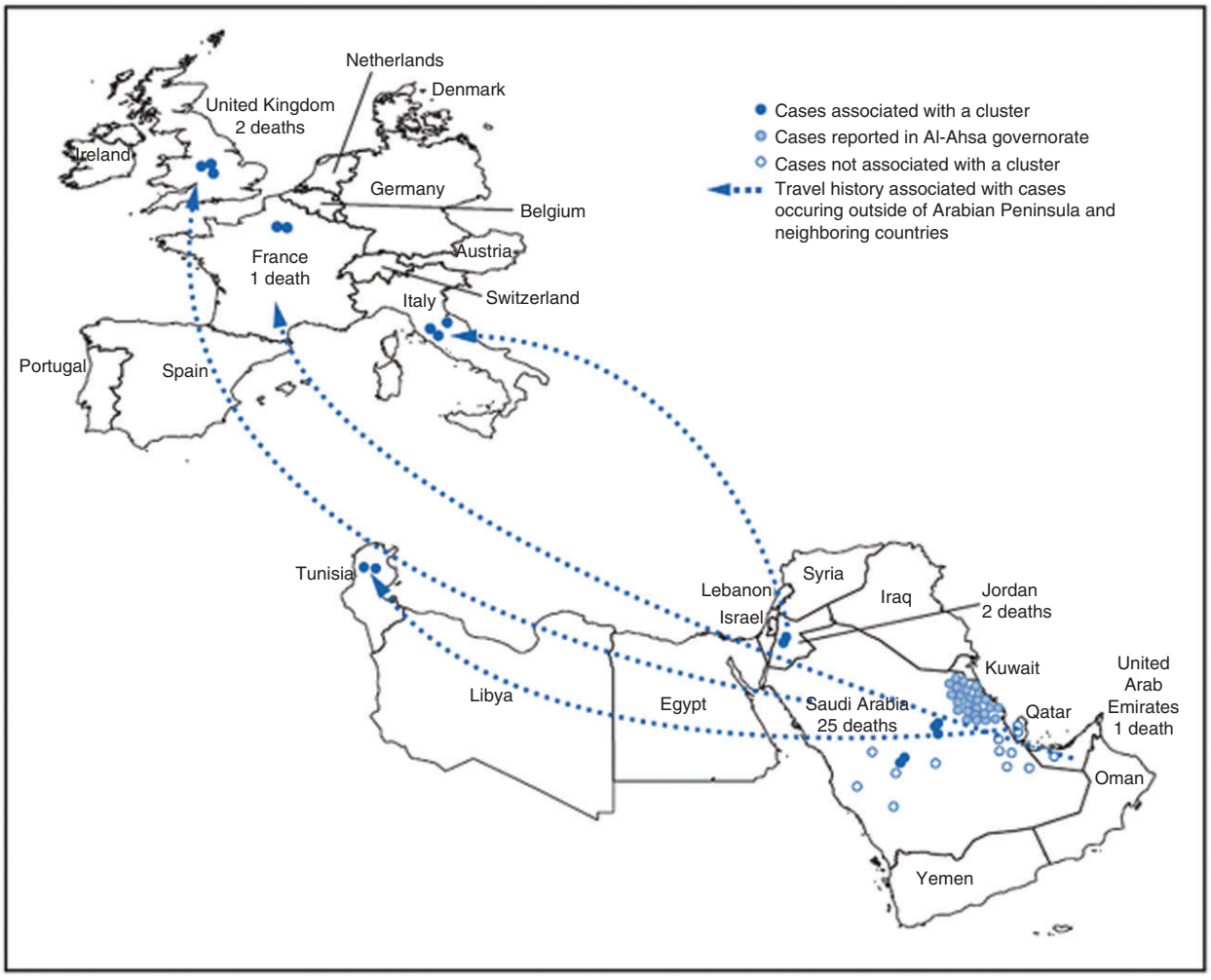

Figure 2 Human infections with MERS-CoV (Middle East respiratory syndrome coronavirus).
Source: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6223a6.htm?s_cid=mm6223a6_w.

Seasonal challenge in the diagnosis of influenza

The influenza diagnosis is an integral part of routine virological diagnostics and is required especially in the winter months to an ever greater extent. In particular, in patients with risk factors (e.g., immunosuppression, pre-existing respiratory conditions) for complications, a specific antiviral therapy with oseltamivir (Tamiflu®, p.o.) or zanamivir (Relenza®, p.i./i.v.) can be initiated. For inpatients, a quick and specific influenza diagnosis also allows for the forming of a cohort, thus minimizing the risk of nosocomial infections [1].

The traditional growing of influenza viruses in cell culture is hardly used in routine testing today. Approximately 1–2 weeks following infection, specific IgG antibodies can be detected serologically. Owing to the cross-reactivity of the antibodies previously formed (previous contact with other influenza viruses), only the increase of titers between two samples obtained at intervals of 2–4 weeks can point to an acute infection. The IgA influenza antibodies indicate a recent infection and are independent of previous influenza virus infections, as they usually disappear approximately 4 weeks after infection. However, IgA antibodies cannot be detected in every patient with an influenza virus infection [16].

