

## Review

# Viral infections in Pakistan: prevalence, factors affecting spread, and recommendations for control

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### Abstract

Pakistan is endemic to a number of viral infections, owing to its humid climate, topographical variation, soaring population, and lack of education and awareness. These viruses may have several different modes of transmission, including respiratory or airborne transmission, sexual transmission, blood-borne, fecal-oral transmission, vector-borne transmission, and transmission following an organ transplant. Although several different microorganisms are responsible for causing these infections, a few viruses are found more commonly in Pakistan and are primarily responsible for causing infections. In this study, we present a review of the most recent studies on different viruses, transmitted through various transmission routes, found commonly in Pakistan, along with the prevalence of each, and recommend control measures required against these viruses.

**Key words:** Virus diseases; Pakistan; risk factors; recommendations.

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### Introduction

Pakistan serves as a hub for a variety of viral pathogens due to its favorable environmental conditions and a high population burden, currently being around 225 million [1]. Due to the increasing population and lack of adequate sanitation, increased proximity among people along with unhygienic conditions allows viruses to have adequate breeding grounds and easy transmission. Moreover, due to the relatively unchecked in and out and outflow of people, viruses are now able to be transmitted across borders.

The common viruses in Pakistan are acquired via a variety of transmission routes. Viruses can be transmitted via a respiratory route which includes airborne and droplet viral particle transmission from an infected to a healthy individual. In addition to this, viruses can spread through direct sexual contact, mixing of body fluids which comprise the blood-borne viral pathogens, ingestion of virions by the fecal-oral route, transmission by having an intermediate vector, and via organ transplantation in patients with the compromised immune system.

The primary aim of this study is to present a review of the most recent studies on different viruses, transmitted through various transmission routes, found commonly in Pakistan, along with the prevalence of each, and recommend control measures required against these viruses. Moreover, this study has tried to bridge the gap between recommended prevention strategies and those which are currently being practiced in this country. Thus, effective strategies can be adopted at a small- and large-scale level, which can prove to be efficacious in allowing Pakistan to deal with the ever-increasing burden of transmissible diseases.

### Methodology

To collect data on predominant viruses, their recommended prevention strategies, and the practices currently adopted by Pakistan, a comprehensive literature review was performed, and recent studies and current data were included. Research studies were searched on PubMed and Google Scholar databases. Specific keywords were used for running the search, which included the name of the virus, “Pakistan”,

“prevalence”, “vaccination” and “prevention”. Articles in English ranging from 1987 to 2020 were included, while case studies, editorials, and articles without full-text availability were excluded. In certain cases where recent prevalence data were not available in research articles, a Google engine search was performed and local websites were cited for recent prevalence data. For recommendations, information available from the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) websites were used, while data regarding the implementation of these prevention strategies in Pakistan was included from research articles as well as local newspapers.

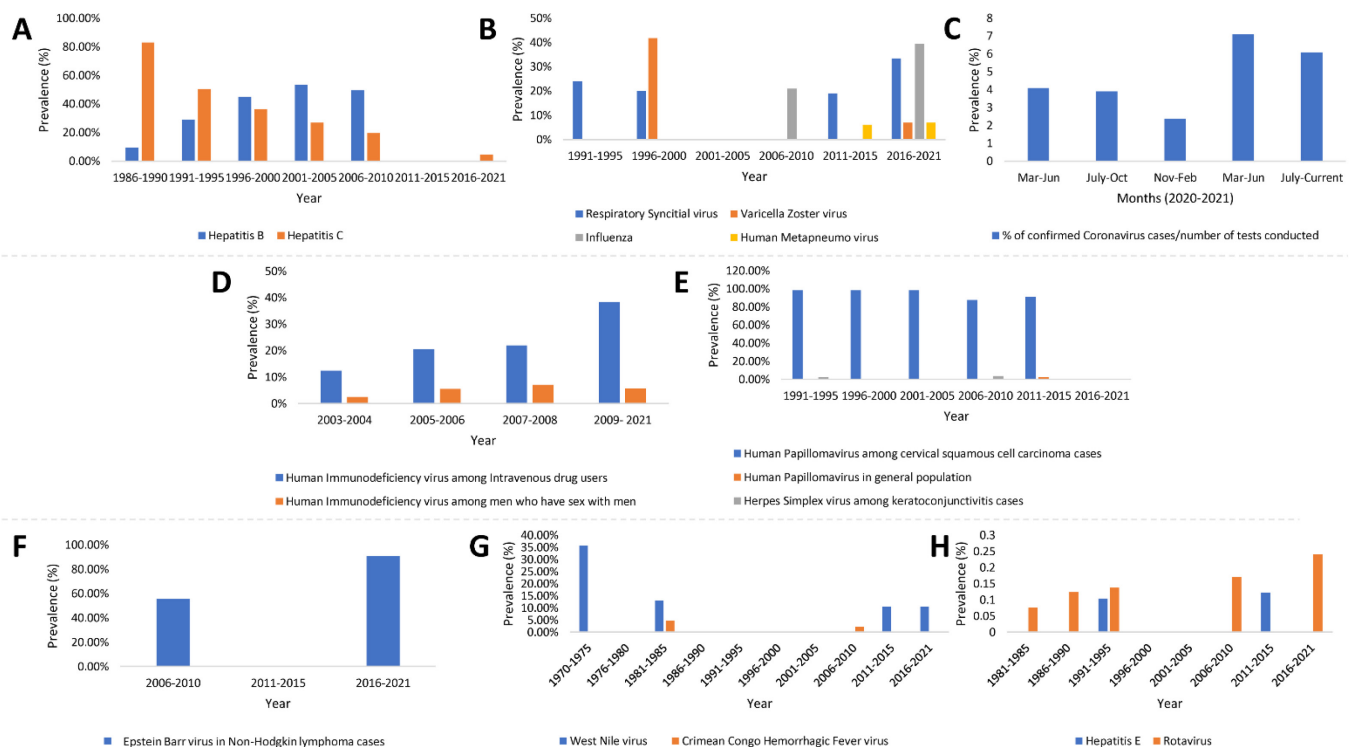
**Results**

Pakistan, throughout the years, has faced viral outbreaks and infections. Tracing back up to the last 50 years, the prevalence of some viral infections has shown an upward trend, others have shown a decreasing trend, while some have remained stable with occasional outbreaks. A graphic representation of these trends has been shown in Figure 1. The current pandemic of

SARS-CoV-2 had its first case in Pakistan on 26 February 2020. With fluctuations in the number of cases till today, a significant increase was noted in May 2021 due to the lifting of restrictions due to the Eid holidays, allowing inter-city and inter-provincial transport [2]. Among the food-borne viruses, a surge of poliovirus cases was observed in 1997 due to poliovirus due to an outbreak in Karachi [3], while for Hepatitis E, the first case was detected in the interval 1950-1955 [4]. In 1972, a sudden increase in infected individuals was noted (250 cases) due to contaminated water supply [4]. The first case of Human Immunodeficiency Virus (HIV) in Pakistan was detected in 1987 and the NACP (National AIDS Control Program of Pakistan) was formed in 1989, while HIV care at the community level started in 2005-2006 [5]. As far as Human Papilloma Virus (HPV) is concerned, the prevalence in the general population has decreased over the years.

