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Flaviviruses of public health concern in South Africa: Present and future threats

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Background: The resurgence and widespread transmission of flaviviruses over the past few decades are particularly concerning.

Aim: This review discusses the structure, aetiology, transmission, detection, diagnosis and prevention strategies for flaviviruses in South Africa.

Setting: Climate change, urbanisation, travel, population growth and changes in viral genetics are all driving the establishment and reemergence of flaviviruses in previously non-endemic areas. Medically important flaviviruses such as dengue, Zika, West Nile and yellow fever have geographically expanded, affecting millions worldwide.

Method: The study was conducted using the search engines, including Google Scholar, PubMed, ScienceDirect and Medline. This review includes published articles on flaviviruses from South Africa and beyond.

Results: Climate change, urbanisation, population growth and changes in viral genetics contribute to the reemergence of flaviviruses. The West Nile virus (WNV) is the most prevalent flavivirus detected in both animals and humans in South Africa. Lesser-known flaviviruses such as Banzi virus (BANV), Bagaza virus (BAGV), Spondweni virus (SPOV), Wesselsbron virus (WSLV) and Usutu virus (USUV) have also been identified in the region, but their current status remains unclear, possibly due to limited surveillance programmes and/or misdiagnosis. Nucleic acid amplification tests, followed by sequencing and serological assays, are commonly employed technologies for surveillance in South Africa. While there are no licensed vaccines for human use against these flaviviruses, licensed vaccines for WSLV and WNV are available for animals.

Conclusion: There is a need to develop molecular diagnostic tools for local strains to prevent misdiagnosis, enhance surveillance programmes, implement preventive measures and facilitate the development of therapeutic agents and vaccines.

Contribution: This review provides insight into the significant health risks that flaviviruses pose to humans and animals. Additionally, it highlights the limitations of diagnostic methods and preventative measures, thereby enhancing the management of these infections.

Keywords: flavivirus; public health; re-emergence; health threat; zoonoses.

Introduction

The world is currently struggling with various anthropogenic factors associated with the emergence and spread of pathogens, such as climate change, urbanisation, globalisation and population growth. Floods and increased temperatures from climate change cause the proliferation of arthropod vectors such as ticks and mosquitoes, increasing the chances of human–vector interactions. Recent spread and outbreaks of chikungunya virus (CHIKV) and Zika virus (ZIKV) underscore the emergence of vector-borne pathogens in previously non-endemic regions. Currently, there is a resurgence of ZIKV and CHIKV infections in some parts of the world; over 12600 cases of ZIKV and 135 654 of CHIKV were reported in America this year. Notably, as of April 2025, South Africa recorded three travel-related cases of CHIKV.[1]

Members of the genus *Orthoflavivirus*, belonging to the family *Flaviviridae*, have a broad geographic distribution with potential for spread and emergence in non-endemic regions. In nature, flaviviruses are primarily maintained and transmitted in a sylvatic or an enzootic cycle between mosquitoes or ticks and birds or non-human primates (NHPs), with spillover to humans and domestic animals in urban transmission. Once flaviviruses have escaped their enzootic cycle, some flavivirus infections, such as ZIKV, are maintained in human populations for years through human-to-human transmissions. Some infections can spill over from animals directly into human populations through drinking raw milk. Over the past decades, flaviviruses such as ZIKV, West Nile virus (WNV), yellow fever virus (YFV) and dengue virus (DENV) have been responsible for outbreaks of diseases worldwide with significant global impact on public health. Approximately 440 000 – 1.3 million cases of ZIKV were recorded in 2015 during the outbreak in Brazil.[2] A total of 200 000 cases and 30 000 deaths due to yellow fever are recorded every year worldwide.[3] In 2024, dengue cases increased twofold from 2023, 14.1 million cases and 9508 dengue-related deaths were reported.[4] Cases of WNV vary annually, and in 2024, WNV was reported worldwide, with notable cases in America. Approximately 2445 human cases of WNV were reported, with 165 confirmed deaths reported in America.[5]

To effectively address the challenges imposed by flaviviruses in public health, it is imperative to deepen our knowledge of flavivirus transmission dynamics, epidemiology, diagnostic methods and available preventive measures. By proactively tackling these aspects, we can better equip ourselves to reduce their effects and safeguard public health. Investing in research and preparedness will not only protect communities but also enhance resilience against future outbreaks.

In South Africa, outbreaks of WNV occur after warmer and wetter periods when mosquito populations increase. Approximately 5–15 human cases are reported annually; however, there are likely additional asymptomatic and undiagnosed cases.[6] West Nile virus is also recognised as a veterinary concern in horses, causing an average of 10 cases of fatal encephalitis each year. Travel-related cases of DENV imported from endemic places have been reported in South Africa, and its principal vectors are prevalent in particular locations, such as KwaZulu-Natal. In addition, there are several lesser-known flaviviruses in the country historically been reported but remain understudied and potentially under-reported. Outbreaks of Wesselsbron virus (WSLV) have been detected in humans and livestock, and WSLV isolates have been obtained from collected mosquitoes. Usutu virus (USUV), Spondweni virus (SPOV), Bagaza virus (BAGV) and Banzi virus (BANV) have been detected in mosquitoes and birds.[7,8,9] Of concern are the recent outbreaks of USUV and WSLV, which were documented in animals and humans in Africa and Europe.[10,11,12,13] This highlights the urgent need for improved surveillance and research efforts in South Africa to better understand the transmission dynamics, epidemiology and potential health risks associated with these flaviviruses.

Factors, including asymptomatic cases, minimal illness and similar clinical presentations among symptomatic infections, result in under-reporting and misdiagnosis of flavivirus infections. For instance, Rift Valley fever virus (RVFV) and WSLV are transmitted by the same vectors and both cause abortions in livestock, with potential for misdiagnosis without having specific laboratory tests to differentiate. In addition, extensive serological cross-reactivity between flaviviruses further complicates accurate diagnosis in both humans and animals. This article aims to review the structure, aetiology, transmission, detection, diagnosis and prevention strategies for flaviviruses in South Africa, ultimately enhancing awareness and improving management of these infections in the region.

Methods

Relevant articles were identified using the search engines Medline, PubMed, Google Scholar and Science Direct, a publisher-specific site for publications. The specific keywords included 'flavivirus', 'public health threat', 'zoonoses' and 're-emergence'. The search only focused on articles written in English, and no restrictions were applied on the type of manuscript and year of publication; systematic reviews, original articles, narrative reviews and meta-analyses were evaluated.

Review findings

Classification of flaviviruses and their antigenic relationships

Flaviviruses are small, ~50 nm in diameter, enveloped, positive-sense and single-stranded RNA viruses. Flaviviruses are arthropod-borne viruses (arboviruses) belonging to the *Flaviviridae* family in the genus *Orthoflavivirus.* Over 70 viral species exist within this genus, and they are classified based on their vectors, that is, tick-borne, mosquito-borne, insect-specific or no known vector[14] as indicated in Table 1. This genus includes medically or veterinary important mosquito-borne flaviviruses such as DENV, ZIKV, YFV, Japanese encephalitis virus (JEV), WNV, USUV and WSLV; tick-borne flaviviruses such as tick-borne encephalitis virus (TBEV), Kyasanur forest virus (KFDV) and Powassan virus (POWV) and as well as lesser known flaviviruses that include BANV, BAGV and SPOV. Flaviviruses are further classified into serocomplexes distinguishable by neutralising antibody reactivity and hence indicate antigenic relationships and serological cross-reactivity based on shared antigen. The amino acid sequence similarity of the envelope (E) protein is approximately 70%–80% for virus species within a serocomplex and 40%–50% between serocomplexes.[15] Serocomplexes with known medical significance include the Dengue virus group, yellow fever virus group, Japanese encephalitis group, Spondweni virus group, Ntaya virus group and mammalian tick-borne viruses (see Figure 1 and Table 1). Figure 1 indicates the relationships of representative members of each complex, based on analysis of the complete polyprotein, indicating antigen relationships. Table 1 shows the geographic distribution of selected mosquito-borne members of each serocomplex relevant to South Africa, selected medically significant tick-borne flaviviruses, an example of an insect-specific flavivirus and one with no known vector.

Flaviviruses are also grouped by their clinical manifestations; however, this depends on the virus and the affected individual. Approximately 80%–85% of flavivirus infections

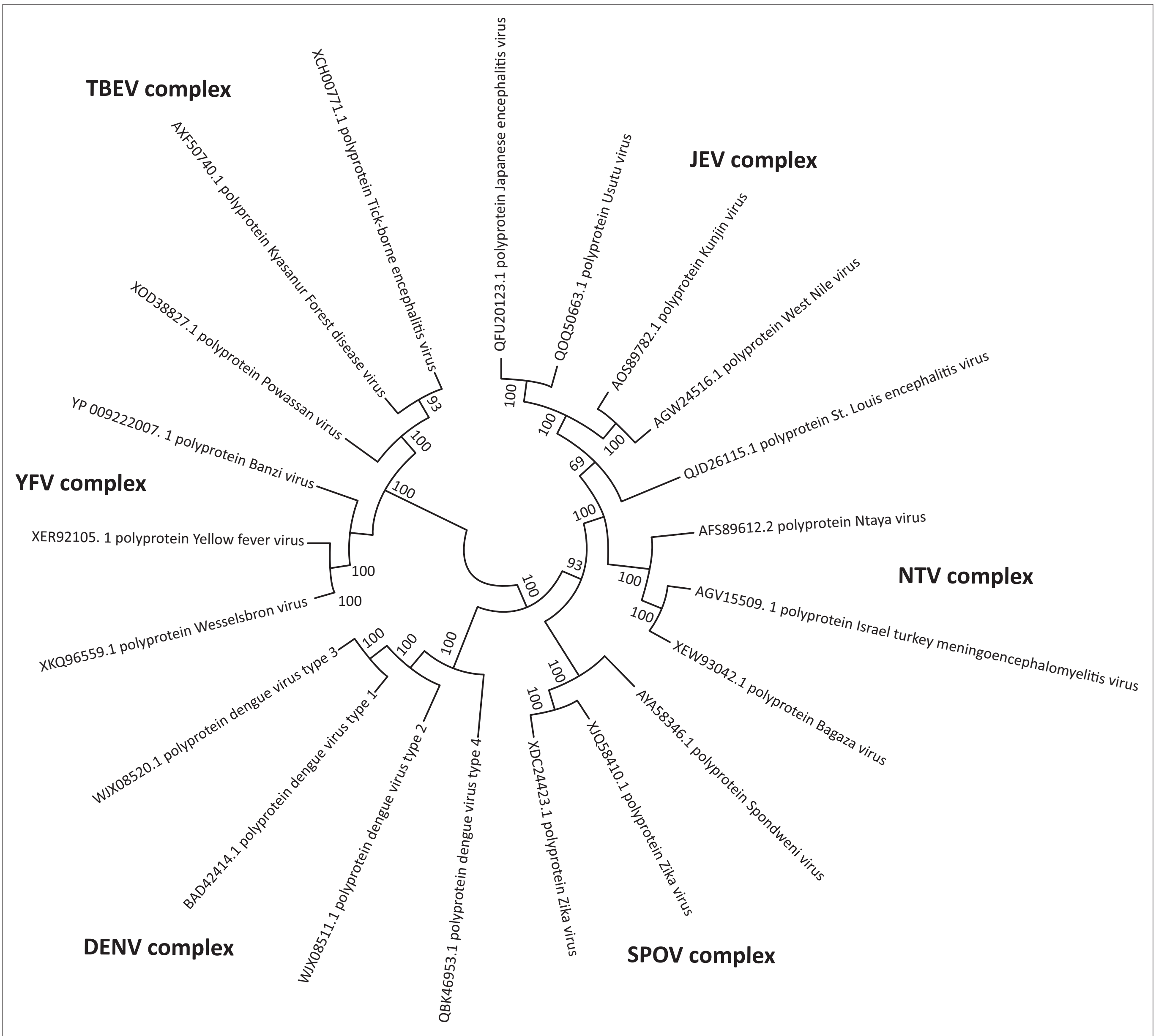
TABLE 1: Geographic distribution of selected members of the family Flaviviridae and their hosts and vectors.