The direct detection of influenza viruses from respiratory material is thus the gold standard for influenza diagnostics. In addition to bronchoalveolar lavage, the double-sided, pooled nasal swab also exhibits good diagnostic sensitivity to detect an influenza virus infection. In routine practice, both molecular biological methods and rapid antigen tests are used, each one with its own

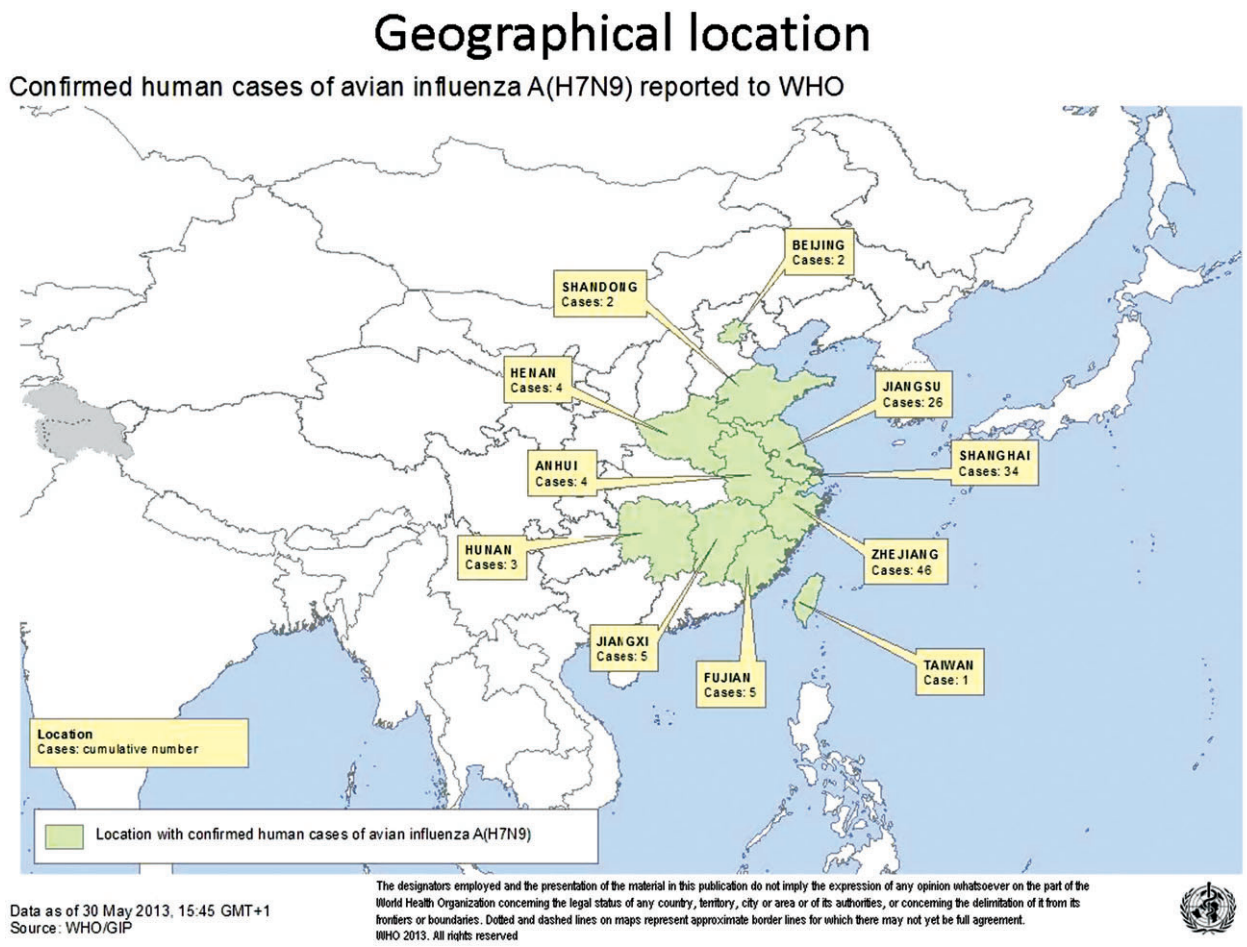

Figure 3 Human infections with influenza virus A/H7N9.
Source: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/06_ReportWebH7N9Number.pdf.

set of pros and cons. In various studies, real-time polymerase chain reaction (PCR) has proved to be the most sensitive method in influenza diagnostics. However, the greater methodological effort, the higher costs and a longer hands-on time can also be a disadvantage compared with the rapid antigen test. Estimates of the diagnostic sensitivity of rapid tests vary greatly and range between 20% and 90%. However, the choice of patients, which were studied can significantly affect the observed positive and negative predictive values. Even improved antigen tests [optimized for the detection of A/H1N1 (2009)] yield a sensitivity of only 79.9% on the third day of illness when compared with the PCR test, the "gold standard", and that sensitivity declines again to 67.3% on the fifth day of illness. The rapid test can therefore be used as a bedside test that provides a first result within minutes, but compared with PCR, cannot rule out an influenza infection due to the lower diagnostic sensitivity [1, 16].

