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COMMENTARY

Proceedings from the Fourth Mesoamerican Symposium "Dr. Roberto Navarro López" on Emerging Zoonotic Disease and Arboviruses: Commenting Insights and Research Findings

Nadia A Fernández-Santos[1,2,*], Mario A Rodríguez-Pérez[1,*], Sofía Segovia-Mancillas[1,3,*], Luis L Rodríguez[4], Sarah A Hamer[5], Gabriel L Hamer[2], Fabián Correa-Morales[6], Susano Medina-Jaramillo[7], Maria Gabriela Palacios-Mendoza[8], Epigmenio Cruz-Aldán[9], Gabriela del Carmen Rodriguez-Dominguez[10], Carlos H Gomez-Hernandez[10], Arturo Larraga-Guillén[11], Irene López González[11], Luis M Rodríguez-Martínez[12], Aldo I Ortega-Morales[13], Ma Isabel Salazar[14], Héctor Enrique Valdez-Gómez[11], Miguel A Márquez Ruiz[15], Maria J Perteguer[16], Benjamín Gastón Gómez-Gordillo[17], Jesús A Aguilar-Durán[1], Ingeborg D Becker Fauser[18], Scott C Weaver[19], Michael J Turell[20], Laura D Kramer[21]*, Jose Guillermo Estrada-Franco[1,*]

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Abstract: Zoonotic pathogens such as arboviruses, arenaviruses, filoviruses, coronaviruses, highly pathogenic Avian Influenza A (H5N1) viruses, vesiculoviruses, and many others are emerging and reemerging worldwide, jeopardizing global veterinary and public health. Parasitic diseases such as visceral and cutaneous leishmaniasis, trypanosomiasis (*Trypanosoma cruzi*), myiasis, and river blindness (*Onchocerca volvulus*) are also paramount for public health in the Americas and elsewhere. In the fall 2024, a group of experts convened in Chiapas, Mexico, for the Fourth Mesoamerican Symposium "Dr. Roberto Navarro López" on Arboviruses and Emerging Zoonotic Diseases. Here, we highlight the importance of some zoonotic pathogens and parasites affecting human health that are being impacted by anthropogenic activities. In this context, there are drivers such as changes in climate and landscape transformations, unsound agricultural practices, and wildlife niche replacement delivering numerous opportunities for zoonotic pathogens to emerge and threaten human health and food security.

Keywords: zoonosis, arboviruses, emerging diseases, re-emerging diseases, symposium, training course

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Introduction

The World Health Organization (WHO) defines a zoonosis as an infectious agent shared between non-human animal and humans. Zoonotic pathogens include bacterial, viral, and parasitic organisms, or prions and can spread among humans and animals through direct contact, food, water, or the environment.[1] Emerging infectious diseases are on the rise in various regions of the world, and especially those associated with zoonotic emergence events in their tropical and subtropical regions. In this picture, several infectious zoonoses are widely spread and known in different regions of the Americas. Pathogen emergence can result in a geographically limited event, sporadic disease outbreaks, or a pandemic. During this process, some pathogens can originate from a sylvatic cycle and then enter an urban cycle that is maintained in humans with no animal reservoirs involved. According to the World Organisation for Animal Health (WOAH), 60% of pathogens that cause human diseases originate from domestic animals or wildlife. Seventy-five percent of emerging infectious human diseases have an animal origin, and 80% of pathogens of bioterrorism concern originate in animals.[2] Currently, high levels of anthropogenic activity, including agricultural intensification, urbanization and other forms of land use change, have led to increased interfaces, where interactions between wildlife, humans and livestock, take place, expanding the risk of cross-species transmission. Pathogen transmission from animals to humans (zoonoses) or even transmission from humans to animals (anthroponosis) needs to be addressed from a multi-sectoral and multidisciplinary angle, which the One Health approach champions. One Health is centered on a systemic understanding of the interdependencies between the health of humans, animals, plants, and the environment and how these can manifest as health threats. Thus, in light of recent human public health crises such as Ebola, Zika and SARS-CoV-2 epidemics, resulting from pathogens of potential animal origin, we underscore the utility of the One Health concept in understanding and confronting global health threats.

Within the scope of the zoonosis, vector-borne pathogens also remain a significant cause of human disease. To reduce their impact, we need to develop a better understanding of their epidemiology, develop sound diagnostic tools, conduct targeted surveillance, learn which species are serving as vectors. What factors are affecting vectorial capacity (eg, vector competence, host feeding preferences, etc.) and develop appropriate preventive and reactive responses. This is often best accomplished by attacking these pathogens where they are enzootic sylvatic cycles before they can spill-over into domestic animal and human populations. However, to do this efficiently, local public health workers (physicians, veterinarians, entomologists, field ecologists, and diagnostic developers) all need to understand the basic concepts involved with pathogen maintenance along with vector biology, such as feeding preferences, oviposition site choice, as well as transmission, surveillance, and diagnosis. Often, these public health workers work independently, rather than collaboratively expanding communication mechanisms, and thus progress has been limited.

Among the alphaviruses in tropical Americas, Venezuelan equine encephalitis virus (VEE) and related viruses in the VEE complex remain among the most important pathogens in the Americas, generating thousands of annual cases from enzootic spillover infections throughout Mexico, Central and much of South America. Unless these cases progress to frank neurologic disease, which occurs only in approximately 5–15%, they are easily misdiagnosed as dengue in the absence of laboratory testing. Equine-amplified epizootics have not been identified since 1995, but spillover enzootic equine cases, some resulting in fatal encephalitis, continue to occur in Mexico as detected by Dr. Roberto Navarro-Lopez et al, since 1993.[3] In 1996, an outbreak of VEE caused a number of equine deaths in coastal Oaxaca, Mexico. Further, a major change occurred recently in alphaviral disease in the Americas when the chikungunya virus was introduced into the Caribbean in 2013 from Asia, followed by another strain into Brazil from Angola in 2014.[3]

Both of these CHIKV strains spread explosively throughout Latin America with millions of cases of severe, often chronic arthralgia. However, the Brazilian strain appears to be more fit for transmission, resulting in most recent cases occurring in Brazil and the Southern Cone of South America. Fortunately, epistatic interactions within both strains inhibited their ability to adapt for transmission by *Aedes albopictus* a generalist able to feed in a large variety of mammals, therefore, it has a much lower vectorial capacity than *Ae*. *aegypti*, limiting the geographic range of the outbreaks to the tropics and subtropics.

In 1996 and after the VEE outbreak associated to a number of equine deaths in Oaxaca, Mexico, Dr. Roberto Navarro-Lopez participated concurrently in 1999 as a member of the Mexican Ministry of Agriculture, with members of

the Mexican Ministry of Health and Zoological Parks organizations in the First International Workshop on Equine Encephalitis in Tuxtla Gutierrez Chiapas.[3]

Dr. Roberto Navarro-Lopez played a key role in the organization and conduct of an event on Equine Encephalitis in Tuxtla Gutierrez in 1999 and was able to galvanize the support of PAHO/WHO by inviting experts in to inform the local workers and to initiate cooperation among institutions. It was a first approach to initiate better collaboration and proved critical for all of these organizations to work together. Thus, the 1999 workshop was so successful, that a second workshop was held in 2004, and it eventually became known as the Mesoamerican Workshop on Arboviruses and Emerging Zoonoses. Unfortunately, Dr. Roberto Navarro-Lopez passed away in 2023, thus the Fourth Mesoamerican "Dr. Roberto Navarro López" Workshop on Arboviruses and Emerging Zoonoses that was held in Tuxtla Gutierrez in September, 2024 was in honor and memory of his tremendous contributions to the field of "Emerging Zoonotic Diseases". These Proceedings and Commentary display our knowledge, in brief, on arboviruses and zoonotic emerging diseases in the American region in honor of Dr. Roberto Navarro by scientists who were associated with him in work.