Pakistan has faced two major Dengue virus outbreaks. In 2017, the country faced a major outbreak in Khyber Pakhtoonkhwa (KPK) province, where more than 18,000 people were infected, of which 86 died. In

**Figure 1.** Summary infographics of prevalent viral infections in Pakistan: The figure shows the prevalence of **A)** Blood-borne viruses Hepatitis B and Hepatitis C at a tertiary care center, **B)** Respiratory borne viral infections: Respiratory Syncytial virus, Varicella Zoster virus, Influenza Human Metapneumovirus, **C)** Respiratory borne viral infection: Coronavirus (represented as the percentage of confirmed cases among the number of tests conducted), **D)** Sexually transmitted Human Immunodeficiency Virus among intravenous drug users and who have sex with men, **E)** Sexually transmitted Human Papillomavirus in the general population and among cervical squamous cell carcinoma cases and Herpes Simplex virus among keratoconjunctivitis cases, **F)** Epstein Barr virus among NHL:non-Hodgkin lymphoma cases, **G)** Vector-borne viruses Crimean Congo Hemorrhagic Fever Virus and West Nile virus, and **H)** Fecal-oral borne Hepatitis E and Rotavirus in Pakistan. All available data in Pakistan has been included in the paper.



the province of Punjab, the worst outbreak occurred in the year 2011, with 21 685 reported cases and more than 350 deaths [3]. The vector-borne Crimean Congo Hemorrhagic Fever virus had its first case in 1970-1975, with an increase in prevalence to 4-5.4% in 1981-1985. An outbreak occurred in 2016 at the time of Eid Ul Adha. Contributing reasons included the advance purchase of animals that were placed and slaughtered in residential areas [6]. Pakistan also had an outbreak of the Chikungunya virus in 2016 [7]. Factors such as warm climate and poor sanitation with open sewage water were the leading causative factors. However, the prevalence has decreased to 7% in 2018 to no cases in the year 2019 [7]. Below we discuss in detail, the viral infections transmitted via different transmission routes.

### *Respiratory droplets*

Some of the infections commonly transmitted via the airborne or respiratory droplet route include chickenpox, influenza, measles, rubella, and coronaviruses.

### Varicella Zoster Virus

Varicella-Zoster Virus (VZV) is a double-stranded DNA virus belonging to the *Herpesviridae* family. Chickenpox is caused by the VZV and has been reported to have a prevalence of 41.8% in Pakistan [8]. Chickenpox occurs more commonly in females than in males, with a prevalence of 45.2% and 39.6%, respectively [8]. Furthermore, its prevalence is associated with increasing age (Supplementary Table 1) [8]. Chickenpox can easily be transmitted by an infected individual to other members of the family, with an 85% chance of transmission. Although the primary mode of transmission of VZV is via inhalation of respiratory droplets, it can also have vertical transmission from mother to child and may also enter the body through the eyes. After infection, VZV proliferates in the tonsils, subsequently infecting T lymphocytes and, thereby, disseminating throughout the body [9]. The primary infection of VZV is chickenpox, which mostly occurs in children and usually resolves on its own. However, with increasing age, this same virus tends to follow a more serious disease course in adults, with a 10-30 times increase in death rate. Apart from chickenpox, VZV is also known to cause Herpes Zoster (shingles), particularly among older individuals (50-60 years), and poor immune defenses. Shingles are known to affect the skin and nervous system and their pathology involves reactivation after the virus has remained latent in the ganglia of sensory neurons due to a prior VZV infection

during childhood [10]. With reactivation, VZV migrates from the ganglia via sensory nerves to related dermatomes and results in the dermatomal distribution of shingles rash. Diagnosis of VZV infection is primarily made based on clinical findings. For laboratory confirmation of infection, PCR is considered the most reliable test [9]. Most cases of VZV infection in healthy individuals are self-limited and therefore, require no treatment. Among the elderly and immunocompromised, VZV is usually treated with intravenous acyclovir for 7-10 days, followed by oral therapy with valaciclovir or famciclovir [9].

### Influenza Virus

Influenza is caused by the Influenza virus, a single-stranded RNA virus, belonging to the *Orthomyxoviridae* family. Influenza viruses can be classified into influenza A and influenza B, which are responsible for causing respiratory tract infections and fever. It is transmitted via respiratory droplets with close person-to-person contact, mainly affecting the upper respiratory system. Due to its variable antigenic nature, influenza epidemics are seen in many parts of the world every year. The overall prevalence of influenza in Pakistan is 24%, with 72% of these infections being Influenza type A. The Majority of these cases are reported to be from the province of Punjab [11]. Furthermore, the influenza virus also has several subtypes. Certain subtypes are found among poultry and are subsequently transmitted to, and cause infection in humans. Some of the common subtypes found among poultry include H5N1 and H7N9 [12]. In recent years, the H7N3 subtype, the most common influenza subtype, has changed to H9N2 and is responsible for a large number of cases in Pakistan. Influenza infection is usually self-limited, consisting of systemic symptoms such as fever, headache, and malaise, along with upper respiratory tract symptoms including cough and sore throat [13]. In such cases, management is symptomatic and anti-viral drugs are not required. Among high-risk groups, with a high susceptibility to complications such as pneumonia, treatment is mainly via neuraminidase inhibitors such as oseltamivir and zanamivir [13].

### Morbillivirus

The causative organism for measles is Morbillivirus, a single-stranded, negative-sense RNA virus, of the *Paramyxoviridae* family. Measles is quite a prevalent infection in Pakistan associated with some degree of mortality, with a subsequently increasing incidence over the years. The disease is characterized

by fever, rash, cough, coryza, and conjunctivitis, along with suppression of the immune system [14]. According to the World Health Organization, 4,386 individuals were infected in 2011, 14,867 in 2012 with 310 deaths, and 25,401 infections with 321 deaths in Pakistan [15]. School-going children are particularly susceptible to measles in Pakistan, with a majority of these children being unvaccinated for measles [16]. A study carried out in 2009 reports a prevalence of 18.77% among school-going children in Islamabad, while another study carried out in 2012 reports a prevalence of 22% among school-going children [17]. In 2019, WHO declared a measles outbreak in several countries, with reported 1,978 cases of measles from Pakistan [18].