Travel-associated viral diseases

There are a variety of travel-associated virus diseases that can often lead to clinical symptoms only after returning home due to their specific incubation periods. A particularly common leading symptom of a virus infection is a fever. Additional symptoms related to dengue virus and chikungunya virus infections, which are diagnosed more and more frequently in travelers, affect the joints [17–21].

Dengue viruses

Dengue viruses belong to the Flaviviridae family and are classified into four serotypes. Dengue viruses are transmitted by mosquitoes in tropical and subtropical countries, most commonly by the species *Aedes aegypti* and *Aedes albopictus* (Figure 4). After a short incubation period, symptomatic progression can be accompanied by

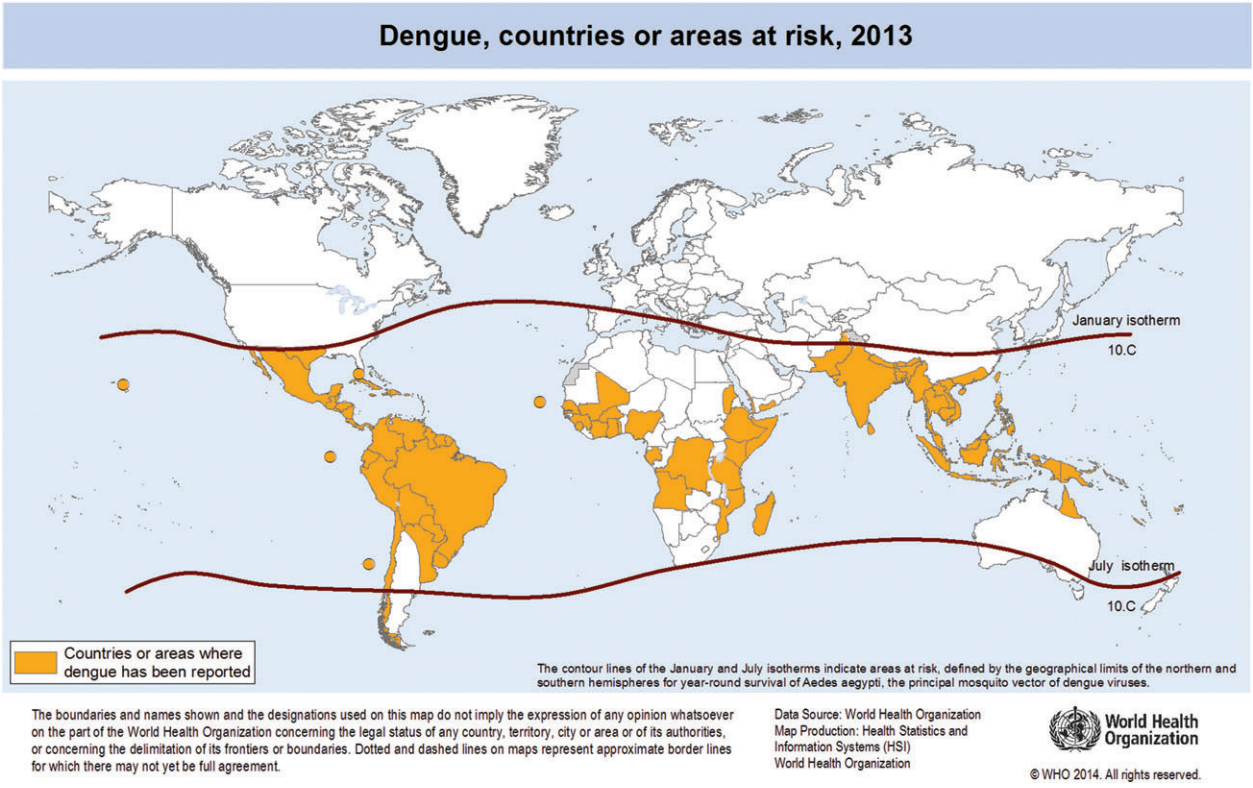

Figure 4 Areas with a high risk of dengue virus transmission.
Source: WHO homepage, international travel and health (http://www.who.int/ith/en/).

sudden fever, followed by headaches, myalgia, arthralgia, conjunctivitis, and erythema (face and trunk with white dermatographism). Severe cases often exhibit a biphasic fever; in this second phase of the illness (duration =3–7 days), one also observes elevated liver enzymes, lymphopenia, thrombocytopenia, and an increase in the hematocrit. The occurrence of a capillary leak syndrome can lead to life-threatening complications. This critical phase can occur 4–7 days after the onset of symptoms and may be accompanied by persistent vomiting, severe abdominal pain, and an enlarged liver. Particularly at risk are young children and people with a secondary dengue virus infection. After having undergone a dengue virus infection, antibodies are formed that provide lifelong immunity against the specific serotype, but cross-protective immunity against other dengue virus serotypes lasts only for a short period of time. The presence of antibodies that bind, but do not neutralize, results, in the case of a dengue virus infection with another serotype, in a significantly more efficient uptake in the viral target cells (monocytes) [17, 18, 20].

The diagnosis can be made in the early stage of infection by means of PCR or NS-1 antigen detection and subsequently by detection of specific IgG and IgM antibodies. The NS-1 antigen is a nonstructural protein that is secreted and is not part of the virion [17–19].