Neglected Tropical Diseases

Leishmaniasis

The protozoan parasite *Leishmania mexicana* can cause cutaneous lesions of various severity, where localized cutaneous leishmaniasis is the more benign form of the disease, characterized by ulcers that form at the site of infected *Lutzomyia* bites. In contrast, diffuse cutaneous leishmaniasis is characterized by non-ulcerated nodules, containing highly infected macrophages that spread throughout the skin, invading the oropharyngeal mucosa during the late stages of the disease.[4]

In addition to the cutaneous forms of the disease, the mucocutaneous and visceral forms of leishmaniasis, caused by other *Leishmania* species, have occasionally been reported in Mexico.[5]

In recent years, patients with an increased clinical severity have been reported in the southeastern part of Mexico.[6] The current study aims to clarify the possible cause of this increased disease severity in two epidemiological scenarios that are currently occurring in the southeastern regions of Mexico:

Scenario 1: the construction of the Maya train, where construction workers are sent into dense jungle areas, exposing them to *Leishmania mexicana* infected *Lutzomyia* bites.[7] Lack of early anti-*Leishmania* treatment leads to high parasite loads and extensive lesions in the patients, which require prolonged treatment regimes.

Scenario 2: many migrants arriving to Mexico from various parts of the world where leishmaniasis is endemic, including South America, have to cross the Darien jungle region between Colombia and Panama.[8] The crossing takes several days where they are exposed to bites of *Lutzomyia* sandflies, that are infected with diverse *Leishmania* species, including those of the *Leishmania* (Viannia) *braziliensis* complex, which are highly virulent and difficult to treat, causing the mucocutaneous form of the disease.

Interestingly, both species (*Leishmania mexicana* and *Leishmania braziliensis*) can cause very severe clinical forms of the disease, yet with opposing immunopathogenic competences: whereas lesions caused by *Leishmania mexicana* have abundant parasites that generate immune "exhaustion" as the disease progresses, lesions caused by *Leishmania braziliensis* generally show low parasite loads, yet very extensive tissue destruction caused by CD8 lymphocytes.[9]

American Trypanosomiasis (Chagas Disease)

Triatomines of the Southern US, Mexico, and Guatemala Chagas Disease Ecology

Chagas disease, caused by infection with the protozoan parasite *Trypanosoma cruzi*, is a neglected disease in the Americas spread by triatomine insects. Over the last 10 years, we have built a community science program through which members of the public have submitted nearly 10,000 triatomines from 28 southern states in the USA.[10] These specimens have been used to show over 50% of adult triatomines are infected with *T. cruzi*. Bloodmeal analysis has revealed dozens of vertebrate species used as hosts for the insects, with domestic dogs being the most common.[11] In recent years, a new community science program in northern Mexico was launched, already with submissions from three countries. Our studies in the Rio Grande Valley of South Texas, at hunting dog kennels in central Texas, and in Reynosa, Mexico, have found dogs to be sensitive indicators of local transmission cycles, with incidence exceeding 30% per year in some settings.[12] Our recent work in Guatemala revealed 16% of dogs were infected in rural communities where

domestic infestations with *Triatoma dimidiata* are common.[13] Finally, new ecological work in Baja California Sur, Mexico, has afforded a collection of *Dipetalogaster maximus* – the world's largest triatomine species – with a very low infection prevalence, likely owing to blood feeding on lizards, which are incompetent reservoirs. We conclude that dogs may be a key tool for managing Chagas disease risk to humans using host-targeted insecticides and that "One Health" approach is needed for combatting this disease.[14–16]

River Blindness (Onchocerciasis)

The control and elimination of neglected tropical diseases (NTDs), which affects over 1 billion people worldwide, is one of the most ambitious goals of the WHO 2030 agenda. For onchocerciasis, the specific target is to eliminate transmission of the parasite *Onchocerca volvulus*. Onchocerciasis is a devastating disease, transmitted by black flies of the family Simuliidae (Diptera). In most affected communities, prior to the implementation of onchocerciasis control programs, a large proportion of the population was blind and the rest were aware that they are condemned to the same end. The disease, which is endemic in Africa and has historically scattered endemic foci in the Americas, is the world's leading second cause of preventable blindness.[17–19]

Colombia and Ecuador were the first countries to have elimination of parasite transmission verified by WHO in 2013 and 2014, respectively, followed by Mexico in 2015 and Guatemala in 2016.[17] The distribution of onchocerciasis in scattered foci in the Americas and the effectiveness in implementing health education and control programs based on the distribution on mass drug administration (MDA) with ivermectin (brand name Mectizan®, donated by Merck) have made these the only four countries in the world, for more than a decade, to receive verification of onchocerciasis elimination by the WHO.[18] In the African continent where onchocerciasis is highly complex and much more disseminated, the WHO has recently, January 2025, verified Niger as the first country in the African Region, and the fifth in the world, to eliminate onchocerciasis.[19]

The Mexican Case

In Mexico, onchocerciasis has historically been endemic in two autochthonous foci: Oaxaca and southern Chiapas or Soconusco. A third focus in northern Chiapas or Chamula was the result of migration of onchocerciasis patients from the Soconusco focus and consisted of imported cases. In the Oaxaca focus, the interruption of parasite transmission was reported after 13 years of bi-annual ivermectin MDA to eligible population at risk communities, while the southern Chiapas focus required 17 years of bi-annual and quarterly MDA to accelerate interruption of parasite transmission. Following the declaration of interruption of transmission, MDA was suspended, and a 3 years post-treatment surveillance was initiated, through O-150 PCR entomological and large-scale IgG4 Ov16 test serological assessments, at the end of which WHO verified the onchocerciasis elimination in Mexico.[20] Since 2016, post-elimination epidemiological verification surveillance has been carried out based on examination for suspected onchocercoma.[19] As this examination is insensitive and inespecific for *O. volvulus* (i.e. palpation of onchorcercomas) in an elimination setting, the WHO has promoted other more effective approaches such as serology using the IgG4 Ov16 recombinant Ov16 antigen test and PCR pool screening of black flies for those countries that has already been verified by WHO, a process which is named post-verification or post-validation surveillance (PVS).[21] However, further development of specific guidance to conduct PVS in those verified countries is urgently needed. A PVS study is currently underway in two historic endemic foci of onchocerciasis in Mexico; PVS tool will also likely be implemented and elsewhere.[19]

Onchocerciasis Post-Elimination and Post-Verification Surveillance

The WHO recommends the implementation of post-elimination surveillance (PES) in any area, foci or country where the transmission has been eliminated. The WHO guidelines indicate that "when an onchocerciasis program establishes a PES system to detect possible resurgence of parasite transmission it should do so in both previously endemic and in non-endemic areas, as well as in areas where imported cases might be expected to occur. Such assessments should be conducted at regular intervals until elimination is verified in all countries in the WHO region concerned, or at least until any risk of recrudescence/reintroduction can be substantially excluded".[19] Thus, in the Americas, large-scale PES and PVS studies should be implemented.