Classification	Serocomplex	Virus	Amplifying host	Vector	Disease associations	Geographic distribution (references)
Mosquito borne	Japanese encephalitis virus complex	WNV	Birds	*Culex (Cx.) quinquefasciatus, Cx. pipiens, Cx. univittatus, Cx. theileri, Cx. modestus, Cx. perexiguus, Cx. neavei*	Causes fatal neurological disease in humans, horses, birds and dogs	Australia, Europe, India, Africa, Caribbean; Madagascar, North and South America[8,20,21,22,23,24,25,26,27,28,29,30,31,32]
		USUV	Various bird species, such as passeriform and strigiform	*Cx. quinquefasciatus, Cx.neavei, Cx.univittatus, Cx. perexiguus, Cx.perfuscus, Cx.pipiens, Aedes (Ae.) caspius, Mansonia(Ma.) africana Ae.albopictus, Anopheles (An.) maculipennis, Coquellittidia aurites*	Epidemic mortalities in Eurasian blackbirds Turdus Merula. Human cases of neurological disease reported	South Africa, Senegal, Kenya, Burkina Faso, Central African Republic, Morocco, Cote d'Ivoire, Uganda Nigeria, Tunisia, European countries such as Brazil, Italy[33,34,35,36,37,38,39,40,41]
		JEV	Wild and domestic birds – egrets and herons	*Cx. pipiens, Cx. quinquefasciatus, Cx. vishnui, Cx. Tritaeniorhynchus, Cx. molestus, Ae. albopictus*	Causes fatal disease in pigs and humans	Asia, the western Pacific and northern Australia[42,43]
		SLEV	Birds – passerine and columbiform species	*Cx. quinquefasciatus, Cx. pipiens, Cx. nigripalpus, Cx. tarsalis*	Human cases with fatalities	USA[44,45]
	Yellow fever virus complex	BANV	Rodents	*Cx. rubinotus*	Human cases of illness; no known mortalities	South Africa, Angola, Namibia, Botswana, Mozambique, Zimbabwe, Tanzania[8,46,47,48,49]
		WSLV	Livestock	*Cx. univittatus Ae. circumluteolus, Ae. caballus, Ae. juppi, Ae. luridus, Ae. mcintoshi, Ae. unidentatus, Ae. (Neomelaniconian) spp.*	Infects humans and causes abortion in sheep, cows and goats	South Africa, Botswana, Zimbabwe, Mozambique, Thailand, Zambia[47,50,51,52]
		YFV	Non-human primates such as monkeys, chimpanzees, baboons, bush babies and spiders	*Haemagogus spp, Ae. aegypti, Ae. albopictus, Ae. africanus, Ae. furcifer/taylori, Ae. luteocephalus, Ae. leucocelaenus, Ae. metallicus, Ae. opok, Ae. vittatus, Ae. simpsoni complex, Sabethes chloropterus*	Infects humans and causes mortalities	West and Central Africa, tropical South America, Caribbean[3,53,54,55,56,57,58,59]
	Ntaya virus complex	BAGV	Unknown	*Cx.univittatus, Cx.guiarti, Cx. ingrami, Cx. tritaeniorhynchus Cx.perfuscus, Cx. thallasius*	Causes mortalities in Partridges and pheasants	South Africa, West and Central Africa, Spain, the Arabian Peninsula, Portugal, India and Spain[9,60,61]
		ITMV	Unknown	*Ae. caspius, Cx. pipiens*	Causes neurological signs in turkeys and some captive and wild pheasants and partridges	Israel and South Africa[62,63]
		Ntaya virus (NTAV)	Unknown	*Cx. spp*	Can infect humans but not known to cause any disease	Zambia, Cameroon, Uganda, Democratic Republic of Congo, Nigeria, Kenya, Romania[64,65,66]
	Spondweni complex	SPOV	Unknown	*Ma. uniformis, Ma. africana, Ae. circumluteolus, Cx.neavei, Ae. cumminsii, Cx.univitattus, Er. silvestris*	Infects humans and causes illness	South Africa, Botswana, Namibia, Nigeria, Angola, Ethiopia,Burkina Faso, Cameroon, Gabon, Mozambique[67,68,69,70]
		ZIKV	Non-human primates	*Ma. uniformis, Ae. africanus, Ae. aegypti, Ae. furcifer, Ae. albopictus, Ae. opok, Ae. jamoti, Ae. flavicollis, Ae. tarsalis, Ae. grahami, Ae. vittatus, Ae. taeniorostris, Ae. fowleri, Ae. dalziella, Ae. minimus, Ae. luteocephalus, Ae. metallicus, Ae. neoafricanus, An. gambiae, Er. uinquevittatus, Er. inornatus*	Infects humans and non-human primates	Asia Pacific, South and Central America, moving into southern areas of North America, East, West and southern Africa[71,72,73,74,75,76,77,78]
	Dengue complex	DENV 1-4	Humans	*Ae. aegypti*	Infects humans and causes fatalities	Widely distributed in most Africa countries, tropical and sub-tropical Asia-Pacific, north- east Australia; South and Central America[71,79,80,81]
Tick borne	Mammalian tick-borne virus complex	POWV	Small to medium mammals	*Ixodes spp.*	Infects and causes fatal cases in humans and mammals such as deer, foxes and monkeys	United States of America, Canada, Russia and Mexicco[82,83]
		KFDV	Rodents, shrews, bats, monkeys	*Haemaphysalis spinigera Haemaphysalis spp., Dermacentor, Rhipicephalus* and *Ixodes genera*	Infects humans and mammals such as deer and domestic ruminants	India[84,85,86]
		TBEV	Small rodents and migratory birds	*Ixodes ricinus, Ixodes persulcatus, Dermacentor reticulatus*	Humans and wild mammals such as deer, sheep, goats and cattle	Italy, Austria, Belarus, Bulgaria, Germany, Czech Republic, Denmark, Estonia, Finland, Croatia, France, Latvia, Lithuania, Hungary, Netherlands, Poland, Russia, including Siberia, as well as in parts of China and Japan[87]
Insect specific	-	Kamiti River virus (KRV)	-	*Ae. mcintoshi*	-	Kenya[88,89]
No known vector	Entebbe bat virus complex	Entebbe bat virus	Bat	Unknown	Unknown	Uganda[90]

Note: Please see the full reference list of the article, Sibanda-Makuvise A, Ndudzo A, Burt FJ. Flaviviruses of public health concern in South Africa: Present and future threats. 2025;40(1), a754. https://doi.org/10.4102/sajid.v40i1.754, for more information.

ITMV, Israel turkey meningoencephalitis virus; WNV, West Nile virus; USUV, Usutu virus; JEV, Japanese encephalitis virus; SLEV, Saint Louis encephalitis virus; BANV, Banzi virus; WSLV, Wesselsbron virus; YFV, yellow fever virus; BAGV, Bagaza virus; DENV, dengue virus; SPOV, Spondweni virus; ZIKV, Zika virus; POWV, Powassan virus; KFDV, Kyasanur forest virus; TBEV, tick-borne encephalitis virus.

are asymptomatic. Some flaviviruses are neurotropic, causing severe neurological syndromes including encephalitis, meningitis and acute flaccid paralysis, for instance, WNV, USUV, JEV and TBEV. Other flaviviruses cause visceral disease, which results in haemorrhagic syndromes, liver failure and vascular compromise, for instance, YFV = virus and POWV.



Note: Phylogenetic analysis was conducted using molecular evolutionary genetic analysis (MEGA-11) software. The complete polyprotein amino acid sequences of flaviviruses were retrieved from the National Center for Biotechnology Information (NCBI) database and aligned using Clustal W. The phylogenetic tree was constructed by using the maximum likelihood method, and the consensus tree representing 200 bootstrap is presented.[16,17,18,19] The external branches represent the NCBI accession number and the virus species. The numbers next to each node represent a measure of support for the node; 100 is maximum support.

SPOV, Spondweni virus; DENV, dengue virus; NTV, Ntaya virus; YFV, yellow fever virus; JEV, Japanese encephalitis virus; TBEV, tick-borne encephalitis virus.

FIGURE 1: Relationship of selected flaviviruses of public health significance, indicating antigenic relationship based on polyprotein analysis.

West Nile virus and Wesselsbron virus in South Africa

West Nile virus was initially detected in 1937 in Uganda in a febrile patient. Thereafter, epidemics have been reported in Europe, Africa, North America, the Middle East and West Asia. West Nile virus is a member of the JEV serocomplex with nine phylogenetic lineages based on genetic sequence variation, WNV lineage 1 to lineage 9 (WNV-L1 – L9). WNV-L1 is divided into clades A, B and C; Clade A includes strains from Africa, Europe, America and the Middle East, and Clade B, also known as Kunjin virus, was isolated in Australia and Clade C includes strains detected in India.[91] WNV-L1 and L2 were detected in South Africa and are of public health concern; they are the most pathogenic, widely distributed and implicated in several outbreaks worldwide.[91,92] In addition to WNV-L1 and L2, L7 also known as Koutango virus (KOUTV) and L8 circulate in Africa.[93]

There are other lineages that have not yet been associated with human and/or animal disease and not isolated in Africa: these include WNV-L3 (Rabensburg virus), detected in the Czech Republic; WNV-L4, isolated and reported in Russia; WNV-L5, isolated in India; WNV-L6, isolated in Spain and WNV-L9, isolated in Austria.[94,95,96] Viruses from these lineages have not yet been associated with human or animal disease.