In 2012, 600 dengue virus infections were reported in Germany, which was about the same number of cases as in 2010 (n=590) (2011: n=288) [22]. In France (Nice) and Croatia, there were also cases of autochthonous dengue virus infections that could not be explained by a person's travel history [20]. To what extent dengue virus infections will also occur in southern Europe cannot be predicted with certainty at the moment, but the next few years will tell. Interestingly, in the summer of 2011, adult female *A. albopictus* species were caught for the first time in Germany, in the upper Rhine Valley [23].

Chikungunya viruses

Chikungunya viruses are part of the Togaviridae family. The name is derived from a Tanzania dialect and means "that which bends up". They are also transmitted by mosquitoes (*A. aegypti* and *A. albopictus*) in Africa and Eurasia (Figure 5). In the past decade there have been several large chikungunya virus outbreaks on island groups in the Indian Ocean and India. As part of these outbreaks, the

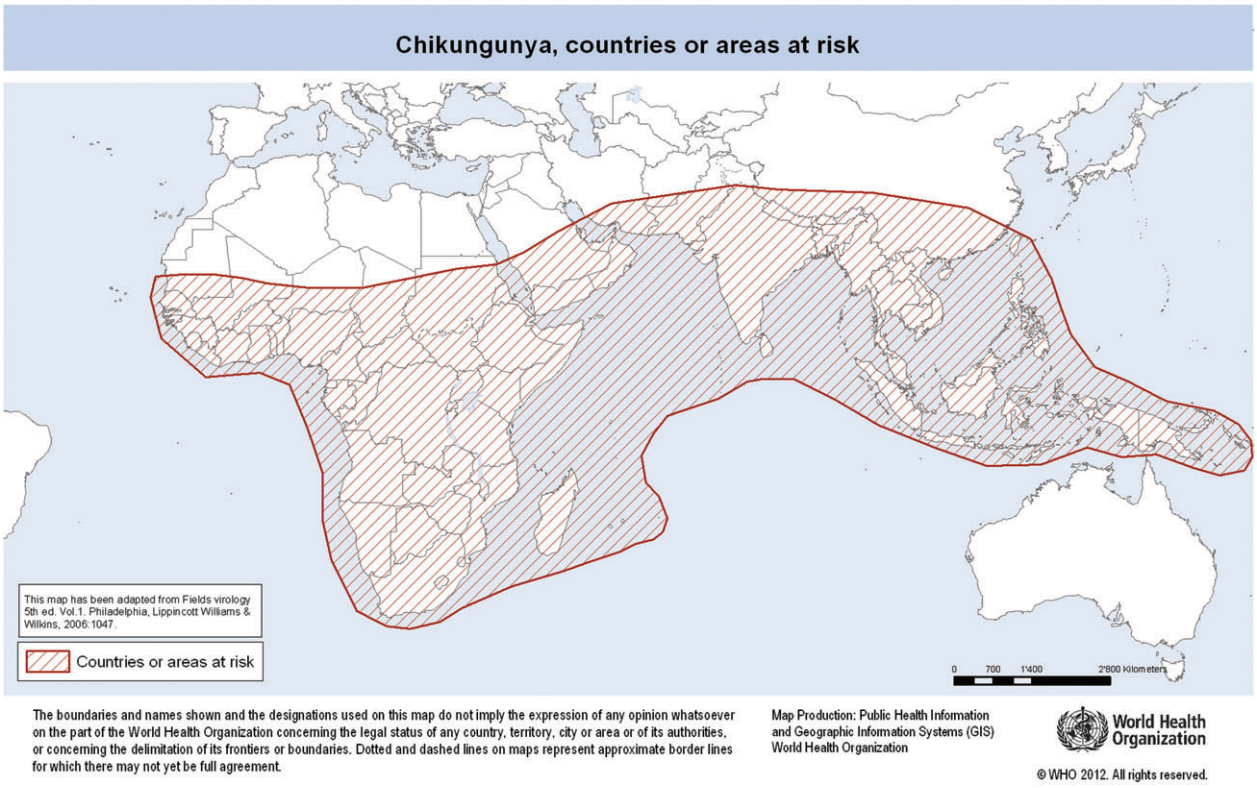

Figure 5 Areas with a high risk of chikungunya virus transmission.
Source: WHO homepage, international travel and health (http://www.who.int/ith/en/).

tropism/preference of the viruses changed in terms of the transmitting mosquito species. A mutation in the envelope protein E1 of the chikungunya virus (A226V) causes a cholesterol-independent virus replication that is associated with improved replication in *A. albopictus*. It also improves transmission by this vector [21]. This mutation was independently selected in populations of different regions of the world, where both vector species occurred simultaneously.

The incubation period of the chikungunya virus infection is 2–7 days. Symptomatic infections are often recognized with fever, headache, conjunctivitis, myalgia, and arthralgia. Arthralgia occurs especially on both sides in the hip region and leads to swollen and tender joints. In 5%–10% of patients, arthralgia may persist for months or even years. Within the first days of illness, chikungunya viruses can be detected in the blood by PCR, whereas specific IgM and IgG antibodies can be detected in the second week of illness [17, 20].