Current WHO entomologic guidelines to verify onchocerciasis elimination require that "a minimum of 6,000 flies in a transmission zone be examined to demonstrate that the upper bound of the 95% confidence interval (CI) of the prevalence of flies with infective L3 larvae is < 0.1% (< 1 infective fly/1,000 flies) in parous flies or < 0.05% (< 1 infective fly/2,000 flies) in all flies.[19–21] Infectivity is determined by performing O-150 PCR on the heads of captured flies and analyzing the results using pool screen software. If this number of flies cannot be captured, demonstrating that the upper bound (UB) with a 95% CI of the estimated ATP is < 20 L3/person/year is an acceptable alternative. If these parameters are above 0.1% or 20 L3/person/year after a post-elimination verification survey, it is an early indicator of recrudescence of parasite transmission". However, in some settings, capturing at least 6,000 flies by human landing collection has been a challenge even after increasing the number of collection days and collecting flies for an additional year. Hence, the Esperanza window trap has proven to be an alternative to increase the yield of fly catching without relying on human collectors as several traps deployed, at least 100 m apart, can be operated by community endemic members.[21,22]

In conjunction with O-150 PCR in black fly studies for stopping MDA and verifying onchocerciasis elimination,[21] WHO also includes the "Ov-16 serology test to determine the presence of IgG4 antibodies in children of less than 10 years of age to detect exposure to the O. volvulus parasite.[18,19] The critical threshold for elimination of transmission is an UB with a 95% CI of less than 0.1% confirmed seropositivity to Ov-16. If it is < 0.1% in the post-verification survey, no further action is required indicating that transmission remains eliminated.[19] In general, a sample size of 2,000 children is required to detect a seroprevalence of < 0.1% at the UB with a 95% CI when none of the samples tested is positive (95%-UBCI = 0.09%). However, if the eligible population of children is less than 1,100, essentially all eligible children should be tested, and the CI should be calculated using statistical methods appropriate for finite populations".[19]

The Global Onchocerciasis Network for Elimination (GONE) was launched on 30 January 2023 by the WHO, Member States, and partners to accelerate progress towards the onchocerciasis targets of the 2030 NTD WHO roadmap. At several GONE meetings, PVS specific guidance was discussed, but no data was available, except from two studies.[17–20] Therefore, further PVS studies are urgently needed to lead the way for the onchocerciasis global international community.[22–24]

Tick-Borne Zoonotic Diseases

Ecological Studies of Rickettsia and Bartonella in Guatemala, Mexico, and USA

The tropical climate and diverse vector community across Guatemala, Mexico, and the southern United States support many vector-borne pathogen transmission cycles, and there is increasing awareness of human disease caused by *Rickettsia* and *Bartonella* pathogens.[25,26] Using a combination of field studies of wildlife and domestic animals and molecular approaches to screen their fleas and ticks, we characterized natural transmission cycles as predictors of human risk. In Texas, we investigated mesomammals and rodents at a large national forest outside a major urban area (Houston).[27] Ticks were commonly infected with several Rickettsial species regarded as endosymbionts, including *R. amblyommatis*. Fleas were most common on Virginia opossum (*Didelphis virginianus*) and included a cat flea (*Ctenocephalides felis*) positive for *Bartonella henselae*, an agent of cat scratch fever.[27,28] In the Rio Grande Valley (RGV) of South Texas, we found a high infestation prevalence of both domestic cats and opossums with fleas, including several infected with *Rickettsia typhi* (agent of murine typhus), *R. felis*, and *Bartonella henselae*.[29,30] Also in the RGV, our studies of ticks removed from wild birds and bird banders showed a great diversity of ticks (including neotropical tick species imported by migratory birds), and *Ehrlichia chaffeensis* infections in *Amblyomma tenellum* ticks that commonly parasitized people.[28,29] In Reynosa, Mexico, we sampled dogs living in six disadvantaged neighborhoods and found 53% of dogs to be infested by ticks, exclusively the brown dog tick (*Rhipicephalus sanguineus)*. Rickettsial infections were overall rare (4%), including *R. amblyommatis, Rickettsia parkeri*, and *Candidatus Rickettsia andeanae*.[31] Finally, in Comapa, Guatemala, we found that dogs may be effective sentinels of flea- and tick-borne disease risk, with agents detected including *R. felis, Candidatus* R. senegalensis, B. *henselae*, and *B. vinsonii* subsp. Berkhoffii. These findings provide an ecological basis for the maintenance of vectors and pathogens and underscore the importance of public and veterinary health surveillance for these pathogens.[32]

Myiasis

This segment of our document is committed to discuss the evidence of a reinfestation and threat mainly associated to animal husbandry and linked to the renewed presence of the screw-worm larvae from *Cochliomyia hominivorax* found in members of these vertebrates between July 2023-November 2024 in at least five countries from the Central America region and Mexico.[33] The threat and medical term called myiasis are closely associated with the parasitic infestation of live vertebrate animals by developing larvae (maggots) of the dipterous fly feeding on tissues or body substances of them.[34] The categorization of myiasis is defined when the dipterous *Cochliomyia* fly seeks open wounds to deposit its eggs and lays them in batches on the dry surfaces at the edge of the laceration of about 2 to 10 days old.[35,36] Then, the larvae hatching from these eggs begin to parasitize the host tissues. A fecund female may produce nearly 3,000 eggs and deposit batches of about 300 eggs.[37,38] *Cochliomyia hominivorax* occurs in tropical and subtropical climates of the Americas.[39,40] Clinical manifestations in animals can be pain, severe itching, restlessness and tissue damage, and in severe cases can lead to death.[41,42] Control of *C. hominivorax* is problematic by the fact that it triggers myiasis not only in cattle but also in domestic animals such as dogs, cats, pets, and other warm-blooded wild animals such as opossums, rabbits, other rodents, raccoons, skunks, and deer.[43] Hence, infestations not only affect animals but also humans and may have similar symptoms, such as wound or ulcer infestations (often *C. hominivorax* larvae). This can lead to rhino myiasis (nasal infestation), in which the larvae damage the cartilage and potentially spread to the cranial cavity.[42,43] In cattle, this picture can become a real problem with its economic impact in livestock production that has been estimated to reach several hundreds of thousands millions US dollars. Annual losses are estimated to exceed $400 million due to myiasis in livestock.[44] The impact on livestock is especially detrimental to cattle, horses, pigs and other animals, causing deaths, reducing productivity, and coupled with high control costs. Historically the *C. hominivorax* infestation problem in the new world was supposed to be eradicated in the Central and North American regions since the 1990s. The achievement of the approach in the North and Central American regions was closely linked to the massive use of the sterile male release technique of *C. hominivorax* that in proper numbers was able to swamp the wild populations.[45,46] Nonetheless, in this scenario, the *Cochlyomia* problem in the South America territory continues to be endemic and with the hidden danger of re-infestations into the North American free fly countries.

Current Situation in Central America and Mexico

Panama, Costa Rica, and Nicaragua report thousands of cases, with risk factors including migration, livestock movement, and illegal animal trafficking. The first alert was given by Panama on July 5, 2023, associated with the high migration trends that occur through the Darién Gap next to Colombia, South America. This was followed by the first reports from Costa Rica notified on July 18th, 2023. Nicaragua followed on April 26th 2024, Honduras on September 16th of the same year, and finally Guatemala in October 2024. Health authorities have indicated that there are more than 40,000 cases of affected animals in these five countries.[46,47] Recently, on November 22, 2024, Mexico reported its first documented case of infested cattle in the southern Chiapas State, bordering Guatemala.[47]

In this regard, it has been identified that the setup in many countries of Central America favors the spreading of the disease, which is closely linked mainly to livestock movement, migration and illegal animal trafficking inasmuch as these banned industries do not monitor whether their animals have injuries or not as they continue to move despite being sick and avoiding essential health veterinary principles. Hence, this scenario becomes the perfect transport for the screw-worm fly.[37]

Thus, in order to protect their livestock production, the affected countries and their Official Agriculture authorities in Central America, Mexico, and the US have implemented several immediate actions, including three approaches as follows: A) Trade restrictions to be imposed by the agriculture authorities to restrict animal imports from the affected countries until further notice; B) Enhanced surveillance in collaboration among agriculture authorities of the affected countries and the not yet affected including Central American countries, Mexico and the US. The main goal is that the partners collaborate to monitor and control their infestations while reestablishing a biological barrier in the Panama Isthmus, and C) Carry out sterile fly releases in order to swamp regions of Central America and Mexico mainly by deploying sterile screw-worm flies in all affected regions to interrupt reproduction cycles and restrict the spread.[41]