#### Rubella virus

Another virus that can be transmitted via the respiratory route is Rubella, which is responsible for causing German measles. It is a single-stranded, positive-sense RNA virus from the *Togaviridae* family. While Rubella primarily causes a self-limited disease, the most feared complication of infection is vertical transmission to the fetus, particularly during the first trimester, known as congenital rubella syndrome [19]. A study conducted among pregnant women revealed a positive Rubella test in 83.4% of the women [20]. Recent data suggests that from 2011 to 2017, Pakistan saw an increase in Rubella cases, with 389 Rubella cases being reported in 2017 with a decrease to 109 cases in 2020 [21].

#### SARS-CoV-2

The novel Coronavirus has recently led to the outbreak of a pandemic, with Pakistan being one of the affected countries [22]. Originating from the province of Wuhan in China, it is a positive-sense RNA virus and belongs to the family *Coronaviridae* [23]. Common symptoms of COVID-19 infection include fever, cough, and shortness of breath [24]. The first case of COVID-19 emerged in Pakistan on the 26<sup>th</sup> of February 2020, and almost a year later, nearly 944,065 people have now been infected in Pakistan. Out of these, 39,905 are active cases while almost 21,828 deaths have been reported, with a fatality rate of 2.3% [25]. According to recent statistics provided by the Pakistani government, more than 10,699 active cases are reported from the province of Punjab while nearly 23,000 active cases are from the province of Sindh [25].

#### Human metapneumovirus and Respiratory Syncytial Virus

Human Metapneumovirus (HMPV) and Respiratory Syncytial Virus (RSV), are single-stranded,

negative-sense RNA from the *Pneumoviridae* family [26]. They are responsible for causing pneumonia, particularly in children, the elderly, and the immunocompromised [27]. In Pakistan, both these viruses have been identified as causative agents for pneumonia among children, with HMPV cases mainly peaking during February, and RSV cases peaking during July [28]. Although RSV and HMPV can cause pneumonia in any age group, studies conducted in Pakistan mainly focus on the pediatric population, while the majority of the cases are reported among children less than 3 months old [29]. Prevalence data for these 2 viruses is also limited and only a few studies report the prevalence of RSV and HMPV among the pediatric population of Pakistan. A study conducted in 2011 identified HMPV in 14.2% of the population and RSV in 17.8% of the population [28]. Another study in 2012 identified RSV to be present in 19% of the pediatric population [29], while a study from 2013 identified RSV in 24% of the pediatric population. However, more recent prevalence data on RSV and HMPV from Pakistan is lacking.

#### *Sexually transmitted*

##### Human Immunodeficiency Virus

The most commonly found sexually transmitted virus in Pakistan is Human Immunodeficiency Virus (HIV) [30]. HIV is a double-stranded RNA retrovirus, belonging to the subgroup lentivirus [31]. It predominantly affects T helper cells, which if left untreated can lead to a state of immunodeficiency known as acquired immunodeficiency syndrome (AIDS). This predisposes the individual to several opportunistic infections and cancers [31]. HIV has a prevalence of less than 0.1% among the general population [30]. According to the most recent national data, an estimate of 0.18 million cases of HIV exist in Pakistan, however, only 39,000 cases are registered with the National AIDS Control Program [32].

Certain high-risk groups are particularly susceptible to HIV infections. A study has reported an estimated HIV infection rate of 38.4% among intravenous drug users, 7.5% among transgender sex workers, 5.6% among male sex workers, 5.4% among homosexual men, and 2.2% among female sex workers [33]. Apart from sexual transmission, HIV is also commonly transmitted through infected blood, vertical transmission, trans-placental transmission, or transmission via breast milk [33].

The diagnosis of HIV can be made by screening for antibodies, followed by running confirmatory tests in case of a positive antibody titer. Additionally, detection

of p24 viral antigen and polymerase chain reaction (PCR) or nucleic acid amplification test (NAT) may also be used [34]. Treatment of HIV includes the use of highly active antiretroviral therapy (HAART), which includes a combination of nucleotide, nucleoside, and non-nucleoside analogs, reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, and integrase inhibitors [34].

#### Human Papilloma Virus

Human Papilloma Virus (HPV) is another common sexually transmitted virus, with its major risk factor being sexual intercourse with different partners, along with inconsistent condom usage and poor immunity. Apart from this, HPV is also transmitted via skin-to-skin contact. It is a double-stranded DNA virus, responsible for causing cervical and penile cancer. HPV types 16 and 18 primarily cause cancer, while HPV types 6 and 11 are associated with benign conditions, including papilloma and warts (*condyloma acuminata*). However, cases occur where there is a subclinical HPV disease and genital lesions are not present. The prevalence of HPV among Pakistani women is reported to be 2.8%. Several studies conducted in Karachi, the largest metropolitan city in Pakistan, have revealed a high prevalence of both HIV and HPV, both of which have been on a steady rise since the 1990s. According to a survey carried out in 2007, 59 out of 60 tested women were positive for HPV, particularly HPV type 16 [35].

#### Cytomegalovirus and Herpes Simplex Virus

Other causative organisms responsible for causing sexually transmitted infections in Pakistan include Cytomegalovirus (CMV) and Human Simplex Virus (HSV). They are double-stranded DNA viruses that belong to the *Herpesviridae* family. Genital herpes is caused by herpes simplex virus 2, while another strain of HSV, HSV-1, is responsible for infections above the diaphragm, such as herpes labialis, cold sores, and encephalitis. On the other hand, HSV-2 is responsible for genital pathologies and as a result can be transmitted to newborns during delivery. Both HSV 1 and 2 can establish latency in sensory ganglia, with HSV 1 in cranial ganglia and HSV-2 in the sacral ganglia. As a result, they can be reactivated at a later point in time. Genital lesions due to HSV-1 are known to occur, however, they have a relatively benign course and have fewer chances of recurrence. A study conducted in 2014 reported a prevalence of 94.5% for CMV and 46.9% for HSV among a cohort of 145 pregnant women [20].

Another study conducted in 2011 reported a prevalence of 3.2% for HSV type 2 infections in Pakistan [36].

Diagnosis of infection can be made via laboratory methods such as viral isolation, Tzank smear, PCR for viral DNA, and serology [37]. Some drugs that can be used for the treatment of HSV include guanosine analogs such as acyclovir and valacyclovir. Viral DNA polymerase inhibitors which include foscarnet and cidofovir may also be used for treatment [37]. For CMV, the antiviral medications ganciclovir and valganciclovir may be used [38].

#### Pox Virus

Molluscum Contagiosum is a common virus resulting in genital warts through sexual transmission in adults. According to a study in the Abbottabad region of Pakistan from January 2010 – December 2014, 8.7% of the sexually transmitted cases had warts due to molluscum contagiosum [39].