West Nile virus is endemic to South Africa, particularly in the Highveld and Karoo regions, where its primary vector, *Cx univittatus*, and avian hosts such as corvids, passerine and raptors are prevalent.[6,29] Other vectors of WNV include *Cx. theileri*, *Cx. pipiens*, Cx. *quinquefasciatus and Aedes spp.* mosquitoes.[26,29,97] Epidemics of WNV are typically triggered by flooding and elevated temperatures, which foster an ideal environment for vector breeding. The largest WNV epidemic in South Africa occurred in 1974, resulting in over 10 000 human cases across a 2500-km² area of the Karoo and Northern Cape Provinces.[28] This was followed by a smaller epidemic between 1983 and 1984, coinciding with the Sindbis (SINV) virus outbreak, in the Witwatersrand-Pretoria region, now known as Gauteng.[98] SINV is an arbovirus from the family *Togaviridae*. In all recorded epidemics, WNV infections were self-limiting and primarily associated with mild febrile illness; symptoms included rash, myalgia and arthralgia, with no reported fatalities. Seroprevalence studies revealed that 55% of humans and 53% of wild birds in the affected areas tested positive for WNV, with some regions reporting seroprevalence as high as 80% to 85%.[29] Prior to these epidemics, sporadic cases were documented in the former Transvaal (Gauteng) and Orange Free State (now referred to as Free State), and serological surveys confirmed widespread WNV distribution among humans and various animal species, including cattle, horses and wildlife.[99,100,101]

West Nile virus outbreaks were recorded on the Highveld in 1974, where over 10 000 febrile cases were reported; in 1984 and 2004, where hundreds of people were affected. In addition to outbreaks, sporadic WNV cases are diagnosed by the National Institute for Communicable Diseases (NICD). Cases are usually confirmed by detecting immunoglobulin M (IgM) antibody; however, serological cross-reactivity must be considered when interpreting results. Cases have been recorded from different provinces in South Africa, for example, in 2017, a veterinarian from Mpumalanga province who frequently performed bird-tagging and animal autopsies, tested positive for WNV-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies by enzyme-linked immunosorbent assay (ELISA).[102] On a separate occasion, a patient from a farm in the Northern Cape province tested positive for WNV.[103]

In addition to the acute cases detected by NICD, seroprevalence studies and vector surveillance have assisted with identifying WNV in South Africa. A seroprevalence study detected WNV antibodies in 7.9% of veterinarians in South Africa, their distribution corresponding with reported cases in WNV-positive animals.[104] A passive entomological survey conducted from 2011 to 2018 detected WNV in *Cx. univittatus, Cx. pipiens, Cx. theileri, Cx. poicilipes, Cx. simpsoni, Cx. bitaeniorhynchus, An. gambiae and Aedes* mosquitoes from Limpopo, Mpumalanga and Gauteng provinces. The study indicated the emergence of potential new mosquito vectors in conservation and peri-urban areas.[8] Vector competency studies are required to confirm the role of these mosquitoes in the transmission and spread of WNV in South Africa.

West Nile virus is neurotropic and affects humans, both domesticated and wild animals and avian species. In humans, approximately 80% of WNV infections are asymptomatic, while 20%–25% develop West Nile fever, a self-limiting condition characterised by high fever, headache, body aches, maculopapular rash, nausea and vomiting.[105] Only about 1% of cases lead to serious neurological complications, including encephalitis, meningitis, myelitis and acute flaccid paralysis.[92,105] Disturbingly, WNV has been detected in 3.5% of unresolved cases of human neurological disease in Gauteng provincial hospitals.[106] In a separate study, WNV was identified as the causative agent in 8% to 11% of hospitalised patients from Mpumalanga and Gauteng provinces presenting with acute febrile and/or neurological symptoms.[107] These findings indicate that WNV is overlooked in neurological cases and should be included in diagnostic tests. Horses are highly susceptible domestic animals and are sentinels for WNV. Clinical signs in horses can range from asymptomatic to severe neurological symptoms due to virus-induced encephalitis.[23,108] In birds, WNV infections are typically asymptomatic, but in the United States of America (USA), species such as blue jays (*Cyanocitta cristata*) and crows (*Corvus brachyrhynchos*) are highly susceptible, often experiencing fatal neurologic disease.[109]

West Nile virus is a zoonotic virus maintained in a sylvatic cycle involving various avian and mosquito species. Migratory birds such as corvids, passerines and raptors are primary reservoirs and amplifying hosts, responsible for long-distance dispersal of WNV. *Cx. univittatus* is the primary vector[109,110] and can transmit WNV to humans, horses and other species through bites. These hosts are considered ‘dead-end’ as the levels of viraemia are below the threshold level required to transmit to arthropods. Migratory birds such as barn swallows, European rollers and bee-eaters play a crucial role in WNV’s geographic distribution. Remarkably, ticks have been found naturally infected with WNV; however, their competency as vectors remains poorly understood.[111] Human-to-human transmission of WNV has been recorded through organ transplants,[112] blood transfusions[113,114] and breast milk,[115] with one report of mother-to-child transmission.[116]

Wesselbron virus was initially isolated from infected sheep in 1955 in the small rural town of Wesselsbron, in the northern district of the Free State, South Africa. This initial outbreak was marked by significant mortality in newborn lambs and abortions in pregnant ewes.[117] Wesselsbron virus causes abortion and the death of newborn offspring in cattle, sheep and goats. In 1956, WSLV antibodies were detected in sheep during an Rift Valley fever (RVF) outbreak in Kroonstad, northeast of Wesselsbron. Since then, WSLV has been identified in various vertebrates, including cows, sheep and humans.

Wesselsbron virus is primarily transmitted by infected *Aedes* mosquitoes, with *Culex*, *Anopheles* and *Mansonia* also implicated. Reservoirs include domesticated ruminants, wild birds and rodents.[118] The levels of viraemia in sheep and cattle can reach threshold levels that are sufficient to infect mosquitoes.[118] Humans can contract WSLV through mosquito bites or handling infected tissues. There are no reports of human-to-human transmission.

Initially, seroprevalence studies indicated that WSLV circulated predominantly in the more tropical parts of South Africa, such as KwaZulu-Natal. Cattle, sheep, goats and donkeys were infected due to the ecological niche of mosquito vectors such as *Ae. circumluteolus*, *Ae. mcintoshi* and *Ochlerotatus juppi*.[71,119] In contrast, more temperate plateau regions such as the Free State, Karoo and Gauteng province had a lower seroprevalence. Similarly, serological studies on humans from KwaZulu-Natal, the southern Cape, revealed WSLV prevalence rates of 32% and 0.7%, respectively. The high variance in the WSLV prevalence rates in two places is attributed to factors such as vector distribution, human activities and environmental conditions. A seroprevalence study, using an indirect IgG ELISA, from the Free State recorded a high seroprevalence rate of 27.6% (54/196) in humans, 19.3% (396/2052) in cattle and 1.1% (1/88) in sheep. Although neutralisation assays were not carried out for accurate confirmation of results, the ELISA used a recombinant ED III antigen, which can differentiate between flaviviruses.[101] The high serodetection rate may have been due to including samples collected after the 2010–2011 RVF outbreak, when there was excessive rainfall that likely favoured increased mosquito populations and potential for other arboviruses, such as WSLV, to occur. Differences in sample collection times and geographic location are possible explanations for the high variance observed in seroprevalences between species. During the 2010–2011 RVF outbreak, two human cases of Wesselsbron disease were reported in South Africa.[120] Like WNV, WSLV is implicated in neurological equine disease.[108]

The actual burden of WSLV is unknown due to serological cross reactivity with other flaviviruses and similar clinical presentation in livestock to RVFV, therefore misdiagnosed if not specifically tested. WSLV and RVFV often occur concurrently and are transmitted by similar vectors.[117] A wide range of domestic animals, such as cattle, sheep, camels, pigs, goats, donkeys and horses, are susceptible to WSLV infection, with WSL disease being particularly significant in sheep in South Africa.[120] In humans, WSLV infection typically manifests as a sudden onset of influenza-like symptoms, including headache, fever, myalgia, rigours and arthralgia.[11]

Flaviviruses known or potentially causing disease in South Africa

Usutu virus (USUV), SPOV, BAGV and BANV are lesser-known flaviviruses previously detected in South Africa, but their current status is unknown.

Usutu virus

Usutu virus is a member of the JEV serocomplex, which is classified into eight distinct genetic lineages; namely, three African (AF1 -3) and five European (EU1-5). Usutu virus is phylogenetically close to WNV, sharing approximately 76% of its amino acid sequences.[121]

Usutu virus is a zoonotic pathogen, first isolated in South Africa in 1959 from *Cx. neavei* mosquitoes. Like WNV, USUV is primarily transmitted and maintained between vectors, that is *Culex* species and birds as the amplifying hosts. Incidentally, USUV is transmitted to humans and animals during their blood meal. Usutu virus isolates have been obtained from various mosquito species, including *Cx. pipiens*, *Cx. perexiguus, Cx. perfuscus, Coquillettidia aurites, Ae. caspius*, *Ae. albopictus,* and *Ma. africana* in other regions.[28,37] A study in Germany demonstrated that USUV can infect bats, making them possible amplifying hosts.[36]

Usutu virus is an endemic virus in the European continent, with phylogenetic studies indicating its introduction from Africa. Usutu virus was first detected in Spain in the 1950s and later detected between 1970 and 1980 in Italy and Austria.[13] It is suspected that long-distance migratory birds such as the babbler (*Sylvia curruca)* and/or the kestrel *(Falco tinnunculus)* introduced USUV into Europe, where resident wild birds such as blackbirds, magpies or sparrows distributed the virus throughout the continent.[35]

Even though avian species are a reservoir for USUV, some avian species, such as Eurasian blackbirds (*Turdus merula*), sparrows (*Passer domesticus*) and great grey owls (*Strix nebulosa*), are highly susceptible to USUV infections.[122] This supports the recent introduction with insufficient time for co-evolution of the virus and the avian species. Usutu virus outbreaks have been recorded in Italy and Austria among grey owls (*Strix nebulosi*), sparrows (*Passer domesticus*), Eurasian blackbird (*Turdus merula*) and other blackbird populations.[13,122] In addition, USUV has been detected in bats, birds, horses and mosquito vectors in European countries such as Spain, Austria, Switzerland, Hungary, the Czech Republic, Germany and Belgium. Locally acquired cases of USUV infection have been reported in Italy, causing meningoencephalitis in immunocompromised human patients.[123,124]

In South Africa, USUV shares the same vectors and hosts with WNV; hence there is potential for this virus to occur. However, in the absence of awareness, absence of diagnostic testing and serological cross reactivity with WNV in surveillance studies resulting in possible misinterpretation of tests, it is unknown if USUV is currently circulating in the country. Symptoms of USUV infections in humans in Europe range from mild or asymptomatic to severe neurological disease. The association of USUV with severe neurological disease warrants further investigation in South Africa.