Within this framework, collaboration of the producers is urged to monitor their animals thoroughly for signs of screwworm infestation involving and observing wounds that enlarge or fail to heal; detecting larvae or eggs around wounds (eggs are creamy white and deposited at wound edges); and behavioral changes indicating discomfort. In the event that any of these events are detected, it is encouraged to the producers to communicate them to the proper Agriculture authorities of their area.[41,46,47]

Viral Pathogens

Structural Biology and Molecular Epidemiologic in Viral Zoonosis

The term zoonosis, used to describe human diseases associated with non-human animals, derives from the Greek words zoon (animal) and nosos (disease). It is defined as any disease that can be transmitted from animals to humans, with animals serving as the primary reservoir. This infection can occur through direct exposure, contact with animals, as well as indirectly through pathogens transmitted by vectors.[48] Although humans have coexisted with wildlife for millennia, certain factors such as recent population growth and the resulting pressures have intensified interactions between people and animals, increasing the risk of transmission of pathogens related to sylvatic disease cycles and the threat of contagion.[49] This behavior of infectious agents can be specifically described through molecular epidemiology, which involves using genetic data to study the geographic distribution of diseases by considering their risk factors and determinants associated with the emergence of outbreaks in human populations, as well as the biological processes at the molecular level.[50] Until now, various frontier approaches have described molecular behavior at the level of gene expression and associated mutations, but still in a preliminary manner regarding how the biochemistry of macromolecules impacts the biological structure of living beings and the associated behaviors in viruses that infect them through their fusion proteins to evade immune responses, emulate entry mechanisms, and eventually control the cells they infect. In this context, the use of structural biology as an approach to epidemiological behavior can be highly valuable for predicting possible adaptations and mutations of viral zoonoses.[51] By providing crucial information about virion structures and their interactions with host cells, significant alterations that influence transmission and pathogenesis can be anticipated. This knowledge enables us to develop effective prevention and treatment strategies long before actual diseases emerge. The ability to foresee these epidemiological events is essential for responding quickly to viral threats and delaying the onset of new epidemics as well as the emergence of endemic conditions for these diseases.[51]

Viral Hemorrhagic Fevers

Arenavirus and Filovirus

Among the viral hemorrhagic fevers (VHF) we find two families, the Arenaviridae and the Filoviridae that share characteristics A) they are enveloped RNA viruses; B) their origin are zoonotic; C) they are found in geographically restricted areas and D) they can spread from person to person by direct contact with symptomatic patients, body fluids, or cadavers, or through inadequate infection control in hospital settings.[52]

The family Arenaviridae contains a unique genus (*Arenavirus*) that comprises 22 viral species including the old world Lyssaviruses and the new world arenaviruses when infecting the humans may result in high death rates. Here, we focus and provide information on the South American new world VHF.[53] Including Junin, Guanarito, and Machupo viruses and their immunological and phylogenetic association with the Ocozocoautla de Espinosa virus isolated in South Mexico in 2012.[54–56] The Argentine hemorrhagic fever disease coupled with Junin virus and found in 1954 and linked with the Cricetid rodent *Calomys musculinus* in the surrounding corn fields of the greater Buenos Aires, Argentina. Likewise, we describe the Bolivian hemorrhagic fever studies conducted by Karl Johnson and his research group in 1958 and linking the rodent *Calomys callosus*, also from the *Cricetidae* family, and Machupo virus in the San Joaquin region of Beni, Bolivia. The description of the Venezuelan hemorrhagic fever in the West region of Venezuela, associated with the rodent *Zygodontomys brevicauda* and Guanarito virus in the 1990s is generally described.[53] The three South American VHF were linked to a serological study, viral isolation and phylogenetic analysis conducted on rodent species of the *Cricetidae* family and collected samples in the North American range of the Tacaribe serocomplex virus and from two States in the US (New Mexico and Texas) and from 10 States of the Mexican Republic ranging from north to south in the country. The immunological analysis of 4,893 Cricetid rodents showed IgG antibodies to *Amapari* virus, a member of the South

American VHF and associated phylogenetically to Ocozocoautla de Espinosa virus (OCEV) from Chiapas, and in the same topology and closely linked to Junin and Machupo virus.[54–56] Rodents obtained in the same viral isolation region pointed to five species of *Peromyscus mexicanus* with positive IgG serology.[54] The need to further explore the putative impact of OCEV in the human population of the region was highlighted.

Within the causes of VHF diseases, we also find that the Filoviridae family encompasses several genera including the genus *Orthoebolavirus* including the six known species of Ebola virus according to the International Committee on Taxonomy of Viruses (ICTV).[57] Thus, we focus on the genus *Orthoebolavirus* and one of its six known species, the species *Orthoebolavirus zairense*. However, the other five, *Orthoebolavirus bundibugyoense, Orthoebolavirus sudanense, Orthoebolavirus taiense, Orthoebolavirus restonense*, and *Orthoebolavirus bombaliense* are not treated here.[58] We centered on the history and first identification of the species *O. zairense* (September 1976) and the West African Ebola pandemic (Dec. 2013-March 2016) that was also associated with the *O. zairense* specie.[58] Ebola virus was first documented in the Yambuku village in northern Zaire (now the Democratic Republic of the Congo) in Africa. In 1976, an unusual mortality at a Belgian Catholic hospital mission began affecting the staff and inhabitants of the village of Yambuku. Infected people showed symptoms beginning with fever, muscle and body aches, headache, cough, and sore throat, followed by vomiting and diarrhea as well as bleeding from the mouth, nose, or internal organs and death.[59,60] Teams of scientists from Europe and the US converged into north Zaire; a group from the Tropical Medicine Institute in Antwerp, Belgium, directed by Dr Peter Piot conducted epidemiological studies in approximately 10 villages surrounding Yambuku in late summer 1976, traced contacts and isolated cases. A virus resembling Marburg virus was isolated from hemorrhagic patients and identified later as a unique new virus named after the nearby river of Ebola in the surroundings of the Yambuku village.[59,60]

In December 2013, a large Zaire Ebola virus outbreak (the largest thus far known) started in rural Guinea (West Africa), then extended to densely populated metropolitan regions in Guinea and to neighboring Liberia and Sierra Leone. It was first recognized in March 2014. Ebola outbreaks involved thousands of people, and about 39% of infected people died.[58] A very small number of infected travelers (including health care workers returning home) have spread Ebola virus to Europe and North America. A few health care workers, who helped treat the infected people in Europe and North America also acquired the infection. Between December 2013 and March 2016, Ebola cases documented in the West African outbreak were 28,646 from three counties and 11,323 deaths (the large majority from Guinea, Sierra Leone, and Liberia) and including other seven countries with 52 deaths including Nigeria, US, Spain and others recorded. Sierra Leone, Guinea, and Liberia were declared free of Ebola in the spring of 2016.