#### *Bloodborne*

Viral hepatitis is the most common blood-borne infection in Pakistan. According to a study conducted in 2010, viral hepatitis accounts for 7.5% of the lethal cases found in Pakistan and is a major cause of hepatic cirrhosis and carcinoma. Among viral hepatitis, Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) account for the majority of the cases.

#### Hepatitis C Virus

HCV is a single-stranded, positive-sense RNA virus belonging to the *Flaviviridae* family of viruses. The majority of patients with acute HCV infection go on to develop chronic HCV infection. A small proportion of these patients also subsequently develop liver cirrhosis, which increases the risk of hepatocellular carcinoma among them. Studies have reported a prevalence of 4.8% for HCV, while 10 million people in Pakistan are reported to be symptomatic or asymptomatic carriers of HCV [40]. Due to the extremely high prevalence of HCV, Pakistan is known to have the second-highest number of HCV infections [41]. According to a study conducted in 2001, HCV accounts for 29% of cases of chronic liver disease and 8% of cases of hepatic carcinoma in Pakistan [42].

Populations with a high prevalence of HCV include patients with chronic liver disease (54.0%), health care providers (5.4%), and pregnant women (5.2%) [43]. HCV is majorly transmitted via blood transfusions and intravenous drug injections, while cases of vertical transmission are much rarer. Other factors that can lead to HCV transmission include hemodialysis,

unprotected sexual intercourse, organ transplantation, tattoos, and ear piercing, as well as sharing razors and toothbrushes. Although HCV accounts majorly for the infectious disease burden, in recent years it has been an increased threat to health as cases of multi-drug resistant HCV are now being reported. Hence, Pakistan has been recommended to follow WHO's target of reducing HCV infections by 80% and fatalities by 65%, by the year 2030 [44].

Diagnosis of HCV infection can be made by detecting HCV IgM and IgG in the patient's serum, or via viral isolation, viral antigens, and RNA detection in serum (11). Treatment for HCV is mainly with interferons [45].

#### Hepatitis B Virus

HBV is a double-stranded DNA virus belonging to the *Hepadnaviridae* family. HBV infection can manifest as acute or chronic infection, fulminant hepatitis, or even hepatocellular carcinoma [46]. It has a prevalence of 2.5% in Pakistan [47], while 7-9 million people are reported to be symptomatic or asymptomatic carriers of HBV [48]. Similar to HCV, the major mode of transmission for HBV is also through transfusion of contaminated blood and intravenous drug injections, however, vertical transmission is more likely to be found in HBV than in HCV infections [49]. Populations reported having an increased prevalence of HBV in Pakistan include patients with chronic liver disease (25.7%), health care providers (6.0%), and army personnel (3.5%) [43]. Laboratory testing for HBV includes IgM and IgG antibodies against the surface or the core protein of the virus, antigen testing for the HBV surface antigen, and PCR for HBV DNA [46].

#### *Fecal-oral*

Common viruses found in Pakistan that are transmitted via the fecal-oral route include poliovirus, Hepatitis E Virus (HEV), rotavirus, adenovirus, enterovirus, and Hepatitis A Virus (HAV).

#### Poliovirus

Poliovirus is a positive sense, single-stranded RNA virus, belonging to the *Picornaviridae* family of viruses. It is responsible for causing polio which is spread via consumption of food and water contaminated with fecal material, and rarely through respiratory droplets of infected persons. Polio affects the central nervous system, leading to partial or complete paralysis, and is reported to have a 5% fatality rate. Pakistan is one of the only three countries in the world where polio infections are still present [50]. Compared

to areas with easy accessibility to the polio vaccine, those with poor accessibility are reported to have 73% more cases of polio [50]. Vaccination against polio continues to be a challenge in Pakistan. This is attributed to several myths against the polio vaccine, including a belief that the polio vaccine contains *non-halal* or religiously prohibited contents and that it can cause infertility. Moreover, the poor governance of polio eradication programs also contributes to the problem. As a result, polio is a significant cause of morbidity and mortality in Pakistan.

In 2011, Pakistan was reported to have more polio cases than any other country in the world, while in 2014, approximately 306 cases of polio were reported in Pakistan, which accounted for 80% of the worldwide cases of polio in that year [51]. Out of these 306 cases, 96% were reported from the province of Khyber Pakhtunkhwa [51]. 2015 and onwards, fewer annual cases of Polio have been reported, however, in 2019 there was an increase in Polio cases with 144 cases being reported. In 2020, 84 cases have been reported so far, the majority of them being from Balochistan [52].

#### Hepatitis E Virus

HEV is a single-stranded RNA virus belonging to the *Hepevirus* family. It is commonly found among pregnant women in Pakistan, with a prevalence of 57-91% among jaundiced pregnant women and a 90% probability of vertical transmission. Among pregnant women, HEV can lead to fulminant hepatic failure [53]. According to a study, Non-A and Non-B hepatitis viruses are more common in pregnancy with a prevalence of 64% among all viral hepatitis cases in pregnancy [54]. Out of the 64% cases, 40% were due to HEV infection, making it the most common cause of viral hepatitis in pregnancy. HEV is more common among children and accounts for 50-60% of acute viral hepatitis infections among children [43].

#### Rotavirus

Rotavirus is the most common cause of gastroenteritis and diarrhea in children, with an overall prevalence of 28.5% among children. In the 6-11 month age group, rotavirus infections have a prevalence of 20% [55]. Rotavirus is a double-stranded RNA virus that belongs to the *Reoviridae* family of viruses and causes most of the infections in the spring and winter seasons. The prevalence of rotavirus in the metropolitan city of Karachi is 13.7%, with G1 and G9 being the most common genotypes, while G2, G3, and G9 are also fairly common [56]. A study conducted in 2013

reported a prevalence of 35% from 2008 to 2009, among children up to 5 years in Lahore [57].

#### *Vector-borne*

Pakistan has a hot and humid climate which favors the growth of several vectors of diseases, including mosquitoes and ticks REF. Some of the viruses commonly transmitted by these vectors include Dengue, Congo Crimean Virus, Chikungunya, and Human West Nile Virus (WNV).

#### Dengue virus

Dengue virus is a positive-sense RNA virus that belongs to the family of *Flaviviridae* and has 4 serotypes, namely DEN-1, DEN-2, DEN-3, and DEN-4. It is commonly transmitted to humans by the bite of an infected female *Aedes aegypti* mosquito at night time. Other vectors for dengue include *Aedes albopictus*, *A. polynesiensis*, *A. scutellaris*, *A. hensilli*, *A. furcifer*, and *A. luteocephalus*. Upon gaining entry into the human body, the virus spreads via the bloodstream to the liver and central nervous system and affects the blood cells involved in the killing of microbes. Dengue is responsible for causing ‘break-bone fever’ and ‘dengue hemorrhagic fever’ (DHF), with the latter being more serious. Break-bone fever consists of fever with generalized weakness, arthralgia, and myalgia. DHF, on the other hand, causes sepsis with shock, hemorrhage, and fever, resulting in possible death [58].