Spondweni virus

Spondweni virus is a zoonotic pathogen transmitted and maintained in a sylvatic cycle between mosquito vectors and NHPs. Spondweni virus was initially isolated from a pool of

Ma. uniformis mosquitoes caught in the subtropical northern KwaZulu-Natal.[65] Initially, SPOV was mostly isolated from *Ae. circumluteolus* and other mosquito vectors, including *Ma. africana, Ae. cumminsi and Er. Silvestris.*[65] Due to its vector biology, the potential for urban SPOV epidemic cycles was deemed low; concurring serological studies in the same region indicated very low seroprevalence.[65]

However, recent epidemiological changes have led to the detection of SPOV in anthropophilic mosquitoes such as *Cx. quinquefasciatus* in Haiti.[125] In South Africa, SPOV has been isolated from *Ae. cumminsi*, *Ae. circumluteolus, Cx. univittatus, Cx. neavi, Er. silvestris, Ma. africana* and *Ma. uniformis*. Vector competency studies demonstrated low infection and dissemination rates in *Cx. quinquefasciatus*, *Ae. albopictus* and *Ae. aegypti post* exposure to moderate oral doses of infectious SPOV, suggesting that the virus can potentially adapt to *Ae. aegypti* as a vector, which would impact the potential for spread.[74] *Ae. aegypti*, a vector for ZIKV, YFV and DENV, is an invasive urban mosquito that breeds in human-made containers, lives close to people and has a blood meal from humans and/or animals, consequently facilitating the urban transmission of arboviruses.

Symptoms of SPOV infection typically present as acute febrile illness, including headache, fever, chills, nausea, myalgia, arthralgia and a maculopapular, pruritic rash.[65,125] While most cases are mild, some can progress to vascular leakage, shock or neurological involvement, particularly in immunocompromised patients. There is an incident of two laboratory staff members who presented with illness after handling infected materials at Ndumu in South Africa.[65] Spondweni virus causes fetal harm in an immunocompromised mouse model, deficient in type I interferon signalling, suggesting potential for similar effects in humans during pregnancy.[126]

Bagaza virus

Bagaza virus is a member of the Ntaya serocomplex, which causes neurological disease in avian species, particularly turkeys and other Phasianidae family. Bagaza virus is closely related to Israel turkey meningoencephalitis virus (ITMV); they have a nucleotide similarity of 96 to >99%.[127] Due to their high similarity index, it has been proposed that ITMV and BAGV are treated as one species.[128]

Bagaza virus was initially isolated in South Africa in 1978 from turkeys exhibiting clinical signs akin to those of the ITMV.[63] It was later detected in dead Himalayan monal pheasants with neurological symptoms in 2016–2017.[128] Recently, BAGV was isolated from mosquitoes collected from an urban site in Bloemfontein.[9] The isolate, VBD 74/23/3, was closely related to ZRU96/16/2, isolated from dead Himalayan monal pheasants with neurological symptoms in 2016–2017 and MP-314-NA-2018, an isolate from mosquitoes in northwestern Namibia with genetic distances of 0.0085 and 0.016, respectively.

Bagaza virus is an emerging pathogen that causes febrile illness in humans. It was known to occur mainly in Africa until its recent discovery from *Cx. tritaeniorhynchus* mosquito pools in India. Concurrently, 15% of patients with acute encephalitis tested positive for BAGV neutralising antibodies,[54] indicating that BAGV might be causing acute encephalitis in these patients.

Banzi virus

Banzi virus was initially isolated in 1956 from a febrile child in South Africa. Subsequent serological studies in northern KwaZulu-Natal indicated that humans were previously infected with BANV, with neutralising antibodies detected using mouse protection tests.[51] Banzi virus is transmitted and maintained in a sylvatic cycle between *Cx. rubinotus* mosquitoes as the primary vector and rodents as natural hosts, with infrequent human feeding. Recent entomological surveys detected BANV in mosquitoes from peri-urban and conservation areas in South Africa.[8] However, information on its clinical presentation in humans and animals is limited, with only one other reported case of febrile illness in Tanzania.

Factors responsible for the emergence of flaviviruses

Flaviviruses are arboviruses with three overlapping transmission cycles: sylvatic, rural and urban, with different vectors and hosts involved in each cycle and location.[129]Arboviruses are transmitted to humans, birds, livestock and NHPs, which act as reservoir or amplifying host through bites of hematophagous vector arthropods such as ticks and mosquitoes. The sylvatic transmission cycle is maintained between the arthropod vector (mosquitoes or ticks) and a reservoir or amplifying host such as NHPs, rodents, birds and bats. In a rural transmission cycle also known as the emergence zone, human activities such as hunting, farming and herding encroach on the sylvatic transmission cycle with humans infected through arthropod bites. The urban transmission cycle involves 'domesticated' mosquitoes, the mosquitoes that breed on stagnant water around human settlements as vectors and humans as hosts.[129]

Many arboviruses, including flaviviruses such as YFV, ZIKV and WNV, have escaped from their sylvatic cycle and spread globally as a result of anthropogenic factors such as climate change, urbanisation, population growth, global travel and most significantly, viral mutations allowing adaptation to new vectors.[130,131] Increased ambient temperatures and flooding as a result of climate change create favourable environments for flavivirus vectors, thereby heightening the risk of vector–human interactions and subsequent infection surges.[132,133] Additionally, migration and/or displacement of people and livestock from one place to another (change in land use) due to climate disasters such as flooding and drought can trigger outbreaks of infectious diseases.

The rising human population, particularly in densely populated cities like Johannesburg, exacerbates the emergence of flaviviruses. Overburdened sewage systems in these urban areas frequently burst, resulting in standing water in the streets, ideal breeding grounds for mosquitoes. In addition, authorities are expanding urban areas into previously protected wild habitats, increasing opportunities for arthropods to interact with wildlife and humans, further elevating the risk of transmission.

Moreover, the international movement of people, livestock and cargo facilitates the dispersal of vectors and pathogens to new regions, leading to the emergence of infections in previously unaffected areas. Changes in viral genetics are particularly concerning, as they could result in the development of new variant strains with heightened virulence and viraemia levels in vertebrates. For example, WNV-L2 was considered less virulent than WNV-L1 before the occurrence of the six amino acid substitutions at E(V159I), NS1 (L338T), NS2A (A126S), NS3 (N421S), NS4B (L20P) and NS5 (Y254F) proteins. Amino acid substitutions resulted in increased virulence, causing serious disease in South Africa and other countries among humans, horses and birds.[134] Genetic mutations also enable viruses to adapt and live in new environments and infect different hosts and vectors, enhancing vector competence and transmission rates. Lastly, the development of pesticide resistance among vectors poses a significant challenge, complicating vector management and contributing to the emergence of flavivirus outbreaks. For instance, insecticide resistance has been recorded in some flavivirus vectors such as *Cx. quinquefasciatus*, *Cx. pipiens*, *Ae. albopictus* and *Ae. aegypt*.[135,136,137,138,139,140,141,142,143]

Detection and diagnosis

In South Africa, arthropod survey studies collect mosquitoes and ticks in January to April and November to December (highly dependent on rains for the year), when it is wet and warm, tick and mosquito season. Arboviral infections such as WNV are recorded by the NICD at this time. During the mosquito and tick season, an arboviral infection should be suspected when a patient presents with flu-like symptoms such as fever, headache, muscle pains and even seizures and neurological signs. The gold standard for accurately diagnosing flavivirus infections is viral isolation in culture. However, this method requires skilled personnel to maintain the virus viability without contamination, is time consuming and necessitates specialised biosafety and containment facilities that are often unavailable in resource-limited settings. Nucleic acid amplification tests (NAAT) have emerged as the most rapid and accurate diagnostic tools, offering the advantage of detecting both viable and non-viable infectious agents.[144] Detection of viral nucleic acids in vectors and tissues from infected humans or animals such as the spleen, brain, serum, liver and cerebrospinal fluid (CSF) is carried out by reverse transcription-polymerase chain reaction (RT-PCR) followed by sequence analysis.[145,146,147,148]

A significant challenge with nucleic acid detection and viral isolation for diagnostic purposes is that viraemia may be very low, as severe symptoms often develop only after viraemia has declined to undetectable levels.[149] Consequently, serological testing of serum and/or CSF for antibodies remains crucial in surveillance studies.[150] However, results must be interpreted cautiously due to potential cross-reactions stemming from antigenic similarities among different flaviviruses. Consequently, a positive viral-specific IgG and IgM test should be confirmed with a neutralising antibody test to prevent misdiagnosis due to serological cross-reactivity. In South Africa, certain private and public laboratories offer both serological and PCR tests for flaviviruses such as ZIKV, DENV, YFV and WNV, at the discretion of the examining doctor. There are various commercially available ELISA kits (Table 2) to detect flaviviruses; however, neutralisation tests are required to accurately differentiate between flaviviruses. These kits are quite expensive and laboratories from low-income countries frequently cannot afford them, and many rely on in-house assays. In South Africa, the NICD offers a diagnostic service based on NAAT and serology, for selected arboviral infections known in the country or with potential to occur in travellers from endemic regions.

Additionally, post-mortem histopathological examinations and immunohistochemistry using formalin-fixed tissue samples of animal tissues such as brain, spleen and liver

TABLE 2: Commercial enzyme-linked immunosorbent assay kits available in South Africa for the detection of flaviviruses.

Kits	Coat protein	Brief description
ID Screen® Flavivirus Competition	pr-E antigen	A competitive ELISA kit that detects anti-pr-E flavivirus antibodies of WNV, JEV, TBEV, JEV, USUV, ZIKV and DENV in multiple species
ID Screen® West Nile Competition multi-species	pr-E antigen	A competitive ELISA kit that detects anti-pr-E antibodies in multiple species. The kit detects WNV, JEV, TBEV, JEV, USUV, ZIKV and DENV antibodies in multiple species, including humans
ID Screen® West Nile IgM Capture	Anti-horse IgM polyclonal antibody	IgM Antibody Capture ELISA (MAC) kit that detects anti-prE IgM antibodies in horse serum and plasma, indicating recent infection
Panadea WNV Lin 1/2 (NS1) Combo IgG ELISA Kit	NS1	This kit is based on the patented IgG FcγR ELISA technology intended for qualitative detection of IgG antibodies against WNV-L1 and WNV- L2
WNV Antibody Test Kit, ELISA, InBios International, Inc	Not stated	A kit designed to detect recent exposure of horses to WNV by detection of IgM antibodies in equine serum to WNV-derived recombinant antigen
West Nile *Detect*™ IgG ELISA	Not stated	A kit used to detect antibodies in human serum to WNV-derived recombinant antigen
Anti-USUV ELISA (IgG), EUROIMMUN	Recombinant USUV structural protein	A semi-quantitative or quantitative *in vitro* assay kit for the detection of human IgG antibodies against USUV in plasma or serum

ELISA, enzyme-linked immunosorbent assay; WNV, West Nile virus; USUV, Usutu virus; JEV, Japanese encephalitis virus; DENV, dengue virus; ZIKV, Zika virus; TBEV, tick-borne encephalitis virus.

from suspected cases can be used for detection, with confirmation by nucleic acid amplification followed by nucleotide sequencing.