Many lessons were learned from the West African pandemic and several new approaches to tackle Zaire ebolavirus outbreaks including advances in epidemiology, diagnostics, vaccines, and treatments have been proposed. They involve novel treatments such as the use of monoclonal antibodies for boosting the immune system of patients. Currently, two FDA-approved treatments are available for Ebola virus patients (species *Orthoebolavirus zairense*).[61] Ebanga (single monoclonal antibody) and Inmazeb (triple monoclonal antibody cocktail).[62] Both of them were approved to treat acute EVD in adult and pediatric patients. Ebanga blocks binding of the virus to the cell receptor, preventing its entry into the cell.[55] Inmazeb monoclonal antibodies can provide important pre- or post-exposure protection against infectious disease for those not yet vaccinated or in individuals that fail to mount a protective immune response after vaccination. Inmazeb (REGN-EB3), a three-antibody cocktail against Ebola virus, lessened disease and improved survival in controlled trials.[62]

In diagnostics for Ebola virus, a novel mechanized diagnostic assay, the Xpert Ebola Assay (Cepheid Inc., Sunnyvale, CA, USA), was recently developed. This assay has been used with the Cepheid GeneXpert System, which is widely used for rapid detection of tuberculosis and rifampin resistance in decentralized settings and then was adapted to process suspected blood samples of EVD patients. The research group *Médecins Sans Frontières* demonstrated that the median time for obtaining results for EVD patients was reduced from 334 min to 165 min.[63]

Despite the fact of the painful deaths associated with the East African pandemic of EVD some clinical advances were obtained. Preliminary studies and supported in emergencies by WHO the use of an rVSV-ZEBOV a recombinant, replication competent vesicular stomatitis virus (VSV)-based candidate vaccine that expresses a surface glycoprotein of Zaire Ebolavirus.[64] The tested outcome of rVSV-ZEBOV in preventing Ebola virus disease in contacts and contacts of contacts was verified in infected patients in Guinea, West Africa.[64] The WHO jointly with various companies, trial

centers, funding agencies, and others have tackled suspected outbreaks of EVD. However, landmark efficacy trials have been carried out and expect to consolidate advances of safety, immunogenicity data including race data for final deployment of not only rVSV-ZEBOV but several other promising vaccines. Several unanswered questions remain and need to be solved including the durability of protection, mechanistic immunological dynamics and chosen deployment strategies. Advances on *Orthoebolavirus zairense* vaccinations are on the rise.

Finally, lessons and advances in prevention strategies of the West African pandemic were reinforced including avoiding contact with sources of infection, counting anyone suspected of having EVD, and potential hosts, reservoirs, and even vector species in endemic countries. Bats are suspected reservoir species for filoviruses (Ebola species, involving suspected species of the West African pandemic, and Marburg virus) and demonstrated thus far that the Egyptian fruit bat *Rousettus aegyptiacus* is a natural reservoir of Marburg virus.

Additional advances on using appropriate PPE mainly on health care providers and others taking care to minimize disease transmission risk, avoiding spread from person to person by direct contact with symptomatic patients, body fluids, or cadavers, or through inadequate infection control in a hospital setting.

Avian Influenza

Avian influenza viruses (AIVs) are highly contagious respiratory viruses of birds, leading to significant morbidity and mortality globally and causing substantial economic losses to the poultry industry. Wild waterfowl (Anseriformes) and other aquatic birds (Charadriiformes) are considered the natural reservoir of 17 of 19 hemagglutinin (H1-16 and H19) and 9 of 11 neuraminidase (N1-9) glycoproteins, externally embedded within the viral envelope.[65,66] The antigenic and genetic diversity of these two glycoproteins is used to determine the AIV subtype.[67] Besides, different combinations of HA and NA have been isolated from more than 125 bird species.[68] AIVs are classified as low-pathogenic avian influenza viruses (LPAIV) and highly pathogenic avian influenza viruses (HPAIV) based on disease severity and lethality in chickens. So far, the HPAIV designation has been restricted to H5 and H7 subtypes.[69] AIVs typically circulate in migratory waterfowl acting as reservoirs but also non-migratory wild birds including endangered ones.[68,70] Spillovers to other wild and domestic birds are common. Likewise, accidental infections in mammals contribute to AIV diversity, raising public concern about potential spillover events to humans. The diversity and phylogenetic patterns of AIVs are mainly determined by geographic barriers and limited by the intercontinental exchange of AIVs via migratory flyways.[71,72] Human exposure to avian influenza viruses can lead to infection and disease, ranging from mild, flu-like symptoms or eye inflammation to severe, acute respiratory disease and/or death.[73]

A novel genotype of HPAI H5N1 arose in 1996 in Southern China and through ongoing mutation, reassortment, and natural selection, has diverged into distinct lineages resulting in the arrival of distinct clades and expanded into multiple hosts reservoir.[74] H5N1 clade 2.3.4.4b viruses have spread through Europe, Asia, Africa and the Americas, displacing some other clade viruses and reassorting with local LPAI viruses to produce a diverse range of genotypes among variable and species-dependent pathogenicities.[75]

In 2021, the H5N1 strain was spread by wild birds, leading to significant mortality among them across most European countries. Its spread to North America in the fall of 2021 took place through the circumpolar pelagic migration routes along Iceland, Greenland, Arctic. It was first detected in a great black-backed gull in eastern Canada in November 2021.[76] Since 2021, there has been an increase in the frequency of detections among non-avian species including both wild terrestrial and marine mammals, particularly scavengers and those with potential proximity to infected birds.[68] As these viruses rapidly spread across North America in 2022, they underwent extensive reassortment with LPAIVs circulating in the region, leading to the emergence of diverse viral genotypes by spring 2022. As of March 2024, livestock and dairy herds have tested positive in Minnesota, Texas, Kansas, Michigan, and other States.[66] While sustained mammalian transmission has not been documented yet, the persistent recirculation of this clade in wild and domestic mammalian populations is concerning for potential virus adaptation and species jumps. Spillovers to terrestrial and marine mammals have occurred, raising concerns about broader cross-species and mammal-to-mammal transmission risks.

Epidemiological Surveillance on Wild Birds in Mexico

The "One Health" concept is highlighted in addressing the problem of avian influenza viruses and its devastating effects recently experienced by commercial poultry, bird and wild mammal populations including a significant increase in human cases. Molecular adaptations of the H5 HP strain have allowed it to modify its genomic capabilities to cross the species barrier. This situation is compounded by a sustained environmental change of human origin. Its effects contribute to the alteration of fragmented ecosystems, expanding the interface between humans and opportunistic, small-scale, and notoriously abundant biota that are highly adaptable to change.[69,70]

Although almost all AI strains develop into low-pathogenic forms, H5 and H7 are significantly lethal with some species particularly susceptible to certain subtypes. Their immune response is driven by the specificity of sialic acid receptors located on membranes of the host's respiratory and digestive tracts. These receptors facilitate the entry of viruses into the cell, releasing their genetic material and subsequent replication. Thus, AI subtypes can be transmitted to different species either through an intermediate host or through adaptive mutations.[67,68]

In Mexico, the Coordination of Epidemiological Surveillance in Wild Birds (CPA-SENASICA) is committed to providing early warnings about the diversity of IAVs present along the main waterfowl migratory flyways. Active surveillance is carried out in avian environments during spring and summer. Samples obtained from wild birds' surveillance are normally based on tracheal and cloacal swabbing but also can include organs collection such as brain, trachea, lungs, spleen and duodenal loop, also serum extraction in some cases, depending on the needs of the investigation. These samples are sent to SENASICA'S official laboratory network for RT-PCR and IHA tests. Eventually, positive results for AIV with a high viral load are candidates for viral isolations, and eventually for phylogenetic studies.[68,70]

Additionally, a telemetry program is in place to determine the movements and the area of use for wintering teals (reservoirs) and resident grackles (carriers).[77] This study aims to establish risky contact rates in wetlands and poultry environments (susceptible). Thus, the determinants of dissemination of AIV by wild birds involve aspects such as: i) Distribution and density of reservoirs, carriers, and susceptible; ii) Mechanisms of pathogen dissemination; iii) Disease intensity and prevalence; iv) Structural barriers; v) Immune response and molecular compatibility.[71,72]