The prevalence of each serotype of Dengue varies with time and place. According to a study conducted in 2006, DEN-2 and DEN-3 were the most prevalent serotypes found in Karachi, while in 2007 DEN-3 was the most prevalent in Karachi [59]. In 2010, the predominant serotypes were DEN-1 and DEN-2, infecting approximately 5050 individuals, of which Sindh had approximately 2000 doubtful and 881 correctly diagnosed cases, Punjab had 1885 cases and Khyber Pakhtunkhwa had 158 cases [59]. In 2019, a major dengue outbreak occurred with a total of 52,485 reported cases and 91 deaths. Among these, the majority of the cases were from Sindh and Pakistan’s capital city Islamabad [60].

Laboratory testing for the Dengue virus includes antibodies and viral antigens [61]. Primary infection is mostly self-limited and does not require treatment. For DHF, currently, there is no treatment. The management of such patients includes supportive and symptomatic measures [61].

#### West Nile Virus

West Nile Virus (WNV) is a single-stranded, negative-sense RNA virus, belonging to the *Flaviviridae* family. It is transmitted to humans through mosquitoes. Signs and symptoms of WNV infection are similar to those of Dengue hemorrhagic fever. Some infected persons develop West Nile Fever, while others may develop symptoms related to the central nervous system, such as seizures and possibly coma [62]. According to a study conducted in 2018 in Pakistan, between 2015 and 2016, 105 of 997 tested patients had IgM antibodies for WNV in their blood [63]. Diagnosis is primarily based on the detection of IgM antibodies in serum or CSF of infected individuals. Treatment of WNV infections is supportive [62].

#### Chikungunya virus

Chikungunya virus is a positive-sense RNA virus belonging to the alphavirus subgroup of the *Togaviridae* family. The presenting complaints of Chikungunya infection are the same as for dengue break-bone fever. Chikungunya infections are spread by the bite of *A. aegypti* and *A. albopictus* mosquito. The larvae of these mosquitos prefer breeding in outdoor ceiling water tanks, air conditioning coolers, and sewage and tire dumps. *A. albopictus* is particularly tolerant to a wide range of temperatures, hence, owing to Pakistan’s climatic condition and lack of sanitation, the vector very easily inhabits water supplies in Pakistan. In contrast to the Dengue virus, the Chikungunya virus is mostly transmitted by the bite of an infected mosquito during daytime hours [64]. Moreover, cases of Chikungunya peak following the monsoon season. Although primarily transmitted by a vector, Chikungunya also has a 48.7% chance of vertical transmission from an infected mother [65]. Studies conducted in 2017 and 2018 have reported Chikungunya infection among 30,000 people in Karachi. Recent data from another study has shown that out of more than 1,500 people tested, 50% were positive for Chikungunya infection [66]. No specific treatment exists for Chikungunya infections. Symptomatic treatment options such as non-steroidal anti-inflammatory drugs (NSAIDs), rehydration therapy, and other antipyretic and analgesic medicines may be used [67].

#### Congo Crimean Hemorrhagic Fever virus

Congo Crimean Hemorrhagic Fever (CCHF) is caused by the CCHF virus which is a single-stranded, negative-sense, enveloped, RNA virus belonging to the *Bunyaviridae* family of viruses. It is either transmitted

via a tick vector called *Hyalomma* or through contact with infected fluids of humans or animals, especially after contact with infected animal blood directly after animal slaughter. It may also be acquired in hospitals through the reuse of infected syringes or via aerosol droplets [68]. Early symptoms of CCHF include fever, chills, head and back pain, loss of balance, and photophobia, while bleeding from orifices usually begins on the fourth day [69]. CCHF is a multi-organ disease, affecting blood vessels, liver, brain, and the reticuloendothelial system. The virus targets the blood vessels of multiple organs and interferes with their diffusion capability. As a result, massive amounts of fluid enter the interstitial space and cause shock and blood loss [70].

Globally, Pakistan has the fourth most reported cases of CCHF. The most common type of CCHF Virus in Pakistan is Asia-1 [68]. The first case of CCHF in Pakistan was diagnosed in 1976 and most of the subsequent cases have occurred in the province of Balochistan, as livestock farming is one of the most common occupations there. The cases show a spike after *Eid-ul-Adha*, an Islamic religious tradition when thousands of animals are sacrificed. While previously, Pakistan experienced CCHF cases seasonally, now disease by this virus happens all year long, and approximately 60 cases of CCHF are reported in a year [71]. According to WHO, Pakistan is endemic to the CCHF virus, where 52 out of 354 screened patients tested positive for CCHF Virus RNA between 2007 and 2013 [72], whereas between 2012 and 2015, a total of 161 positive cases were reported [69].

Depending on disease transmission, CCHF infections may be associated with high mortality rates. Although specific treatment options for CCHF are still scarce, recent advances have led to the use of serum obtained from individuals who recovered from the disease along with the use of IV Ribavirin, both of which may prove to be beneficial for the treatment of CCHF [73].

#### *Organ transplantation*

Viruses commonly transmitted as a result of organ transplantation include Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), HBV, HCV, HDV, HEV, and HIV. Immunosuppression as a result of organ transplantation as well as decreased T-cell immunity due to immunosuppressant drugs administered to organ transplant patients is the major risk factor for acquiring these infective organisms.

#### Cytomegalovirus

CMV is a double-stranded DNA virus that belongs to the family of *Herpesviridae* and can be transmitted through a variety of modes such as vertical transmission, blood, and salivary contact, sexual contact, and organ transplantation. Among immunocompromised patients, CMV causes heterophil negative mononucleosis which presents with fever, weakness, and the presence of abnormal white blood cells in the blood. In bone and kidney transplant patients, CMV is responsible for causing hepatitis and pneumonitis and has a 100% fatality rate. According to a study conducted in 2009 in Pakistan, out of 36 patients receiving organs, 4 develop diseases from CMV [74]. Several recent advances have been made for the prevention and treatment of CMV infection among organ transplant recipients [75], however, these options are currently not available for use in Pakistan.