Preventative measures and therapeutic strategies

Preventing flavivirus infections in humans and animals primarily relies on effective mosquito vector control programmes. In addition, mosquito bite prevention measures, including repellents, sleeping under nets, putting animals in enclosed structures during the night and avoiding low-lying wetland areas, can significantly reduce their interaction with vectors. Veterinary and laboratory personnel handling infected or potentially infected tissues and materials must wear personal protective equipment, such as gloves, masks and face shields, to prevent contact with the virus and should avoid procedures that aerosolise the virus. In some countries, blood donors returning from WNV-afflicted areas are excluded to avoid transmission of WNV during blood transfusion. Although rare, human-to-human transmission has been recorded for WNV in the USA.[151]

Continuous use of repellents and insecticides has raised challenges such as resistance and environmental harm. Therefore, biological control measures such as gene editing to obtain sterile male mosquitoes are being evaluated in Tanzania, while Wolbachia-based approaches have been adopted by countries such as Indonesia, Vietnam, Malaysia, Thailand and Taiwan.[152] *Wolbachia pipientis,* an obligate intracellular mosquito parasite, uses two mechanisms to reduce the transmission of arboviruses: it induces cytoplasmic incompatibility and produces an infertile progeny from an uninfected female and a *Wolbachia*-infected male, thus reducing the vector population. *Wolbachia* infection also blocks the replication of pathogens such as viruses in the mosquito, reducing the efficiency of vectors.

Flavivirus vaccine development is a critical concern, especially for ZIKV and DENV, due to the complexity of the immune response and the severity of the disease outcomes. At present, Q-denga (TAK-003), a live attenuated vaccine containing DENV 1–4 serotypes for children between 9 and 16 years, is available for DENV. There are also highly effective and safe vaccines against some flaviviruses, such as YFV and JEV, for humans. The live attenuated 17D-204 YF vaccine developed by weakening the Asibi yellow fever strain through serial passaging of cell cultures has been used since 1938. The vaccine provides lifelong protection for 80%–90% people within 10 days of vaccination. Even though there are antigenic similarities in flaviviruses, the 17D-204 YF vaccine does not offer reliable cross-protection against ZIKV, DENV and WNV. However, some flavivirus vaccines provide some cross-protection to related viruses and/or within the same serocomplex in other models. For instance, WNV vaccination offers protection against USUV disease in mice[153] and also, JEV and Saint Louis encephalitis virus (SLEV) vaccination provided protection to lethal WNV challenge in a hamster model.[154]

Currently, there are no licensed vaccines for humans against WNV, WSLV, USUV, BAGV, SPOV or BANV. However, a number of WNV vaccines have gone through human clinical trials, such as VRC-WNVDNA020-00-VP, HydroVax-00, Chimerivax-WNV02 and rWN/DEN4Δ30 and a DNA vaccine encoding WNV prM/E. Among these, ChimeriVax-WNV02 is the most promising candidate assessed in phase II clinical trials.[155] Currently, there is an FLAVIVACCINE project that aims to develop a broad-spectrum, mosquito saliva-targeted vaccine that protects against multiple flaviviruses.

For veterinary use, a live, attenuated WSLV vaccine exists for non-pregnant animals, providing lifelong immunity.[117] WNV vaccines, including West Nile-Innovator DNA vaccine (Fort Dodge Animal Health, commercialised by Pfizer) and Recombiteck Equine West Nile Virus Vaccine, produced by Merial-Sanofi Aventis,[156,157] available for use in South Africa.

Currently, there are no specific antivirals against flaviviruses. In general, antivirals are designed to interfere with critical stages of the virus life cycle, that is, replication, protein synthesis, assembly, entry and virus egress. Research in this field is ongoing, with a lack of funding to assess antivirals through all the clinical stages a major obstacle. A number of compounds have been evaluated for the antiviral efficacy only in *in vitro*, *in vivo* and first-stage clinical trials. Notably, ZIKV-Ig (ZIKV), TY014 (YFV) and Tyzivumab (ZIKV) were assessed in first-stage clinical trials.[158] Some Food and Drug Administration (FDA)-approved drugs, such as Dasabuvir, Efavirenz and Tipranavir inhibit replication of multiple flaviviruses *in vitro.*[159] Dasabuvir, an antiviral drug against the hepatitis C virus (HCV), demonstrated strong and antiviral effects against ZIKV, WNV and TBEV in Vero cells. Tipranavir, an antiviral for treating human immunodeficiency virus (HIV) infection, had antiviral effects against ZIKV and TBEV in Vero cells. Another antiviral agent for treating HIV, efavirenz, demonstrated antiviral effects against TBEV, WNV, ZIKV, DENV and YFV in Vero cells.[159] Interestingly, targeted therapies, including monoclonal antibodies directed against the flavivirus surface E glycoprotein and non-structural protein 1 (NS1), have been shown to be effective against WNV in mice.[160]

Recommendations

To effectively address the public health threats posed by flaviviruses in South Africa, several key recommendations should be implemented. Initially, it is essential to establish and maintain robust One Health surveillance programmes that monitor the prevalence and spread of flaviviruses in both human and animal populations.[161] Such surveillance should include syndromic screening for lesser-known flaviviruses such as BANV, BAGV, WSLV and USUV. In addition, investing in the continuous development and validation of molecular diagnostic tools tailored to detect local strains of flaviviruses is crucial. This investment will help reduce misdiagnosis and improve the accuracy of surveillance efforts. Public awareness campaigns should also be implemented to educate the public and healthcare professionals about flavivirus transmission, symptoms and preventive measures. Emphasising the importance of avoiding arthropod bites and using protective clothing can significantly reduce infection rates.

Furthermore, prioritising research and development of effective vaccines for humans against flaviviruses is vital, particularly for those that currently lack licensed vaccines. Investigating the potential for cross-protection among flavivirus vaccines, as observed with USUV and WNV, should also be a focus. A One Health approach with collaboration between public health and veterinary services is essential to ensure a comprehensive approach to flavivirus management. This includes implementing vaccination programmes for animals and monitoring zoonotic transmission. Additionally, clinical guidelines to diagnose and manage flavivirus infections should be regularly reviewed and updated to incorporate the latest research findings and technological advancements.

Conclusion

Flaviviruses present significant public health threats in South Africa, with the potential for re-emergence and rapid spread exacerbated by environmental and societal changes. While WNV remains the most recognised flavivirus,[24,162] the presence of lesser-known variants highlights the need for heightened vigilance. Effective surveillance, accurate diagnostic tools and public education are crucial in mitigating the impact of these viruses. Moreover, the development of vaccines and therapeutic agents is essential to protect both human and animal health. By adopting a proactive and collaborative approach, South Africa can better prepare for and respond to the challenges posed by flavivirus infections, ultimately safeguarding public health and economic stability.

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Competing interests

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Authors' contributions

A.S.-M. was responsible for the conceptualisation, methodology, investigation and writing of the article. A.N. was involved with the writing, reviewing and editing of the article. F.J.B. contributed to investigation, validation, reviewing, editing, supervision and provision of resources.

Ethical considerations

This study was conducted as a desktop review, where information was obtained from publicly available data and literature. The study did not require formal ethical approval involving human and/or animal subjects. Instead a waiver of ethical review (UFS-HSD2024/0148/2910) was obtained from the University of Free State Human Research Ethics Committee. The waiver was granted on the grounds that the study did not involve interaction with human participants, collection of personal data, or any procedures that could pose risk or harm to individuals.

The research was conducted in full accordance with the ethical principles outlined in the Declaration of Helsinki, as revised in 2013, ensuring respect for the integrity of data sources and the responsible use of information.

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Data availability

The data that support the findings of this study are available from PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (https://scholar.google.com/).

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References

1. National Institute for Communicable Diseases. Chikungunya cases rise: South African travellers urged to take care [homepage on the Internet]. 2025 [cited 2025 Jul 18]. Available from: https://www.nicd.ac.za/chikungunya-cases-rise-south-african-travelers-urged-to-take-care/
2. Samarasekera U, Triunfol M. Concern over Zika virus grips the world. Lancet. 2016;387(10018):521–524. https://doi.org/10.1016/S0140-6736(16)00257-9
3. Mutebi JP, Barret AD. The epidemiology of yellow fever in Africa. Microbes Infect. 2002; 4(14):1459-1468. https://doi.org/10.1016/S1286-4579(02)00028-X
4. Haider N, Hasan MN, Onyango J, et al. Global dengue epidemic worsens with record 14 million cases and 9,000 deaths reported in 2024. Int J Infect Dis. 2025;158:107940. https://doi.org/10.1016/j.ijid.2025.107940
5. Vector Disease Control International. West Nile virus. 2025 [cited 2025 Jul 18]. Available from: https://www.vdci.net/vector-borne-diseases/west-nile-virus-education-and-mosquito-management-to-protect-public-health/
6. Venter M, Swanepoel R. West Nile virus lineage 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. Vector Borne Zoonotic Dis. 2010;10(7):659–664. https://doi.org/10.1089/vbz.2009.0230
7. Jupp PG, Kemp A. Studies on an outbreak of Wesselsbron virus in the Free State Province, South Africa. J Am Mosq Control Assoc. 1998;14(1):40–45.
8. MacIntyre C, Guarido MM, Riddin MA, et al. Survey of West Nile and banzi viruses in mosquitoes, South Africa, 2011–2018. Emerg Infect Dis. 2023;29(1):164.
9. Sekee TR, Bubuluma R, Van Jaarsveldt D, Bester PA, Burt FJ. Multiplex PCR method for MinION sequencing of Bagaza virus isolated from wild caught mosquitoes in South Africa. J Virol Methods. 2024;327:114917. https://doi.org/10.1016/j.jviromet.2024.114917
10. Bakonyi T, Erdélyi K, Ursu K, et al. Emergence of Usutu virus in Hungary. J Clin Microbiol. 2007;45(12):3870–3874. https://doi.org/10.1128/JCM.01390-07
11. Diagne MM, Faye M, Faye O, et al. Emergence of Wesselsbron virus among black rat and humans in Eastern Senegal in 2013. One Health. 2017;3:23–28. https://doi.org/10.1016/j.onehlt.2017.02.001
12. Savini G, Monaco F, Terregino C, et al. Usutu virus in Italy: An emergence or a silent infection? Vet Microbiol. 2011;151(3–4):264–274. https://doi.org/10.1016/j.vetmic.2011.03.036