To reinforce the One Health concept, the CPA maintains inter-institutional agreements with the Ministries of Environment and Health. On the one hand, samples from ducks are coordinated during the hunting season, as well as the attendance of notifications. Positive cases of HP AIV in wild birds are reported to the Institute of Epidemiological Diagnosis and Reference for human tracing. Finally, ongoing advocacy is being carried out to activate reporting mechanisms in cases of sick or dead birds.[73]

SARS-CoV-2 (COVID-19)

Transmission at the Human-Domestic and Wild Animal Interface

Five years ago, SARS-CoV-2 emerged from wild animal origins, entered human populations, and spread globally. Our One Health research program detected relatively frequent active SARS-CoV-2 infections among pets in households with active human COVID cases in Texas through the rise and fall of the ancestral SARS-CoV-2 lineages and the Alpha and Delta variants (8.5% pets were qPCR-positive 2020–2021).[78] This was followed by far fewer cat and dog infections with the Omicron variant (3% qPCR-positive in 2022). The three animals that were positive in our Omicron-era longitudinal household investigations were dogs each infected with a different lineage of Omicron for which viral genome sequences in the dogs matched identically to the infected humans in the house that we sampled at the same time or 1 week earlier.[79,80] Similar variation in viral spill-over from humans to animals during waves of different variants of concern occurred among captive white-tailed deer. In fall 2021, we reported the first findings of infection among farmed deer, in which over 94% of sampled animals on one farm were positive, and repeated sampling confirmed the persistence of neutralizing antibodies for at least 13 months.[81] Since spring 2023, 37% of deer across 14 Texas ranches were positive for neutralizing antibodies and 6% had RT-qPCR positive swabs. In one facility sampled in November 2023, 15 female deer were sampled, and all had neutralizing antibodies to SARS-CoV-2, with endpoint titers of 1:10–1:640. Eleven deer had RT-qPCR-positive respiratory swabs; one also had a positive rectal swab. Six out of 11 respiratory swabs yielded infectious virus with replication kinetics displaying lower growth 24h post infection in vitro when compared to seven

Omicron lineages isolated from humans in Texas in the same period. However, virus growth was similar between groups at 48h and 72h, suggesting no strong attenuation of deer-derived virus.[82] All deer viruses clustered in the XBB Omicron clade, but with distinct mutations compared to contemporaneous viruses detected in humans. Future work at these and other deer facilities will include testing agricultural animals and wildlife to understand the risk of onward enzootic transmission of SARS-CoV-2.[81]

Vesicular Stomatitis (Rhabdovirus)

Vesicular stomatitis is a disease of cattle caused by members of the genus *Vesiculovirus* (family Rhabdoviridae), four of which are called "vesicular stomatitis viruses (VSV)".[83] Clinical disease progresses as severe vesiculation and/or ulceration of the tongue, oral tissues, feet, and teats and results in substantial loss of productivity. Except for its occurrence in horses, it is clinically indistinguishable from foot and mouth disease.[84] Unlike foot and mouth disease, it is highly contagious to humans and can cause a temporarily debilitating illness. Vesicular stomatitis occurs seasonally each year in the southeastern United States, southern Mexico, all of Central America, and northern South America, and emerges from tropical areas to cause sporadic epidemics in colder climates during the summer months.[85] *Vesiculoviruses* are transmitted by arthropods. However, unlike other arboviruses, VSV does not produce sustained viremias in domestic animals and apparently depends on horizontal transmission between co-feeding insects on animals that serve as amplifiers of infection without necessarily being infected. Vesicular stomatitis can affect people showing a typical influenza-like illness with symptoms including fever, muscle aches, headache and malaise. Thus, it can be transmitted from animals to humans through direct contact with infected animals or their secretions. Usually, the health implications in the vertebrate host are negligible.[83,86]

Arthropod-Borne Viral Diseases (Arbovirus)

Most arboviruses responsible for diseases in humans or domestic animals are members of one of three families: Flaviviridae, Bunyaviridae, and Togaviridae.[87]

Arbovirus Vectors in Mesoamerica

Various species of mosquitoes have historically been involved in the transmission of arboviruses in Mesoamerica.[86,88] Some species of mosquitoes, such as *Ae. aegypti* and *Ae. albopictus*, are vectors of DENV, chikungunya (CHIKV), Zika (ZIKV), and yellow fever (YFV) viruses,[89,90] while other species are important vectors of other viruses.[48] See Table 1 for

Table 1 Arbovirus Vectors in the Americas

Virus	Vectors
Alphaviridae (Alphaviruses)	
Venezuelan equine encephalitis	*Aedes angustivittatus, Ae. scapularis, Ae. taeniorhynchus, Psorophora ferox, Culex erraticus, Cx. spissipes, Cx. taeniopus, Mansonia titillans.*
Western equine encephalitis	*Ae. vexans, Ae. triseriatus, Ps. columbiae, Cx. quinquefasciatus, Cx. tarsalis*, and *Cs. inornata.*
Eastern equine encephalitis	*Ae. vexans, Ae. taeniorhynchus, Ae. triseriatus, Psorophora columbiae, Cx. nigripalpus, Cx. salinarius, Culiseta melanura, Coquillettidia perturbans.*
Madariaga virus	*Ae. serratus Cx. pedroi, Ps. albigenu, Ps. ferox*

(*Continued*)

Table 1 (Continued).

Virus	Vectors
Chikungunya virus	*Ae. aegypti, Ae. albopictus*
Flaviviridae (Flaviviruses)	
St. Louis encephalitis	*Ae. vexans, Cx. nigripalpus,*
	Cx. quinquefasciatus, Cx. restuans,
	Cx. salinarius, Cx. tarsalis
West Nile virus	*Ae. vexans, Cx. nigripalpus, Cx. pipiens* (USA and Canada)
	Cx. quinquefasciatus, Cx. restuans,
	Cx. salinarius, Cx. tarsalis,
	Cs. inornata
Dengue viruses	*Ae. aegypti, Ae. albopictus*
Zika virus	*Ae. aegypti, Ae. albopictus*
Yellow fever virus	*Ae. scapularis, Ae. aegypti, Ae. albopictus,*
	Haemagogus mesodentatus,
	Sabethes chloropterus

a list of important viruses found in the Americas and their most important vectors. Geographic distribution studies of these species in the American continents are paramount for entomologic surveillance and control.[49]

Molecular Determinants for Arbovirus Infection on Aedes and Culex Mosquitoes

In order to fully understand the dynamics of arboviruses transmitted by mosquitoes that affect humans, we face the great challenge of elucidating, at a molecular level, how the interactions occur and lead to mosquitoes becoming infected with specific viruses and transmitting them efficiently.[90] Arboviruses are transmitted through different cycles, some of them involve multiple hosts, adding complexity to the challenge. Certain mosquitoes, such as *Culex* and *Aedes*, are highly competent vectors for numerous viruses responsible of major human diseases such as dengue, chikungunya, yellow fever, mayaro, west equine encephalitis, west nile, japanese encephalitis, Oropuche, Usutu, among others.[51] These mosquitoes exhibit unique characteristics in terms of their feeding habits, host-seeking behavior and response to visual and olfactory cues.[90] However, the molecular basis of vector competence remains to be explored in depth. Elements such as the vector gut microbiome including not only to extracellular bacteria but also to the different endosymbionts and mosquito specific viruses need to be thoroughly characterized in vectors such as *Aedes* and *Culex* mosquitoes, particularly to comprehend populations exhibiting different susceptibilities to viruses.[91,92] Finally, detailed genetic analysis of immune-related molecules in mosquitoes must be integrated into vector competence studies.