#### Epstein-Barr virus

EBV is a double-stranded DNA virus belonging to the family of *Herpesviridae* and is usually transmitted through contact with the saliva of an infected person, as well as through organ transplantation. Among the vast majority of individuals, EBV leads to the development of infectious mononucleosis, which is a self-limited disease [76]. In kidney transplant patients, it causes abnormal B cell proliferation, smooth muscle tumors, and B cell lymphomas in recipients of organ transplants and are responsible for viral diseases in 1-3% of liver transplant patients [77]. In particular, EBV leads to a condition known as post-transplant lymphoproliferative disorder (PTLD), which is a type of neoplastic disorder [78]. Detection of EBV among post-transplant patients can be made via PCR testing [78]. Statistics have shown that out of 1467 patients undergoing organ transplants, 20 patients acquired EBV post-transplant [79]. However, despite being a common cause of post-transplant infections, there are no statistics available for EBV infection among transplant patients in Pakistan.

#### Hepatitis C virus

Hepatitis C is a significant cause of viral infection in patients receiving liver transplants. According to a survey, 35% of liver transplant recipients developed viral diseases [80]. HCV causes infection in 18-20% of recipients of non-infected donors, while in recipients of HCV positive donors, it is responsible for causing 67-96% of infections [81]. Apart from HCV, HEV also accounts for cases of hepatitis after organ transplantation.



Patients with renal failure receiving dialysis as well as those receiving kidney transplants are both at risk of acquiring infections. Patients on dialysis are immunodeficient and require frequent injections, making them prone to receiving infections either through direct contact or through the use of non-sterile hospital equipment. HBV, HCV, HDV, and HIV are some of the viruses commonly transmitted to dialysis patients [82]. Patients receiving renal transplants are at a 15% risk of developing infections and are most likely to develop these infections 3-6 months post-transplant. Within the first 2 months, infection with HSV 1 and 2 occur most commonly, in the next 2-6 months, infection with CMV and hepatitis viruses is most common, while after 6 months, common infectious agents include VZV, HBV, HCV, HIV, EBV, CMV, HSV, Rabies virus, Adenovirus, West Nile Virus, polyomaviruses (BK and JC) and Parvovirus [81].

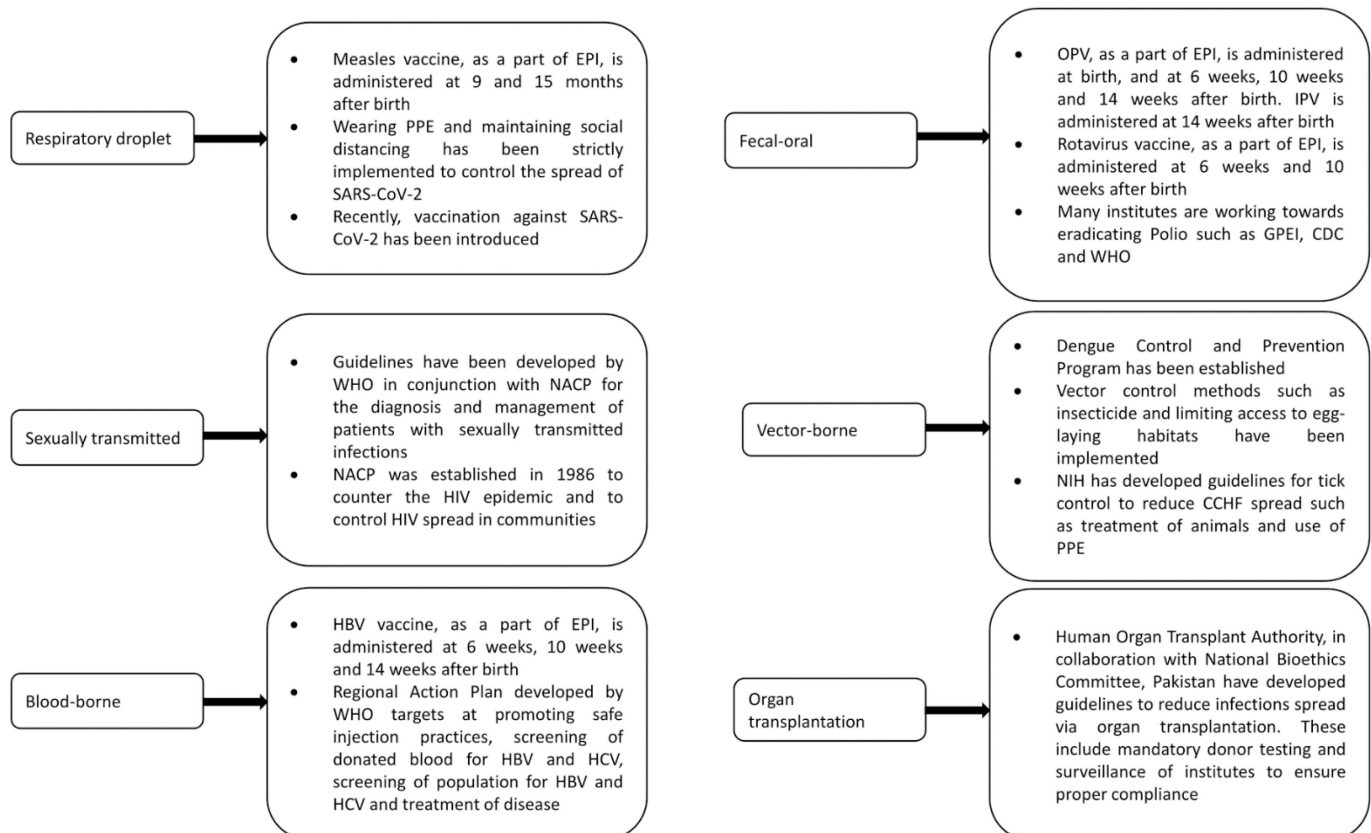
*Animal bites*

Rabies

Rabies is a zoonotic infection, caused by the Rabies virus belonging to the *Rhabdoviridae* family of viruses. It is a negative sense, single-stranded RNA virus, which is primarily transmitted to humans by the bite of a rabid animal or through infected saliva [83]. Rabies may exhibit a variety of clinical manifestations, leading to fatality in 100% of unvaccinated individuals. Pakistan is reported to be among the top countries with endemic Rabies infection, accounting for nearly 2,000-5,000 rabies deaths per year [84]. More recent data has shown that there are nearly 100,000 cases of rabies annually in Pakistan [84]. Approximately, 98% of these are due to bites from rabid dogs, and most cases are among teenage males during the summer months.

Diagnosis of rabies is either made clinically or via post-mortem analysis of brain and cerebellum samples [83]. As of yet, there is no cure for rabies. Management includes supportive care, and disease can be prevented by immediate post-exposure prophylaxis in the form of one dose of rabies immunoglobulin at the bite site and

**Figure 2.** Viral infection control and management framework of Pakistan. Viral infection control and management framework of Pakistan. Key transmission routes and control measures are given. Key: EPI-Expanded Program on Immunization; OPV- Oral Polio Vaccine; PPE- Personal protective equipment; IPV- Inactivated Polio Vaccine; WHO- World Health Organization; GPEI- Global Polio Eradication Initiative; NACP- National AIDS Control Program; NIH- National Institute of Health, Islamabad; CDC- Centers for Disease Control and Prevention.