It is assumed that there are 17–53 imported chikungunya cases a year in Germany [23, 24], although probably not all infections are diagnosed, given the mild nature of the illness. A chikungunya outbreak occurred in Italy in 2007, leading to 205 documented infections [25]. The detected chikungunya viruses were phylogenetically linked to viruses in a previous outbreak in the Indian Ocean. In addition, two autochthonous chikungunya virus infections were observed in France, after a girl nearby had returned home from Asia with a diagnosed chikungunya infection [20].

New strategies for preventing HIV-1 infections

According to the current estimate of the prevalence and incidence of HIV-1 infections (end of 2012) by the Robert Koch Institute (RKI), there are 78,000 people with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) in Germany [26]. The estimated number of individuals in whom a HIV-1 infection has not yet been diagnosed amounts to 14,000 people (approx. 18% of HIV-1-positive individuals). It is estimated that there were 3400 new infections in Germany in 2012 [26]. Particularly during an acute HIV-1 infection, the HIV-1 viremia is high, thus patients can transmit HIV-1 to other people very easily. Thus, the HIV-1 PCR is positive a few days prior to the

serological assays. HIV-1-specific antibodies can usually be found 3–6 weeks after infection. The use of serological assays detecting antigen (HIV-1 protein: p24) and antibodies can bring forward the diagnosis time of the acute HIV-1 infection by a few days. Because most assays do not distinguish between the detection of antigen or antibodies, the immunoblot, as a confirmation test, can still be negative, whereas the specific HIV-1 PCR is not. Owing to the particularly high infectivity of patients with acute HIV-1 infection, even clusters of transmitted HIV-1 variants carrying drug resistance mutations have been observed. Overall, the frequency of transmitted drug resistance in treatment-naive patients is 10% in Germany [27].

The sexual transmissibility of HIV-1 is particularly high in anal intercourse, so that MSM (men who have sex with men) pose a special risk group for the transmission of a HIV-1 infection [28]. Meanwhile, however, as many as approximately 20% of new HIV-1 infections in Germany are being transmitted heterosexually. In addition, there is a clear correlation between the probability of transmission of HIV-1 and the presence of other sexually transmitted diseases, such as syphilis and genital herpes. In Germany, too, rising numbers of syphilis proceeded to an increase of new HIV-1 infections after 2000. One study has shown that early treatment of the HIV-1-positive partner significantly reduces the transmission risk of HIV in discordant couples (different HIV status) [29]. HIV-1 was transmitted in only one case where the HIV-1-infected partner had started an antiretroviral treatment independently of the specific immune status. These findings underscore the new concept of treatment as prevention starting antiretroviral treatment independent of the immune status and concomitant factors to decrease the risk of transmitting HIV-1.

Another approach for the prevention of HIV-1 transmission, which has been studied by several groups, is based on pre-exposure prophylaxis (PrEP) [30–33]. Thus, as several studies have demonstrated, the prescription of tenofovir (TDF) and/or Truvada [tenofovir (TDF) + emtricitabine (FTC)] for HIV-1-negative individuals at high risk of acquiring HIV-1 infection has led to a significant risk reduction (44% [33], 67% [31], 62% [32]). Low drug levels were identified in all studies as the main cause for the failure of PrEP and were even associated with the complete lack of protection in the study by Van Damme et al. [30]. Furthermore, the Baeten et al. study found resistant variants at the time of diagnosis, which had probably been selected due to the irregular intake of prophylactic drugs [31].

Thus, and not surprisingly, the success of this approach for the prevention of HIV-1 depends very much on the compliance of the patient. In cases of inadequate compliance, however, this approach could actually lead to the selection of drug-resistant variants. Another question that remains is whether the established diagnostic assays can retain their high sensitivity and specificity in this situation for patients who, due to their irregular intake of pills, went on to become HIV-1-infected. Finally, concurrent sexually transmitted diseases can significantly increase the likelihood of transmission of HIV-1 and could possibly even jeopardize the protective effect of PrEP.

Prevention and diagnosis of HPV diseases

Human papillomavirus (HPV) is a non-enveloped DNA virus and infects epithelial cells of the skin and mucous membranes [34]. A HPV infection results in the dysregulation of the cells own proliferation machinery and, consequently, in increased cell divisions of the infected cell. As such, HPV causes various malignant and benign tumors. There are over a hundred different HPV types, some of which are associated with specific human diseases. A distinction is made between low-risk types (6, 11, 42, 43, 44), such as the triggers of benign genital warts, and high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) associated with the emergence of malignant tumors. HPV is commonly detected by means of PCR or hybridization on a swab or extracted material. There is a wide range of diagnostic assays available to detect a specific HPV type or different groups of HPV types (e.g., high or low risk). The strong cross-reactivity of the different HPV antibodies currently prevents the use of serological assays in routine diagnostics. Cervical cancer is most commonly caused by HPV16 (60%) and HPV18 (10%). A similar picture emerges for anal cancer (HPV16: 75% and HPV18: 3%). The frequency of HPV-associated oropharyngeal cancer has increased significantly in recent decades, and is now up to 72% of all head and neck tumors. In contrast, genital warts are not a life-threatening disease, but are diagnosed at least once in the live of up to 10% of all sexually active men and women. There is no specific antiviral therapy to treat a HPV infection. Warts and HPV infections of the cervix with only mild dysplasia often heal completely within 6 months to 2 years. At the CIN2+ stage (moderate to severe changes of the cervix), a cervical biopsy (conization or excision from the cervix) may become necessary. Depending on the extent and localization, genital warts must also be removed surgically. Attempts of local treatment with cytostatic drugs (5-fluorouracil) or immune modulators (interferons, imiquimod) may sometimes