Arbovirus Diagnosis

Diagnostic assays provide the results upon which decisions are made on treatment of patients, captive and domestic animals, determination of which viruses are circulating in nature, and what measures need to be taken to control spread of infection. But there are many challenges to diagnostics today.[93] To begin with, there are more than 500 arboviruses, of which 100 cause disease. Worldwide movement of these viruses is increasing with increased numbers of people traveling at higher speeds than in the past, exacerbating diagnostic techniques based on the geographic location of the presenting patient. Many endemic pathogens have similar disease profiles (signs and symptoms) making it difficult to distinguish among them clinically . In addition, there is cross-reactivity between viruses in antibody assays and with some viruses, there is long lasting IgM.[94–96] Interpretation is further complicated by the phenomenon of "original antigenic sin".[49] Here, we describe laboratory tests available for diagnosis of human cases as well as field sample testing. It described molecular and classical assays for mosquito-borne viruses, the advantages and problems of both, basic protocols of select assays, and interpretation of results.

To determine the etiology of these diseases when a febrile patient presents to a clinic, laboratory-based diagnostic tests are needed. The specific test is selected based on sensitivity, specificity, speed, what specimen is available, and cost. It has been shown, for example, that whole blood is the best specimen for WNV detection.[94–96] Diagnostics in humans

focus on acute antibody (IgM) in serum/csf, seroconversion IgG and/or neutralizing antibody in acute/convalescent paired specimens, or non-structural 1 antigen in dengue cases, or active infection: virus or viral RNA in serum/csf.[94–96] In order to know which test to perform, the clinician must be knowledgeable about the pattern of each virus infection. For example, with dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV), the extrinsic incubation period (EIP) is 8–12 days and the intrinsic incubation period (IIP) 3–14 days (average 4–7). The EIP is the day from when the mosquito takes an infectious blood meal from human number 1 to when it transmits to human number 2 by bite. The IIP is from the day the mosquito feeds on a viremic human until it is capable of transmission . Viral RNA and RCR detection can be conducted from day 1–7 with these viruses and serological assays for IgM initially, then IgG subsequently.[94–97]

Detection of encephalitis viruses that do not undergo human amplification, such as WNV, is different as viruses can be detected in the blood only before the onset of clinical encephalitis approximately day 8–9. Recognition of non-specific symptoms such as fever, headache, myalgia may lead to early testing of blood, especially if an outbreak is ongoing, but otherwise may not be implemented in time.[95] Interpretation is further complicated by low titers of specific IgM reported in some patients for more than 12 months, attesting to the presence of IgM not systematically associated with a recent infection.[95] In the case of positive IgM results, it is necessary to confirm the diagnosis by a plaque-reduction neutralization test (PRNT), which is able to detect specific WNV-neutralizing antibodies and to differentiate between closely related flaviviruses. Two serum samples are required for accurate results, an acute sample collected during acute infection (0–45 days) and a convalescent sample collected after recovery (3–7 weeks). The test is also performed with several different challenge arboviruses depending on patient location and travel history. Knowledge of onset date and serum collection date are also essential for accurate interpretation of results.[95]

Because of cross reactions, particularly with flaviviruses, screening serologic assays to detect antibody collected ≥4 days after illness onset such as the IgM or IgG enzyme-linked immunosorbent assay (ELISA) must be followed by a PRNT as described above to detect virus-specific neutralizing antibodies.[91]

Diagnostic assays are also important in environmental surveillance. In such studies, assays are conducted to detect infectious virus or viral RNA in arthropod vectors (mosquito, tick, sandfly for example) in the case of vector-borne diseases, or the investigator can test for seroconversion in sentinel chickens or alternative animals. High throughput assays are needed because of the high numbers of samples.[95,98]

Flavivirus

Dengue Virus (DENV)

According to the WHO about four billion people worldwide are at risk of dengue infections and roughly 390 million new cases of the illness are documented annually.[99,100] Two mosquitoes from the *Stegomyia* subgenus of *Aedes, Ae. aegypti* (the most important vector) and *Ae. albopictus* are known for their efficient dengue virus transmission and are prevalent all year-round in 130 countries from all continents where dengue is endemic.[88,101] Unquestionably, both mosquito species are involved in dengue transmission cycles although *Ae. aegypti* is more anthropophilic and therefore the most epidemiologically important vector in dengue epidemics, at least in the Americas. Yet, recently dengue epidemics have been reported from the European continent and observations note cases in the Mediterranean region due to the widespread distribution of *Ae. albopictus*. The American continent is currently (2022–2024) experiencing the greatest number of dengue cases. The WHO reports that there are 11,500, 000 documented cases, with 7,000 deaths as of August 2024 worldwide and about 90–95% are clinical cases registered in the Americas.[102] Chiefly among them Brazil is reporting, as of August 2024, the highest number of cases, with 9.4 million clinical cases and 5,189 deaths.[102] Mexico historically has experienced outbreaks associated with each of the four dengue serotypes.[99,103] For instance, between 2011–2014 most dengue clinical cases reported to the Mexican Health Ministry were dengue serotype 1; many of them were located in coastal regions of the Gulf coast, Pacific, and the Yucatan Peninsula. During 2018–2019 dengue cases were mainly linked to subtype dengue 2 and clustered in a region largely located in the south of the country in the two Mexican States of Oaxaca and Chiapas.[103] The onslaught of this dengue outbreak also affected coastal areas of the States of Veracruz and Tamaulipas in the Gulf region and Guerrero Michoacan and Jalisco coastal settings.[99] Most of the affected regions are routes of human migration, both external and internal, and important trade paths of goods and services. In 2021, the lowest recorded number of dengue cases was reported to the Mexican Health Ministry in

decades and unquestionably linked to the outcome of the COVID-19 pandemic when most people remained in their homes with few vector contacts outside their premises. In 2022, a new pattern of dengue cases began to arise, and dengue subtype 3 became the predominant subtype in 2023. By the summer of 2024, dengue 3 became the leading cause of dengue illness in Mexico with the difference that the strains circulating are very aggressive and lethal cases have increased exponentially as compared with the summer of 2023 (23 deaths as of August 30th versus 128 deaths in August 30th 2024). Further, the disease has expanded to larger metropolitan cities such as Guadalajara in Jalisco state and Monterrey in Nuevo Leon state with the distinctive condition that now there is transmission in localities above 1800 meters altitude, originally considered the highest altitude for dengue transmission to occur.[99] The outbreak of dengue 3 subtype is expected to continue causing havoc in different regions of the Mexican Republic until the rainy season ends in the middle of November. Because of this, the Mexican Health Ministry has implemented control and surveillance activities declaring a state of dengue emergency and readiness all over the country since May 2024. These include among others, source reduction campaigns, larvicide and adulticide events to eliminate breeding activities of the vector known to circulate in different areas, surveillance undertakings monitoring temporal and spatial dynamics of the mosquito populations through the use of ovitrap collections, implementing viral isolation studies in mosquito pools to determine the dengue serotype circulating in different areas, performing insecticide resistance assays to launch biological and chemical mosquito control alternate activities, and even the use of new technologies such as the use of *Wolbachia*-infected mosquitoes for their control.[100,104]

Alphavirus in the Americas

Within the *Togaviridae* and according the International Committee on Taxonomy of Viruses (ICTV), the family encompasses a single genus, the alphaviruses.[105] The alphaviruses genus includes about 30 species, many of them are vertebrate pathogens spread by mosquitoes and moving otherwise within vertebrate host amid humans, non-human primates, equids, pigs, amphibians, reptiles, birds and rodents. Numerous alphaviruses are pathogens of public health and veterinary concern. Among the most important alphavirus disease pathogens of veterinary and public health interest in the Americas we found Venezuelan equine encephalitis, eastern equine encephalitis, Madariaga, western equine encephalitis, and chikungunya.[106]