5 doses of rabies vaccine within 28 days of being bitten. Moreover, pre-exposure prophylaxis is recommended among those who frequently come in contact with potentially infected animals [83].

## Recommendations

A few safety recommendations and preventive measures have been proposed by several different health organizations and different researchers. Some of these recommendations are common for each mode of transmission, while others are more specific to different viruses. Despite having a framework for the control of viral infections in Pakistan (Figure 2), the prevalence of certain viral infections is on the rise.

### *General preventive measures*

The following recommendations are common for most modes of transmission:

- Regular hand washing and sanitization;
- Follow proper hygiene practices and avoid touching eyes or mouth;
- Avoid sharing personal items such as nail cutters and razor blades;
- Avoid contact with others during the infective phase of the disease;
- Vaccination and immune globulin therapy, both pre-and post-exposure to a virus, particularly in high-risk areas;
- In hospitals, patients with contagious infections should be kept in isolation, and health professionals attending to them should wear proper personal protective equipment (PPE) and should be up to date with their vaccination schedule. Anti-microbials can be administered as prophylaxis for nosocomial infections;
- Community education programs and advertisement campaigns with influential personalities, celebrities, and religious leaders, to spread awareness regarding viral transmission and diseases. This will also encourage people to seek professional help earlier in the course of the disease;
- Surveillance teams in different areas to keep track of the prevalent viruses and the number of fatalities it causes in that area, as well as to assess the success of an intervention program;
- Regular insecticide spraying, use of full sleeve clothes and sleeping nets, regular cleaning of water supplies, and covering stagnant water sources;

- In case of an outbreak, the guidelines laid by WHO regarding outbreaks should be followed. Other measures include [85]:
  - a) The widespread availability of testing facilities;
  - b) Evacuation of the area;
  - c) Effective communication between the public and the authorities, educating people about the outbreak, and other preventive measures which can be taken;
  - d) Educating health professionals regarding the disease ;
  - e) Body temperature checking of people entering the country through airports and seaports;
  - f) Isolation of infected individuals;
  - g) Wards to admit infected patients should be made away from the city;

Besides, the CDC also set up a few guidelines to prevent the spread of infectious diseases via immigrants. These include:

- All travelers should be vaccinated for mumps, measles, rubella, diphtheria, tetanus, pertussis, polio, and varicella;
- Most travelers should get vaccines for Hepatitis A and Typhoid;
- Travelers from endemic areas should be vaccinated for Hepatitis B, rabies, malaria, and yellow fever.

### *Guidelines and recommendations for specific modes of transmission*

Recommendations specific for each mode of transmission, vaccine/vaccination status, and practices in Pakistan are summarized in Supplementary Table 1 [86-123].

## Vaccinations in Pakistan

Despite several prevalent infections and high infection rates, Pakistan generally has a low rate of immunization. Studies have shown that among children less than 1-year-old, only 30.8% received complete vaccinations, while the rest were either not completely vaccinated or not vaccinated at all [124]. Moreover, vast differences in rates of vaccination have been observed between different Pakistani provinces. Punjab has the highest rates of childhood immunization while Balochistan has the lowest [125]. In recent years, although the immunization rates in Punjab and Khyber Pakhtunkhwa have improved, Sindh and Balochistan have witnessed a decline in their rates of childhood

immunization [126]. Furthermore, it has been noted that the vaccination rates among adults are significantly lower than even those of children, despite a majority of adults considering adulthood vaccination to be important. In Supplementary Table 1, we summarize the vaccines available for the different viral infections and their availability and usage in Pakistan.

## Conclusions

Pakistan is home to several viral infections, some of which are more prevalent than others. If the recommendations suggested by WHO and CDC are followed, such infections can be controlled.

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**Annex – Supplementary Items****Supplementary Table 1.** Key modes of transmission, specific recommendations for prevention, vaccine/vaccination status, and recommendations being implemented in Pakistan.

<b>Mode of transmission</b>	<b>Key recommendations for prevention</b>	<b>Vaccines Available</b>	<b>What is being practiced in Pakistan</b>
<b>Respiratory droplet</b>	<ul style="list-style-type: none"> <li>-Varicella-Zoster, MMR, and influenza vaccines to be administered</li> <li>-Immunoglobulins to be given to premature infants, newborns with a mother infected during the delivery week, pregnant women, and as post-exposure prophylaxis</li> <li>-The intramuscular protective serum prevents infection in 91% of people if administered within a day of interaction with an infected individual</li> <li>-Contact precaution and isolation of patients to negative air pressure rooms</li> <li>-Promote a healthy diet and Vitamin A intake</li> <li>-Individuals with parotitis to be isolated for at least 5 days</li> <li>-Amantadine and rimantadine can be used as influenza prophylaxis among family members of infected individuals</li> </ul>	<ul style="list-style-type: none"> <li>-Live weakened vaccines are available and 1 dose of them is effective in preventing all types of VZV infections.</li> <li>-Live weakened influenza vaccines are available although they are not seen to be very effective in children.</li> <li>-Among adults, however, a high dosage of influenza vaccine is effective.</li> <li>-MMR (measles, mumps, rubella) vaccine is another live, weakened vaccine shown to be very effective in preventing these viral infections.</li> <li>-Although no vaccine is currently available, the development of several vaccines against SARS-CoV2 is underway, which will most likely reduce the morbidity and mortality due to the virus.</li> <li>-Although several trials have been conducted for vaccine development, there is currently no vaccine available against RSV and HMPV.</li> </ul>	<ul style="list-style-type: none"> <li>-In Karachi, 7.2% of children below 2 years of age received</li> <li>-Varicella-Zoster vaccine while 6.6% of the children were vaccinated against influenza.</li> <li>-Some statistics for vaccinations in adulthood: 11.5% received MMR vaccine; 7.5% received influenza vaccine; 1.4% received VZV vaccine</li> <li>-52% of children below 2 years of age in Karachi received measles vaccine at 9 months of age, while only 32% received the 2<sup>nd</sup> dose of measles vaccine</li> <li>-No data available regarding prophylactic measures and contact precautions</li> </ul>
<b>Sexually transmitted</b>	<ul style="list-style-type: none"> <li>-Surveillance systems to ensure proper adherence to antiretroviral therapy and to eliminate HIV taboo aspect</li> <li>-Identifying HIV patients and limiting their sexual contact</li> <li>-Anti-retroviral therapy for pregnant HIV women as well as for 4-6 weeks to children born to HIV positive mother</li> <li>-Promoting age-appropriate sex education and the use of condoms by men or Tenofovir gel by women</li> <li>-Use of pre-and post-exposure prophylaxis within 72 hours</li> <li>-Circumcision. which decreases the risk of males getting HIV by 60%</li> <li>-Infected mothers to undergo a cesarean section and take precautions while breastfeeding</li> </ul>	<ul style="list-style-type: none"> <li>-No vaccines are currently available for HIV prevention.</li> <li>-Vaccines against HPV are available and recommended to be administered.</li> <li>-Although several clinical trials are being conducted and quite a few vaccine models have been trialed, there is currently no vaccine available for CMV and HSV.</li> </ul>	<ul style="list-style-type: none"> <li>-Only 24,000 individuals in Pakistan have access to anti-retroviral therapy through 35 HIV treatment centers</li> <li>-Only 7 centers for HIV therapy are present in Sindh province, all of these being in the major cities only</li> <li>-A study from 2017 has reported that no mothers of HIV positive children received antiretroviral therapy, nor were any of the children administered therapy as prophylaxis</li> <li>-98% of people with HIV use condoms inconsistently with regular sexual partners, while 63% of HIV positive people use condoms inconsistently with casual sexual partners</li> <li>-No data available regarding prophylactic measures</li> <li>-More than 75% of males in Pakistan receive circumcision</li> <li>-Breastfeeding has been identified as a risk factor among 79% of HIV positive children</li> </ul>