improve the clinical situation. Given how very limited the treatment options are, prevention of HPV infections is of particular importance. Two vaccines are currently available (Gardasil®, Merck, HPV types: 6, 11, 16, 18 and Cervarix®, GlaxoSmithKline, HPV types: 16, 18), which differ in their composition. Gardasil® not only protects against HPV types 16 and 18, but also against HPV types 6 and 11 (quadrivalent vaccine). In Cervarix®, AS04 is used as an adjuvant to enhance the body's immune response. The basic immunization consists of three vaccinations 0–2–6 and/or 0–1–6 months apart.

Australia launched its national HPV vaccination program in April 2007. In the first phase, girls aged 12/13 years were vaccinated with the quadrivalent HPV vaccine Gardasil® [35]. This program achieved a vaccination rate of 73% in the target group. In a recent study samples of women aged 18–24 years who underwent a gynecological consultation were examined for the prevalence of HPV types 6, 11, 16, and 18. The prevalence of HPV types differed between samples obtained before and after the start of the vaccination program. The incidence of the HPV types 6, 11, 16, and 18 had decreased significantly (28.7% vs. 6.7%), both in the vaccinated and in non-vaccinated subjects, respectively, after the start of the vaccination program. Also, the frequency of oncogenic HPV types that are not included in the HPV vaccination decreased slightly from 38.6% to 30.8%. Previously, it had already been shown that heterosexual boys in the age groups in which the girls were vaccinated with the quadrivalent vaccine were protected significantly from genital warts [36].

In America and Canada, the HPV vaccination with the quadrivalent HPV vaccine has been recommended for young males and young adults since the end of 2011 [37]. In examining a similar recommendation for Australia, the current data have been compiled and evaluated. In men, too, the HPV infection is mostly transient. However, the HPV infection does not reach a peak prior to the age of 25 years, but is similarly high across all age groups and only increases with the number of sexual partners. Studies have already shown that the quadrivalent vaccine efficiently protects boys and girls from genital warts. HPV-associated tumors in men affect the penis, the anus, and the oral cavity, but are generally much less common than cervical cancer. An exception is anal cancer in HIV-1-positive MSM. By far the largest proportion of HPV-associated cancers in men is caused by HPV types 16 and 18. Although a direct effect of the HPV vaccination on the incidence of HPV-associated tumors in men has not been shown so far, it is tempting to speculate so, because the number of HPV infections in young male adults was significantly reduced by the vaccination. Overall, the HPV vaccination for young males and young adults in Australia was classified as useful. This was explained by the direct protection of the vaccinated against HPV infections. The indirect effects of the HPV vaccination of boys, which may lead to a further decrease of HPV infections in women, due to herd immunity, would not have been sufficient on its own for this recommendation. In Germany, the Standing Committee on Vaccinations at the RKI (STIKO) continues to recommend the HPV vaccination for girls aged 12–17 years before the first sexual intercourse.

In addition, the screening guideline for cervical cancer in America was revised in 2012 (Figure 6) [38]. According to this, no screening of women under 21 years is recommended, because the prevalence of cervical cancer is very low in this age group, and conspicuous findings in the past have often led to further unnecessary diagnostic tests and treatments in this group of women. For women aged 21–29 years, cytological screening every 3 years is sufficient, because up to 80% of sexually active women in this age group suffer a HPV infection that heals on its own within 2 years in 90% of cases. After the age of 30 years, a cytological screening can be done every 3 years, or a combined screening (cytological and HPV-PCR) every 5 years. A meta-analysis of studies comparing HPV-PCR tests and cytological tests has shown a higher sensitivity of HPV-PCR in diagnosing a CIN3 lesion particularly in women over the age of 30 years [39]. In addition, a negative HPV-PCR had a highly predictive value for an equally inconspicuous screening at the next routinely scheduled examination. No further screening is recommended for women aged 65 years and older who have undergone adequate screening for cervical cancer and who do not have an elevated risk. A prospective study in Sweden [40] has shown that women diagnosed with cervical cancer during HPV screening between 1999 and 2001 exhibited a better cure rate than women who had not been diagnosed during the screening program. This significant difference remained even after the data were adjusted for the stage of the cervical cancer. In Germany, HPV-PCR is primarily performed to clarify conspicuous cytological tests, but there is a discussion underway to restructure screening tests [S2 guidelines: prevention, diagnosis and treatment of HPV infections and preinvasive lesions of the female genitals (Guidelines Register No. 015/027)].