13. Weissenböck H, Chvala-Mannsberger S, Bakonyi T, Nowotny N. Emergence of Usutu virus in Central Europe: Diagnosis, surveillance and epizootiology. In: Knols BCJ, Takken B, editors. Emerging pests and vector-borne diseases in Europe. Wageningen: Wageningen Academic, 2007; p. 153–168. https://doi.org/10.3920/9789086866267_011
14. Nelson AN, Ploss A. Emerging mosquito-borne flaviviruses. mBio. 2024; 15(12):e02946-24. https://doi.org/10.1128/mbio.02946-24
15. Chang HH, Huber RG, Bond PJ, et al. Systematic analysis of protein identity between Zika virus and other arthropod-borne viruses. Bull World Health Org. 2016;95(7):517. https://doi.org/10.2471/BLT.16.182105
16. Saitou N. and Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–425.
17. Zuckerkandl E. and Pauling L. Evolutionary divergence and convergence in proteins. In: Bryson V, Vogel HJ, editors. Evolving genes and proteins. New York, NY: Academic Press, 1965; p. 97–166.
18. Tamura K, Stecher G, Kumar S. MEGA 11: Molecular evolutionary genetics analysis Version 11. Mol Biol Evol. 2021;38(7):3022–3027. https://doi.org/10.1093/molbev/msab120
19. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985;39:783–791. https://doi.org/10.2307/2408678
20. Jupp PG. The ecology of West Nile virus in South Africa and the occurrence of outbreaks in humans. Ann NY Acad Sci. 2001;951:143–152. https://doi.org/10.1111/j.1749-6632.2001.tb02692.x
21. Petersen LR, Marfin AA. West Nile virus: A primer for the clinician. Ann Intern Med. 2002;137:173–179. https://doi.org/10.7326/0003-4819-137-3-200208060-00009
22. Marfin AA, Gubler DJ. West Nile encephalitis: An emerging disease in the United States. Clin Infect Dis. 2001;33:1713–1719. https://doi.org/10.1086/322700
23. Venter M, Human S, Zaayman D, et al. Lineage 2 West Nile virus as cause of fatal neurologic diseases in horses, South Africa. Emerg Infect Dis. 2009;15(6):877–884. https://doi.org/10.3201/eid1506.081515
24. Burt FJ, Grobbelaar AA. Leman, Anthony FS, Gibson GVF, Swanepoel R. Phylogenetic relationships of southern African West Nile virus isolates. Emerg Infect Dis. 2002;8(8):820–826. https://doi.org/10.3201/eid0808.020027
25. Venter M, Human S, Van Niekerk S, Williams J, Van Eeden C, Freeman F. Fatal neurologic disease and abortion in mare infected with lineage 1 West Nile virus, South Africa. Emerg Infect Dis. 2011;17(8):1534–1536. https://doi.org/10.3201/eid1708.101794
26. Engler O, Savini G, Papa A, et al. European surveillance for West Nile virus in mosquito populations. Int J Environ Res Public Health. 2013;10:4869–4895.
27. Botha EM, Markotter W, Wolfaardt M, et al. Genetic determinants of virulence in pathogenic lineage 2 West Nile virus strains. Emerg Infect Dis. 2008;14(2): 222–230. https://doi.org/10.3201/eid1402.070457
28. Ochieng C, Lutomiah J, Makio A, et al. Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007–2012. Virol J. 2013;10:140.
29. McIntosh BM, Jupp PG, Dos Santos I, Meenehan GM. Epidemics of West Nile and sindbis viruses in South Africa with Culex (Culex) univittatus Theobald as vector. S Afr J Sci. 1976;72(10):295–300.
30. McIntosh B. The epidemiology of arthropod-borne viruses in southern Africa [unpublished DSc dissertation]. University of Pretoria; 1980.
31. Hollidge BS, Gonzalez-Scarano F, Soldan SS. Arboviral encephalitides: Transmission, emergence, and pathogenesis. J NeuroImmune Pharmacol. 2010;5:428–442. https://doi.org/10.1007/s11481-010-9234-7
32. Patsoula E, Vakali A, Balatsos G, et al. West Nile virus circulation in mosquitoes in Greece (2010–2013). Biomed Res Int. 2016;2016:2450682. https://doi.org/10.1155/2016/2450682
33. Nikolay B, Diallo M, Boye CS, Sall AA. Usutu virus in Africa. Vector Borne Zoonotic Dis. 2011;11:1417–1423. https://doi.org/10.1089/vbz.2011.0631
34. Ashraf U, Ye J, Ruan X, Wan S, Zhu B, Cao S. Usutu virus: An emerging flavivirus in Europe. Viruses. 2015;7:219–238.
35. Engel D, Jöst H, Wink M, et al. Reconstruction of the evolutionary history and dispersal of Usutu virus, a neglected emerging arbovirus in Europe and Africa. MBio. 2016;7(1):e01938-15. https://doi.org/10.1128/mBio.01938-15
36. Cadar D, Becker N, Campos RdM, Borstler J, Jost H, Schmidt-Chanasit J. Usutu virus in bats, Germany, 2013. Emerg Infect Dis. 2014;20:1771–1773. https://doi.org/10.3201/eid2010.140909
37. Nikolay B, Diallo M, Faye O, Boye CS, Sall AA. Vector competence of Culex neavei (Diptera: Culicidae) for Usutu virus. Am J Trop Med Hyg. 2012;86(6):993–996. https://doi.org/10.4269/ajtmh.2012.11-0509
38. Fros JJ, Miesen P, Vogels CB, et al. Comparative Usutu and West Nile virus transmission potential by local Culex pipiens mosquitoes in north-western Europe. One Health. 2015;1:31–36.
39. Lecollinet S, Blanchard Y, Manson C, et al. Dual emergence of Usutu virus in common blackbirds, eastern France, 2015. Emerg Infect Dis. 2016;22(12):2225. https://doi.org/10.3201/eid2212.161272
40. Vazquez A, Jimenez-Clavero M, Franco L, et al. Usutu virus: Potential risk of human disease in Europe. Euro Surveill. 2011;16(31):19935. https://doi.org/10.2807/ese.16.31.19935-en
41. Williams MC, Simpson DI, Haddow AJ, Knight EM. The isolation of West Nile virus from man and of Usutu virus from the bird-biting mosquito Mansonia aurites (Theobald) in the Entebbe area of Uganda. Ann Trop Med Parasitol. 1964;68: 367–374. https://doi.org/10.1080/00034983.1964.11686258
42. Campbell GL, Hills SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: A systematic review. Bull World Health Organ. 2011;89:766–774, 774A–774E. https://doi.org/10.2471/BLT.10.085233
43. Samy AM, Alkishe AA, Thomas SM, Wang L, Zhang W. Mapping the potential distributions of etiological agent, vectors, and reservoirs of Japanese Encephalitis in Asia and Australia. Acta Trop. 2018;188:108–117. https://doi.org/10.1016/j.actatropica.2018.08.014
44. Rodrigues SG, Nunes MR, Casseb SM, et al. Molecular epidemiology of Saint Louis encephalitis virus in the Brazilian Amazon: Genetic divergence and dispersal. J Gen Virol. 2010;91:2420–2427. https://doi.org/10.1099/vir.0.019117-0
45. Reisen WK. Epidemiology of St. Louis encephalitis virus. Adv Virus Res. 2003;61:139–183. https://doi.org/10.1016/S0065-3527(03)61004-3
46. Smithburn KC, Kokernot RH, Heymann CS, Weinbren MP, Zentkowsky D. Neutralizing antibodies for certain viruses in the sera of human beings residing in northern Natal. S Afr Med J. 1959;33:555–561.
47. Jupp P. Mosquitoes of southern Africa: Culicinae and Toxorhynchitinae. Hartebeespoort: Ekogilde Publishers; 1996.
48. Jupp PG, McIntosh BM, Anderson D. Culex (Eumelanomyia) rubinotus Theobald as vector of Banzi, Germiston and Witwatersrand viruses IV: Observations on the biology of C rubinotus. J Med Entomol. 1976;12:647–651. https://doi.org/10.1093/jmedent/12.6.647
49. McIntosh BM, Jupp PG, Dos Santos ISL, Meenehan GM. Culex (Eumelanomyia) rubinotus Theobald as vector of Banzi, Germiston and Witwatersrand viruses I. Isolation of virus from wild populations of C. rubinotus. J Med Entomol. 1976;12:637–640.
50. Gould EA, De Lamballerie X, Zanotto PM, Holmes EC. Origins, evolution, and vector/host coadaptations within the genus Flavivirus. Adv Virus Res. 2003;59:277–314. https://doi.org/10.1016/S0065-3527(03)59008-X
51. Kokernot RH, Szlamp EL, Levitt J, McIntosh BM. Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of the Caprivi strip and Bechuanaland protectorate. Trans R Soc Trop Med Hyg. 1965;59(5):553–562. https://doi.org/10.1016/0035-9203(65)90158-6
52. Smithburn KC, Paterson HE, Heymann CS, Winter PAD. An agent related to Uganda S virus from man and mosquitoes in South Africa. S Afr Med J. 1959; 33(46):959–962.
53. Bryant JE, Homes EC, Barrett AD. Out of Africa: A molecular perspective on the introduction of yellow fever virus into the Americas. PLoS Pathog. 2007:3(5):3e75. https://doi.org/10.1371/journal.ppat.0030075
54. Huang Y-LS, Higgs S, Horne KM, Vanlindingham DL. Flavivirus-mosquito interactions. Viruses. 2014;6:4703–4730. https://doi.org/10.3390/v6114703
55. Germain M, Cornet M, Mouchet J, et al. Recent advances in research regarding sylvatic yellow fever in West and Central Africa. Bull Inst Pasteur. 1982;80:315–330.
56. Haddow AJ, Smithburn KC, Dick GWA, Kitchen SF, Lumsden WHR. Implication of the mosquito Aedes (Stegomyia) africanus Theobald in the forest cycle of yellow fever in Uganda. Ann Trop Med Parasit. 1948;42(2):218–223. https://doi.org/10.1080/00034983.1948.11685365
57. Christian KA, Iuliano AD, Uyeki TM, et al. What are we watching- top global infectious disease threats, 2013–2016: An update from CDC's global disease detection operations center. Health Secur. 2017;15(5):1–10.
58. Fontenille D, Diallo M, Mondo M, Ndiaye M, Thonnon J. First evidence of natural vertical transmission of yellow fever virus in Aedes aegypti, its epidemic vector. Trans R Soc Trop Med Hyg. 1997;91:533–535. https://doi.org/10.1016/S0035-9203(97)90013-4
59. Tukei PM, McCrae AWR. Natural history of yellow fever vectors and reservoirs: Studies in East Africa. Cah ORSTOM Ser Ent Med et Parasitol. 1972;10(2):159–161.
60. Sudeep AB, Bondre VP, Mavale MS, et al. Preliminary findings on Bagaza virus (Flavivirus: Flaviviridae) growth kinetics, transmission potential & transovarial transmission in three species of mosquitoes. Indian J Med Res. 2013;138(2): 257–261.
61. Bondre VP, Sapkal GN, Yergolkar PN, et al. Genetic characterization of Bagaza virus (BAGV) isolated in India and evidence of anti-BAGV antibodies in sera collected from encephalitis patients. J Gen Virol. 2009;90(11):2644–2649. https://doi.org/10.1099/vir.0.012336-0
62. Barnard BJH, Buys SB, Du Preez JH, Greyling SP, Venter HJ. Turkey meningoencephalitis in South Africa. Pretoria: Government Printer; 1980.
63. Braverman Y, Davidson I, Chizov-Ginzburg A, Chastel C. Detection of Israel turkey meningo-encephalitis virus from mosquito (Diptera: Culicidae) and Culicoides (Diptera: Ceratopogonidae) species and its survival in Culex pipiens and Phlebotomus papatasi (Diptera: Phlebotomidae). J Med Entomol. 2003;40(4): 518–521. https://doi.org/10.1603/0022-2585-40.4.518
64. Antipa C, Girjabu E, Iftimovici R, Drăgănescu N. Serological investigations concerning the presence of antibodies to arboviruses in wild birds. Virologie. 1984;35(1):5–9.
65. Dilcher M, Sall AA, Hufert FT, Weidmann M. Full-length genome sequence of Ntaya virus. Virus Genes. 2013;46:162–164. https://doi.org/10.1007/s11262-012-0825-7
66. Davidson I. A new look at avian flaviviruses. Israel J Vet Med. 2015;70(2):3–8.
67. McIntosh BM, Kokernot RH, Peterson HE, De Meillon B. Isolation of Spondweni virus from four species of culicine mosquitoes and a report of two laboratory infections with the virus. SAMJ. 1961;35(31):647–650.
68. Wolfe MS, Calisher CH, McGuire K. Spondweni virus infection in a foreign resident of upper Volta. Lancet. 1982;2(8311):1306–1308. https://doi.org/10.1016/S0140-6736(82)91511-2