	-Vaccines for HPV to be administered -Regular pap smears for women aged 21 to 65 at 3-year intervals		-0.7% of adults received the HPV vaccine in Karachi -Data from 2019 shows that only 2% of women had undergone pap smears
<b>Bloodborne</b>	-Mandatory screening of donated blood products through tests such as EIA or RIA and proper syringe disposal along with the use of clean syringes at health care facilities -3 doses of HBV vaccine to be administered to infants, high-risk individuals, and health care workers -Pregnant women having HBV DNA greater than 200,000 IU/ml should be given antiviral drugs such as lamivudine, telbivudine, and tenofovir in the third trimester	-3 doses of HBV vaccine are to be administered to infants, high-risk individuals, and health care workers.  -No vaccine is currently available against HCV.	-In 2000, 77.4% of the blood was screened for HIV while 86.8% of the blood was screened for HBV  -59-73% of children in Pakistan between 12-23 months of age receive the Hepatitis B vaccine -62.2% of adults received HBV vaccine while 47.1% received HCV vaccine  -No data available regarding prophylactic measures for HBV infection
<b>Fecal-oral</b>	-Vaccines and post-exposure prophylaxis to be administered. Individuals traveling to disease-endemic areas should also be vaccinated  -Following guidelines laid down by the Global Polio Eradication Initiative (GPEI), including: Children older than 1 year should be vaccinated at least 4 times with Oral Polio Vaccine (OPV); Routine poliovirus testing in children younger than 15 years -Early detection of HEV and prevention of vertical transmission among pregnant women -Infants with diarrhea should be kept in separate day-care centers	- Following guidelines have been laid down by the Global Polio Eradication Initiative (GPEI): Children older than 1 year should be vaccinated at least 4 times with Oral Polio Vaccine (OPV) -Routine poliovirus testing in children younger than 15 years.  -Vaccine against HEV is available and is shown to be effective in preventing infection. -RotaTeq and Rotarix are both live vaccines available against Rotavirus.	-45.4% received vaccinations for Hepatitis A  -7.2% of children vaccinated against rotavirus  -Overall, 79% of the children were vaccinated against polio.  -In Pakistan, there is currently no vaccine available to protect against HEV. -25% of public schools and 41% of private schools in Karachi provided vaccinations to children according to the Expanded Programme on Immunization (EPI), particularly for Polio
<b>Vector-borne</b>	-Administration of Dengue vaccine  -Use of mosquito repellent lotion, permethrin coated clothes, acaricides for tick elimination, indoor residual spraying -Introduction of larvivorous fish (guppy and gambusia) and/or Bacillus Thuringiensis bacteria in water supplies	-A live, weakened vaccine Dengvaxia is available against dengue, however, it provides effective protection only in dengue seropositive individuals. Several potential vaccines against WNV have been under research, however, so far no vaccine is commercially available. -No vaccine is available against the Chikungunya virus.  -A project for the development of the CCHF vaccine is currently underway, but so far no vaccine is commercially available.	-In Pakistan, supportive treatment is provided for Dengue patients. There are currently no vaccines available in Pakistan to protect against Dengue  -48% of the people in Karachi use mosquito sprays to prevent Dengue infection  -No preventive measures are being taken in Pakistan with regards to safe animal sacrifice and proper disposal



	<p>-The sacrifice of CCHF infected animals to be conducted far away from residential areas and to practice proper disposal of the bodies</p> <p>-Avoid consumption of unpasteurized milk and raw meat to reduce disease spread</p>		
<b>Organ transplant</b>	<p>-Screening and vaccination of organ recipients for HBV, HCV, HIV, influenza, Herpes Zoster, pneumonia, and screening of organ donors for HBV, HCV, HIV, EBV, VZV, and WNV</p> <p>-Anti-viral therapy and immunoglobulins to be administered to recipients who test positive for any virus</p> <p>-IL-2 receptor antagonists along with antibodies to be used as post-transplant prophylaxis</p> <p>-Transplant organs to be stored in very cold environments pre-surgery</p> <p>-Post-transplant, patients should be kept in aseptic environments, with positive-pressure systems</p>	<p>-No vaccine is currently available against EBV infection, although several recommendations in this regard have been made.</p>	<p>-No data is available for screening and vaccination.</p> <p>-A study from Pakistan has demonstrated that prophylactic management with oral valganciclovir results in low CMV infection rates in kidney transplant patients</p>
<b>Animal bite</b>	<p>-Vaccination of dogs for rabies is the most effective measure for rabies prevention.</p> <p>-Awareness campaigns regarding the behavior of dogs, prevention of bites, and early intervention of wound care.</p> <p>-Pre-exposure prophylaxis is only recommended for at-risk individuals such as laboratory personnel working with rabies virus.</p> <p>-Immediate post-exposure prophylaxis protocol includes washing the bite area with water, administering rabies vaccine, and rabies immunoglobulin.</p>	<p>-Current recommendations are subcutaneous administration of 2 ml anti-rabies vaccine in adults and 1.5 ml in children for 14 days, followed by booster doses on days 24, 34, and 104.</p>	<p>-TNVR (Trap, Neuter, Vaccinate, and Release) campaigns for stray dogs in Pakistan are useful in controlling the stray dog population and thus reducing the spread of Rabies via stray dog bites</p>