In addition to HPV-associated cervical cancer, other HPV-associated cancers have grown in significance in recent years (penile carcinoma, anal carcinoma, carcinoma in the oropharyngeal area). Both approved HPV vaccines are highly effective in the prevention of infections caused by HPV16 and HPV18. The HPV vaccination of boys is medically useful and can help preventing

Population	Women ages 21 to 65	Women ages 30 to 65	Women younger than age 21	Women older than age 65 who have had adequate prior screening and are not high risk	Women after hysterectomy with removal of the cervix and with no history of high-grade precancer or cervical cancer	Women younger than age 30
Recommendation	Screen with cytology (Pap smear) every 3 years. Grade: A	Screen with cytology every 3 years or co-testing (cytology/HPV testing) every 5 years. Grade: A	Do not screen. Grade: D	Do not screen. Grade: D	Do not screen. Grade: D	Do not screen with HPV testing (alone or with cytology). Grade: D
Risk Assessment	Human papillomavirus (HPV) infection is associated with nearly all cases of cervical cancer. Other factors that put a woman at increased risk of cervical cancer include HIV infection, a compromised immune system, in utero exposure to diethylstilbestrol, and previous treatment of a high-grade precancerous lesion or cervical cancer.					
Screening Tests	Screening women ages 21 to 65 years every 3 years with cytology provides a reasonable balance between benefits and harms. Screening with cytology more often than every 3 years confers little additional benefit, with large increases in harms. HPV testing combined with cytology (co-testing) every 5 years in women ages 30 to 65 years offers a comparable balance of benefits and harms, and is therefore a reasonable alternative for women in this age group who would prefer to extend the screening interval.					
Timing of Screening	Screening earlier than age 21 years, regardless of sexual history, leads to more harms than benefits. Clinicians and patients should base the decision to end screening on whether the patient meets the criteria for adequate prior testing and appropriate follow-up, per established guidelines.					
Interventions	Screening aims to identify high-grade precancerous cervical lesions to prevent development of cervical cancer and early-stage asymptomatic invasive cervical cancer. High-grade lesions may be treated with ablative and excisional therapies, including cryotherapy, laser ablation, loop excision, and cold knife conization. Early-stage cervical cancer may be treated with surgery (hysterectomy) or chemoradiation.					
Balance of Harms and Benefits	The benefits of screening with cytology every 3 years substantially outweigh the harms.	The benefits of screening with co-testing (cytology/HPV testing) every 5 years outweigh the harms.	The harms of screening earlier than age 21 years outweigh the benefits.	The benefits of screening after age 65 years do not outweigh the potential harms.	The harms of screening after hysterectomy outweigh the benefits.	The potential harms of screening with HPV testing (alone or with cytology) outweigh the potential benefits.
Other Relevant USPSTF Recommendations	The USPSTF has made recommendations on screening for breast cancer and ovarian cancer, as well as genetic risk assessment and *BRCA* mutation testing for breast and ovarian cancer susceptibility. These recommendations are available at http://www.uspreventiveservicestaskforce.org/.					

Figure 6 Recommended cervical cancer screening in the USA.

HPV-associated diseases. HPV-PCR will become increasingly more important in the diagnosis of CIN2 and CIN3 lesions, especially in women who are older than 30 years.

New aspects of hepatitis E virus infection

The hepatitis E virus (HEV) belongs to the Hepeviridae virus family. It is a non-enveloped virus with a positive strand RNA genome. Four HEV genotypes associated with infections in humans can be distinguished. HEV replicates in the liver and is transmitted via the fecal-oral route. HEV is often detectable in blood 1–2 weeks before the onset of symptoms. Excretion in the stool starts somewhat offset from the viremia and is detectable for 3–4 weeks. The virus becomes mostly undetectable after jaundice subsides and the transaminases have normalized. HEV-specific antibodies become positive in most cases shortly after the onset of symptoms [41, 42].

HEV was first described in the 1970s in connection with an outbreak of acute hepatitis in India, which was transmitted via the fecal-oral route but was not caused

by hepatitis A virus infections (Figure 7). Hepatitis E viruses share the route of transmission with hepatitis A and also the clinical presentations as acute hepatitis. The incubation period of 6–7 weeks for HEV is followed by an uncharacteristic pre-icteric phase with fever, nausea, and vomiting. The icteric phase does not differ from the icteric phase of other forms of viral hepatitis and regresses within weeks [43]. In contrast to the often mild or even asymptomatic course of the disease observed in immunocompetent people, HEV infection in pregnant women, especially in the last trimester, may be fatal. The serious complications of a HEV infection during pregnancy were previously documented in the earliest known outbreak of HEV infections. It is estimated that up to 70,000 deaths are caused every year by HEV infections worldwide [44].

In Europe and North America, HEV infection had long been perceived as a travel disease, often due to contaminated drinking water. However, this is true only for HEV infections with genotype 1 and genotype 2, which only cause human infections and are endemic in many regions of the world with limited hygienic conditions. In contrast, HEV genotype 3 is a zoonotic disease that occurs in Europe and North America, which can infect pigs, deer, wild boars, mongooses, shellfish, and rodents [43]. There are also several case reports about HEV transmissions to people due to the consumption of raw venison, pork liver, or pork sausage. One plasma donation each of 7986 and 4525 in Sweden and Germany, respectively, tested positive for HEV [45]. Because a plasma pool requires up to 3500 plasma donations, a contamination of 10% of plasma pools is not surprising.