69. Haddow AD, Woodall JP. Distinguishing between Zika and Spondweni viruses. Bull World Health Organ. 2016;94:711–711A. https://doi.org/10.2471/BLT.16.181503

70. Kokernot RH, Smithburn KC, Weinbren MP, De Meillon B. Studies on arthropod-borne viruses of Tongaland. VI. Isolation of Pongola virus from Aedes (Banksinella) circumluteolus Theo. S Afr J Med Sci. 1957;22:81–92.

71. Gudo ES, Lesko B, Vene S, et al. Seroepidemiologic screening for zoonotic viral infections, Maputo, Mozambique. Emerg Infect Dis. 2016;22(5):915–917.

72. Dick GWA, Haddow AJ. Uganda S virus: A hitherto unrecorded virus isolated from mosquitoes in Uganda. (1). Isolation and pathogenicity. Trans R Soc Trop Med Hyg. 1952;46(6):600–618. https://doi.org/10.1016/0035-9203(52)90021-7

73. Haddow AD, Schuh AJ, Yasuda CY, et al. Genetic characterization of Zika virus strains: Geographic expansion of the Asian lineage. PLoS Negl Trop Dis. 2012; 6(2):e1477.

74. Jouannic J-M, Friszer S, Leparc-Goffart I, Garel C, Eyrolle-Guignot D. Zika virus infection in French Polynesia. Lancet. 2016;387:1051–1052. https://doi.org/10.1016/S0140-6736(16)00625-5

75. Lanciotti RS, Lambert AJ, Holodniy M, Saavedra S, Signor LCC. Phylogeny of Zika virus in western hemisphere, 2015. Emerg Infect Dis. 2016;22(5):933–935.

76. Paixao ES, Barreto F, Teixeira Mda G, Mda C. History, epidemiology, and clinical manifestations of Zika: A systematic review. Am J Public Health. 2016;106: 606–612. https://doi.org/10.2105/AJPH.2016.303112

77. Faye O, Freire CC, Iamarino A, et al. Molecular evolution of Zika virus during its emergence in the 20th century. PLoS Negl Trop Dis. 2014;8:e2636.

78. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. N Engl J Med. 2016;374:1552–1563.

79. Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: Full circle? Comp Immunol Microbiol Infect Dis. 2004;27:319–330. https://doi.org/10.1016/j.cimid.2004.03.013

80. Messina JP, Brady OJ, Scott TW, et al. Global spread of dengue virus types: Mapping the 70 year history. Trends Microbiol. 2014;22(3):138–146.

81. Amarasinghe A, Kuritsky JN, Letson GW, Margolis HS. Dengue virus infection in Africa. Emerg Infect Dis. 2011;17(8):1349–1354. https://doi.org/10.3201/eid1708.101515

82. Deardorff ER, Nofchissey RA, Cook JA, et al. Powassan virus in mammals, Alaska and New Mexico, USA, and Russia, 2004–2007. Emerg Infect Dis. 2013;19(12):2012.

83. Carrasco M, Horiszny J. A fatal case of Powassan virus Encephalitis. J Brown Hosp Med. 2024;3(3):117405.

84. Sadanandane C, Gokhale MD, Elango A, Yadav P, Mourya DT, Jambulingam P. Prevalence and spatial distribution of Ixodid tick populations in the forest fringes of Western Ghats reported with human cases of Kyasanur forest disease and monkey deaths in South India. Exp Appl Acarol. 2018;75:135–142. https://doi.org/10.1007/s10493-018-0223-5

85. Munivenkatappa A, Sahay RR, Yadav PD, Viswanathan R, Mourya DT. Clinical &epidemiological significance of Kyasanur forest disease. Indian J Med Res. 2018;148:145–150. https://doi.org/10.4103/ijmr.IJMR_688_17

86. Tandale BV, Balakrishnan A, Yadav PD, Marja N, Mourya DT. New focus of Kyasanur forest disease virus activity in a tribal area in Kerala, India, 2014. Infect Dis Poverty. 2015;4:12. https://doi.org/10.1186/s40249-015-0044-2

87. Charrel RN, Attoui H, Butenko AM, et al. Tick-borne virus diseases of human interest in Europe. Clin Microbiol Infect. 2004;10(12):1040–1055.

88. Crabtree MB, Sang RC, Stollar V, Dunster LM, Miller BR. Genetic and phenotypic characterization of the newly described insect flavivirus, Kamiti River virus. Archiv Virol. 2003;148:1095–1118. https://doi.org/10.1007/s00705-003-0019-7

89. Sang RC, Gichogo A, Gachoya J, et al. Isolation of a new flavivirus related to cell fusing agent virus (CFAV) from field-collected flood-water Aedes mosquitoes sampled from a dambo in central Kenya. Archiv Virol. 2003;148:1085–1093.

90. Kading RC, Kityo R, Nakayiki T, et al. Detection of Entebbe bat virus after 54 years. Am J Trop Med Hyg. 2015;93(3):475. https://doi.org/10.4269/ajtmh.15-0065

91. Sule WF, Oluwayelu DO, Hernández-Triana LM, Fooks AR, Venter M, Johnson N. Epidemiology and ecology of West Nile virus in sub-Saharan Africa. Parasit Vectors. 2018;11:1–10.

92. Beck C, Leparc Goffart I, Franke F, et al. Contrasted epidemiological patterns of West Nile virus lineages 1 and 2 infections in France from 2015 to 2019. Pathogens. 2020;9(11):908.

93. Fall G, Di Paola N, Faye M, et al. Biological and phylogenetic characteristics of West African lineages of West Nile virus. PLoS Negl Trop Dis. 2017;11(11): e0006078.

94. Bakonyi T, Hubálek Z, Rudolf I, Nowotny N. Novel flavivirus or new lineage of West Nile virus, central Europe. Emerg Infect Dis. 2005;11(2):225. https://doi.org/10.3201/eid1102.041028

95. Hubálek Z, Rudolf I, Bakonyi T, et al. Mosquito (Diptera: Culicidae) surveillance for arboviruses in an area endemic for West Nile (lineage Rabensburg) and Ťahyňa viruses in central Europe. J Med Entomol. 2010;47(3):466–472.

96. Vazquez A, Sánchez-Seco MP, Ruiz S, et al. Putative new lineage of West Nile virus, Spain. Emerg Infect Dis. 2010;16(3):549. https://doi.org/10.3201/eid1603.091033

97. Rappole JH, Derrickson SR, Hubalek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. Emerg Infect Dis. 2000;6(4):319.

98. Jupp Blackburn NK Thompson DL, Meenehan GM PG. Sindbis and West Nile virus infections in the Witwatersrand-Pretoria region. S Afr Med J. 1986;70(2): 218–220.

99. Guthrie AJ, Howell PG, Gardner IA, et al. West Nile virus infection of Thoroughbred horses in South Africa (2000–2001). Equine Vet J. 2003;35(6): 601–605. https://doi.org/10.2746/042516403775467180

100. Mathengtheng L, Burt FJ. Use of envelope domain III protein for detection and differentiation of flaviviruses in the Free State Province, South Africa. Vector Borne Zoonotic Dis. 2014;14(4):261. https://doi.org/10.1089/vbz.2013.1407

101. Steyn J, Botha E, Stivaktas VI, et al. West Nile virus in wildlife and nonequine domestic animals, South Africa, 2010–2018. Emerg Infect Dis. 2019;25(12):2290.

102. National Institute for Communicable Diseases. A case of West Nile fever in Mpumalanga Province, South Africa [homepage on the Internet]. Communicable Diseases Communiqué; 2017 [cited 2025 Jan 20];16(7):3. Available from: https://www.nicd.ac.za/wp-content/uploads/2017/03/West-Nile-fever.pdf

103. National Institute for Communicable Diseases. Clusters of arboviral infections – West Nile and Sindbis fevers in South Africa [homepage on the Internet]. 2017 [cited 2024 Dec 16]. Available from: https://www.nicd.ac.za/clusters-of-arboviral-infections-west-nile-and-sindbis-fevers-in-south-africa/

104. Van Eeden C, Swanepoel R, Venter M. Antibodies against West Nile and Shuni viruses in veterinarians, South Africa. Emerg Infect Dis. 2014;20(8):1409.

105. David S, Abraham AM. Epidemiological and clinical aspects on West Nile virus, a globally emerging pathogen. Infect Dis. 2016;48(8):571–586. https://doi.org/10.3109/23744235.2016.1164890

106. MacIntyre C, Lourens C, Mendes A, et al. West Nile Virus, an underdiagnosed cause of acute fever of unknown origin and neurological disease among hospitalized patients in South Africa. Viruses. 2023;15(11):2207.

107. Venter M, Van Eeden C, Williams J, et al. Arboviruses associated with neurological disease in animals in South Africa and their zoonotic potential in humans. Int J Infect Dis. 2014;21:184. https://doi.org/10.1016/j.ijid.2014.03.804

108. Bertram FM, Thompson PN, Venter M. Epidemiology and clinical presentation of west nile virus infection in horses in South Africa, 2016–2017. Pathogens. 2020;10(1):20.

109. Vidaña B, Busquets N, Napp S, Pérez-Ramírez E, Jiménez-Clavero MÁ, Johnson N. The role of birds of prey in West Nile virus epidemiology. Vaccines (Basel). 2020;8(3):550. https://doi.org/10.3390/vaccines8030550

110. Kulasekera VL, Kramer L, Nasci RS, et al. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. Emerg Infect Dis. 2001;7(4):722.