In addition, it has been discovered that HEV can not only cause acute hepatitis in immunosuppressed people with no history of travel but also lead to chronic hepatitis. Thus, when elevated transaminase levels are observed, HEV must be considered as differential diagnosis particularly for immunosuppressed patients. The most reliable test for diagnosing a HEV infection is the detection of HEV-RNA in the blood. The serological detection of IgM antibodies can, at best, be done 3–4 days after the onset of jaundice and may remain positive for several months. IgG antibodies already occur shortly after the IgM antibodies [41]. The seroprevalence of IgG antibodies in Europe varies between 0.3% and 52.5%, with Greece, the Netherlands, Italy, and northern France having a low seroprevalence, and England, Denmark, Moldova, and southwest France a high prevalence. In a recently published study, however, it was shown that the HEV-IgG seroprevalence primarily depended on the assay used in these studies [46]. Two hundred healthy healthcare professionals and 30 patients

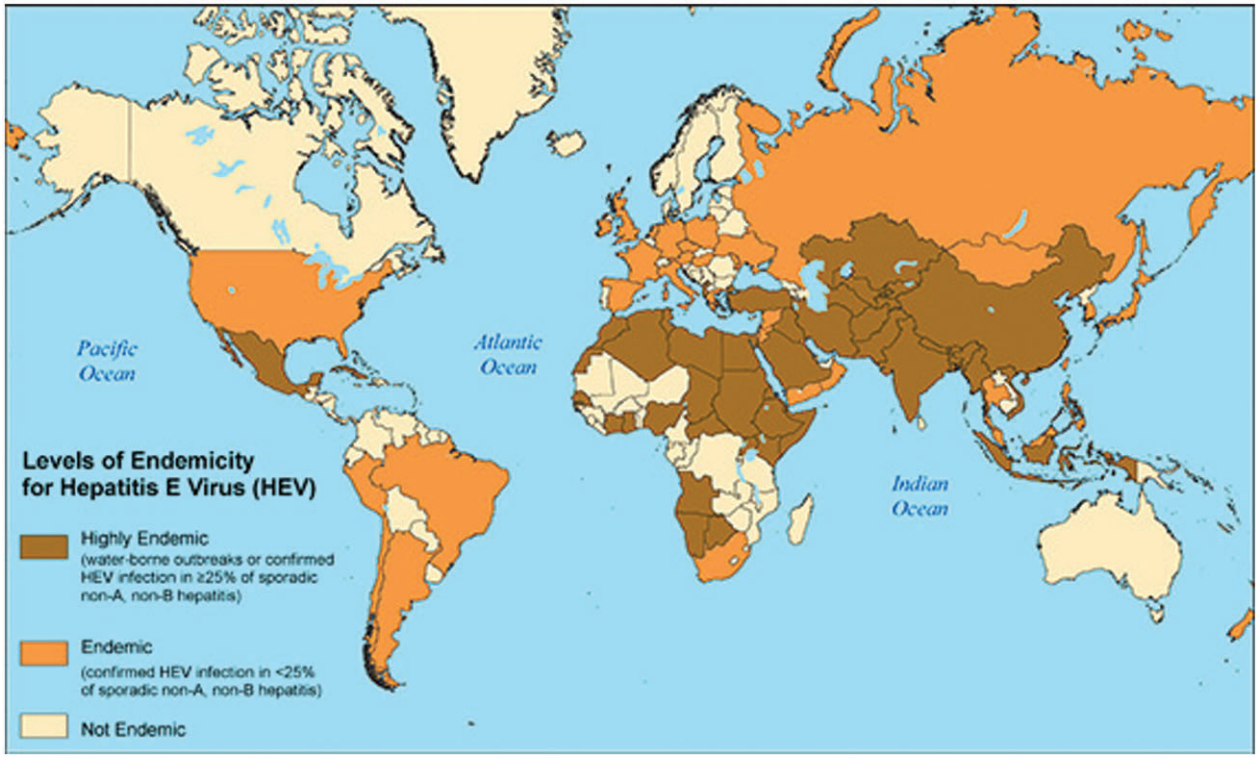

Figure 7 Spread of hepatitis E virus infections.
Source: http://www.cdc.gov/hepatitis/HEV/HEVfaq.htm.

with laboratory-confirmed acute HEV infection were tested using three HEV-IgG assays. The first group exhibited a seroprevalence of 4.5%, 18.0%, and 29.5%, respectively. In the second group, IgG antibodies were found in 83.3%, 96.7%, and 100%, respectively. Interestingly, the seroprevalence of HEV-IgG in European studies correlated very well with the performance of the specific assays in this study. This demonstrates the need for the standardization of serological assays used in routine diagnostics to screen for HEV genotype 1 and genotype 3 infections.

HEV infections represent a major public health challenge all over the world. Although scientific evidence of risk groups and transmission paths has existed for a long time, there are still a significant number of annual HEV infections and HEV-associated deaths. In Europe and North America, HEV must not only be perceived as a travel disease but also as a zoonotic disease that can go hand-in-hand, particularly in immunosuppressed patients, with the clinical picture of hepatitis (acute or chronic). The diagnostic limitations that currently still exist should lead to the judicious use of serological markers and HEV-PCR when clarifying elevated transaminase levels especially in immunosuppressed patients.

Conflict of interest statement

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