111. Lwande OW, Venter M, Lutomiah J, et al. Genetic Diversity of West Nile virus Isolated from the tick, Rhipicephalus pulchellus, in Kenya. Int J Infect Dis. 2014;21:229–230. https://doi.org/10.1016/j.ijid.2014.03.899

112. Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003;348(22): 2196–2203.

113. Montgomery SP, Brown JA, Kuehnert M, et al. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion (Paris). 2006;46(12):2038–2046.

114. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med. 2003;349(13): 1236–1245. https://doi.org/10.1056/NEJMoa030969

115. Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics. 2007;119(3):e666–e671.

116. World Health Organization. West Nile virus [homepage on the Internet]. Geneva: WHO; 2017 [cited 2025 Jan 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/west-nile-virus

117. Weiss KE, Haig DA, Alexander RA. Wesselsbron virus-a virus not previously described, associated with abortion in domestic animals. Pretoria: The Government Printer; 1956 [cited 2025 Jul 25]. Available from: http://hdl.handle.net/2263/58648

118. The Center for Food Security and Public Health. Iowa State University. Wesselsbron disease [homepage on the Internet]. Ames, IA: Iowa State University [cited 2025 Mar 11]. Available from: https://www.cfsph.iastate.edu/Factsheets/pdfs/wesselsbron.pdf

119. Kokernot RH, Smithburn KC, Paterson HE, De Meillon B. Further isolations of Wesselsbron virus from mosquitoes. SAMJ. 1960;34:871–874.

120. Weyer J, Thomas J, Leman PA, Grobbelaar AA, Kemp A, Paweska JT. Human cases of Wesselsbron disease, South Africa 2010–2011. Vector Borne Zoonotic Dis. 2013;13(5):330–336. https://doi.org/10.1089/vbz.2012.1181

121. Nikolay B, Fall G, Boye CS, Sall AA, Skern T. Validation of a structural comparison of the antigenic characteristics of Usutu virus and West Nile virus envelope proteins. Virus Res. 2014;189:87–91.

122. Rijks JM, Kik ML, Slaterus R, et al. Widespread Usutu virus outbreak in birds in the Netherlands, 2016. Eurosurveillance. 2016;21(45):30391.

123. Cavrini F, Gaibani P, Longo G, et al. Usutu virus infection in a patient who underwent orthotropic liver transplantation, Italy, August–September 2009. Euro Surveill. 2009;14:19448. https://doi.org/10.2807/ese.14.50.19448-en

124. Pecorari M, Longo G, Gennari W, et al. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. Eurosurveillance. 2009;14(50):19446. https://doi.org/10.2807/ese.14.50.19446-en

125. White SK, Lednicky JA, Okech BA, Morris Jr JG, Dunford JC. Spondweni virus in field-caught Culex quinquefasciatus mosquitoes, Haiti, 2016. Emerg Infect Dis. 2018;24(9):1765.

126. Jaeger AS, Weiler AM, Moriarty R V, et al. Spondweni virus causes fetal harm in Ifnar1−/− mice and is transmitted by Aedes aegypti mosquitoes. Virology. 2020;547:35–46.

127. Fernandez-Pinero J, Davidson I, Elizalde M, Perk S, Khinich Y, Jimenez-Clavero MA. Bagaza virus and Israel turkey meningoencephalomyelitis virus are a single virus species. J Gen Virol. 2014;95(4):883–887.

128. Steyn J, Botha EM, Lourens C, Coetzer JAW, Venter M. Bagaza virus in Himalayan monal pheasants, South Africa, 2016–2017. Emerg Infect Dis. 2019;25(12):2299. https://doi.org/10.3201/eid2512.190756

129. Valderrama A, Díaz Y, López-Vergès S. Interaction of Flavivirus with their mosquito vectors and their impact on the human health in the Americas. Biochem Biophy Res Commun. 2017;492(4):541–547. https://doi.org/10.1016/j.bbrc.2017.05.050

130. Gould E, Pettersson J, Higgs S, Charrel R, De Lamballerie X. Emerging arboviruses: Why today? One Health. 2017;4:1–13.

131. Liang G, Gao X, Gould EA. Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control. Emerg Microbes Infect. 2015;4(1):1–5.

132. Liao H, Lyon CJ, Ying B, Hu T. Climate change, its impact on emerging infectious diseases and new technologies to combat the challenge. Emerg Microbes Infect. 2024;13(1):2356143.

133. McMichael C. Climate change-related migration and infectious disease. Virulence. 2015;6(6):548–553. https://doi.org/10.1080/21505594.2015.1021539

134. McMullen AR, Albayrak H, May FJ, Davis CT, Beasley DWC, Barrett ADT. Molecular evolution of lineage 2 West Nile virus. J Gen Virol. 2013;94(2):318–325.

135. Brogdon WG, McAllister JC. Insecticide resistance and vector control. Emerg Infect Dis. 1998;4(4):605.

136. Dusfour I, Thalmensy V, Gaborit P, Issaly J, Carinci R, Girod R. Multiple insecticide resistance in Aedes aegypti (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. Mem Inst Oswaldo Cruz. 2011;106:346–352. https://doi.org/10.1590/S0074-02762011000300015

137. Ishak IH, Jaal Z, Ranson H, Wondji CS. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors Aedes aegypti and Aedes albopictus from Malaysia. Parasit Vectors. 2015;8:1–13.

138. Lima EP, Paiva MHS, De Araújo AP, et al. Insecticide resistance in Aedes aegypti populations from Ceará, Brazil. Parasit Vectors. 2011;4:1–12. https://doi.org/10.1186/1756-3305-4-5

139. Marcombe S, Mathieu RB, Pocquet N, et al. Insecticide resistance in the dengue vector Aedes aegypti from Martinique: Distribution, mechanisms and relations with environmental factors. PLoS One. 2012;7(2):e30989.

140. Marcombe S, Farajollahi A, Healy SP, Clark GG, Fonseca DM. Insecticide resistance status of United States populations of Aedes albopictus and mechanisms involved. PLoS One. 2014;9(7):e101992. https://doi.org/10.1371/journal.pone.0101992

141. Norris LC, Norris DE. Insecticide resistance in Culex quinquefasciatus mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. J Vector Ecol. 2011;36(2):411–420.

142. Vontas J, Kioulos E, Pavlidi N, Morou E, Della Torre A, Ranson H. Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. Pestic Biochem Physiol. 2012;104(2):126–131.

143. Yanola J, Chamnanya S, Lumjuan N, Somboon P. Insecticides resistance in the Culex quinquefasciatus populations from northern Thailand and possible resistance mechanisms. Acta Trop. 2015;149:232–238.

144. Cantera JL, White H, Diaz MH, et al. Assessment of eight nucleic acid amplification technologies for potential use to detect infectious agents in low-resource settings. PLoS One. 2019;14(4):e0215756. https://doi.org/10.1371/journal.pone.0215756

145. Elizalde M, Cano-Gómez C, Llorente F, et al. A Duplex quantitative real-time reverse transcription-PCR for simultaneous detection and differentiation of flaviviruses of the Japanese encephalitis and Ntaya serocomplexes in birds. Front Vet Sci. 2020;7:203.

146. Patel P, Landt O, Kaiser M, et al. Development of one-step quantitative reverse transcription PCR for the rapid detection of flaviviruses. Virol J. 2013;10:1–11. https://doi.org/10.1186/1743-422X-10-58

147. Kuno G. Universal diagnostic RT-PCR protocol for arboviruses. J Virol Methods. 1998;72(1):27–41.

148. Faye M, Seye T, Patel P, et al. Development of real-time molecular assays for the detection of Wesselsbron virus in Africa. Microorganisms. 2022;10(3):550.

149. Viral MV, Virus K, RRV RR, (2nd section). Transfusion. 2024;64:S19–S207. https://doi.org/10.1111/trf.17630

150. Kuno G. Serodiagnosis of flaviviral infections and vaccinations in humans. Adv Virus Res. 2003;61:3–65. https://doi.org/10.1016/S0065-3527(03)61001-8

151. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med. 2003;349(13):1236–1245.

152. Utarini A, Indriani C, Ahmad RA, et al. Efficacy of Wolbachia-infected mosquito deployments for the control of dengue. N Engl J Med. 2021;384:2177–2186. https://doi.org/10.1056/NEJMoa2030243

153. Salgado R, Hawks SA, Frere F, Vázquez A, Huang CYH, Duggal NK. West Nile virus vaccination protects against Usutu virus disease in mice. Viruses. 2021;13(12):2352.

154. Tesh RB, Da Rosa AP, Guzman H, Araujo TP, Xiao SY. Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. Emerg Infect Dis. 2002;8(3):245. https://doi.org/10.3201/eid0803.010238

155. Monath TP, Liu J, Kanesa-Thasan N, et al. A live, attenuated recombinant West Nile virus vaccine. Proc Natl Acad Sci USA. 2006;103(17):6694–6699. https://doi.org/10.1073/pnas.0601932103

156. El Garch H, Minke JM, Rehder J, et al. A West Nile virus (WNV) recombinant canarypox virus vaccine elicits WNV-specific neutralizing antibodies and cell-mediated immune responses in the horse. Vet Immunol Immunopathol. 2008;123(3–4):230–239. https://doi.org/10.1016/j.vetimm.2008.02.002

157. Ng T, Hathaway D, Jennings N, Champ D, Chiang YW, Chu HJ. Equine vaccine for West Nile virus. Dev Biol (Basel). 2003;114:221–227.

158. White J, Tunga P, Anderson DM, et al. Results of a double-blind, randomized, placebo-controlled Phase 1 study to evaluate the safety and pharmacokinetics of anti-Zika virus immunoglobulin. Am J Trop Med Hyg. 2021;105(6):1552. https://doi.org/10.4269/ajtmh.20-1578

159. Stefanik M, Valdes JJ, Ezebuo FC, et al. FDA-approved drugs Efavirenz, Tipranavir, and Dasabuvir inhibit replication of multiple flaviviruses in vero cells. Microorganisms. 2020;8(4):599. https://doi.org/10.3390/microorganisms8040599

160. Lai H, Engle M, Fuchs A, et al. Monoclonal antibody produced in plants efficiently treats West Nile virus infection in mice. Proc Natl Acad Sci USA. 2010;107(6):2419–2424. https://doi.org/10.1073/pnas.0914503107

161. Agliani G, Giglia G, Marshall EM, Gröne A, Rockx BH, Van den Brand JM. Pathological features of West Nile and Usutu virus natural infections in wild and domestic animals and in humans: A comparative review. One Health. 2023;16:100525. https://doi.org/10.1016/j.onehlt.2023.100525

162. Venter M, Pretorius M, Fuller JA, et al. West Nile virus lineage 2 in horses and other animals with neurologic disease, South Africa, 2008–2015. Emerg Infect Dis. 2017;23(12):2060. https://doi.org/10.3201/eid2312.162078