Eastern Equine Encephalitis

Eastern equine encephalitis virus (EEEV; *Togaviridae, Alphavirus*) is an enveloped virus with a single-stranded, positive-sense RNA genome approximately 11 kb in length, and is closely related to western equine and Venezuelan encephalitis.[107,108] The virus is predominantly found in the Atlantic and Gulf Coast states of the US and northern Mexico, and has also been reported in the Caribbean.[108,109] Occasionally, EEE cases are reported in the Midwestern and Great Lakes regions. North American EEEV strains are relatively genetically conserved.[107]

The virus is predominantly maintained in nature in freshwater hardwood swamps through a cycle involving the mosquito *Culiseta melanura*, *Culex (Melanoconion) spp*. and passerine birds that serve as the primary reservoir hosts of the virus, sustaining its presence in nature.[109–111] Mosquito species other than *Cs. melanura*, such as *Aedes, Coquillettidia*, and *Culex* spp, can serve as bridge vectors, transmitting the virus to incidental hosts, especially humans and horses.[109–111]

EEE cases are most common during late summer and early fall in the northeastern US and mid-Atlantic states, coinciding with peak mosquito activity. The mechanism by which EEEV survives the winter in temperate regions is not fully understood, as infected adult mosquitoes are unlikely to survive the cold temperatures. Understanding how the virus persists through the winter is crucial for predicting and managing the risk of human and animal infections in the following transmission season. The most like method is Vertical transmission in vector species. In this context several hypotheses have been proposed:

- Hibernating hosts: Some research suggests that certain vertebrates, such as reptiles and amphibians, might act as overwintering reservoirs for the virus.[112,113]
- Mosquito larval overwintering after becoming infected in the fall – this hypothesis only has negative results.[114]
- Re-introduction from warmer southern foci by migrating birds moving north from the Southeast, particularly Florida where year-round transmission occurs.[110]

- Recrudescence of virus in latently infected birds that reactivates in the spring.[115]

Human infections with EEEV are rare and range from asymptomatic to severe. Symptomatic disease typically begins with flu-like symptoms, such as fever, headache, and myalgia. In severe cases, patients develop encephalitis, characterized by altered mental status, seizures, and coma. The case-fatality rate for severe cases is high (around 33%), and survivors often suffer from long-term neurological complications, including cognitive impairment, seizures, and motor deficits. EEEV infection in horses also causes encephalitis, with signs similar to those in humans. Mortality rates in horses are high, often exceeding 90%.[116] An equine vaccine exists to protect horses against EEEV infection. Equine cases of EEE are more frequent than human cases, with outbreaks often occurring in areas where the virus is enzootic.

According to the CDC, an average of fewer than 10 human cases is reported annually in the United States, with the number varying year to year.[117] Factors such as outdoor activities, proximity to swampy areas, and lack of preventive measures increase the risk of exposure. Factors such as climate, rainfall, and temperature can significantly influence mosquito populations and the incidence of EEE.

Effective control requires an integrated approach that combines mosquito control, surveillance, personal protection, and, potentially in the future, human vaccination. A thorough understanding of the virus's ecology, epidemiology, and molecular biology is essential for developing and implementing effective control strategies.

Research into EEE has underscored the importance of early detection and response to outbreaks. Surveillance programs that monitor mosquito populations and bird reservoirs are crucial for predicting and mitigating the spread of EEEV. Public health initiatives focus on educating communities about the risks and prevention strategies, particularly in areas with high mosquito activity.

Advancements in diagnostic methods have improved the ability to detect EEEV infections promptly, allowing for timely medical intervention.[118] Treatment for EEE in humans involves supportive care to manage symptoms, as there are no specific antiviral treatments available for the virus. Physicians emphasize the importance of preventing mosquito bites through personal protective measures and community-wide efforts to control mosquito populations.

EEEV remains a significant public health threat, causing severe neurological disease in humans and equids. Effective control requires an integrated approach that combines mosquito control, surveillance, personal protection, and, potentially in the future, human vaccination. A thorough understanding of the virus's ecology, epidemiology, and molecular biology is essential for developing and implementing effective control strategies. Continued research is needed to improve our ability to predict and prevent EEEV outbreaks and to develop better diagnostic and therapeutic tools.

Madariaga Virus

Madariaga virus (MADV) belongs to the eastern equine encephalitis (EEE) complex. Prior to 2010, MADV comprised Central/South American strains, ie, lineages 2–4 of EEEV but was separated as a distinct virus in 2010.[119,120] It circulates in Central/South America and exhibits distinct evolutionary and ecological features compared to the North American EEEV (lineage 1).[120] Because of the relatively few cases reported in horses or humans, it was believed that MADV was much less virulent than EEEV. However, recent findings of severe disease in both humans and equines indicate that MADV can be highly virulent.[121,122]

However, its enzootic cycle appears to closely resemble that of the Venezuelan equine encephalitis virus (VEEV), a virus that has rodents as the main enzootic hosts and various *Culex* (*Melanoconion*) species are enzootic vectors.[123] Most of the isolations of MADV have been obtained from *Cx*. (*Mel*). *pedroi* from Peru.[124,125] Unfortunately, very little is known about the epidemiology of MADV including the identity of the actual vertebrate amplifying hosts and vectors, or even its complete distribution.[126] Additional studies are needed.

Western Equine Encephalitis Virus: A Summary of Decline, Resurgence, and Research Imperatives

Western equine encephalitis virus (WEEV: family *Togaviridae*, genus *Alphavirus*) is historically responsible for major neurological disease outbreaks in humans and equids across the Americas;[127] yet it presents a compelling paradox. While its activity has dramatically declined (basically disappeared) in North America since the late 20th century, with the last human cases reported around 1998–1999 and no detection in US mosquito surveillance since 2008, it experienced

a major re-emergence in South America (Argentina, Uruguay, Brazil) in 2023–2024, causing hundreds of equine outbreaks and over 100 confirmed human cases, including fatalities after nearly 40 years quiescence.[128,129] This divergence underscores WEEV's retained epidemic potential under favorable conditions.

Epidemiology & Ecology: WEEV circulates in an enzootic cycle involving mosquito vectors and avian reservoir hosts. In North America, *Cx. tarsalis* mosquitoes and passerine birds (sparrows, finches) are primary hosts, with transmission often linked to irrigated agriculture.[127] Humans and equids are typically dead-end hosts. The North American decline is likely multifactorial, potentially involving widespread equine vaccination, reduced equine populations, changes in water management, and possibly a loss of intrinsic viral virulence. In contrast, the recent South American outbreaks have implicated the floodwater mosquito *Ae. albifasciatus* as a key vector, potentially linked to climatic factors like El Niño enhancing vector populations. The specific reservoirs in South America may include birds, rodents, and bats. The large susceptible equine population (due to lapsed mandatory vaccination in Argentina) was also a critical factor in the 2023–2024 resurgence. Phylogenetic studies suggest that this recent outbreak involves WEEV strains that have been circulating in the Southern Cone of South America at least since the 1950s [128]

Molecular Virology & Pathogenesis: WEEV is an ancient recombinant virus (EEEV-like non-structural/capsid genes, Sindbis-like envelope genes).[130] While genetically conserved overall, distinct lineages exist. North American strains have diversified [Groups A, B (B sublineages B1, B2, B3)], with B3 becoming dominant during the decline.[131] South American strains, including the 2023–2024 outbreak lineage, form a separate group related to older regional isolates, suggesting distinct evolutionary pressures.[128] Crucially, recent research identified key cellular receptors: Protocadherin 10 (PCDH10) used by both mammalian and avian strains, and Very Low-Density Lipoprotein Receptor (VLDLR) used by some virulent ancestral strains. Contemporary North American WEEV strains appear to have acquired mutations abolishing efficient binding to human/equine PCDH10 while retaining avian binding, potentially explaining reduced spillover and mammalian virulence. South American strains seem to retain mammalian PCDH10 binding capacity.[132,133] Pathogenesis involves initial replication, viremia, and potential CNS invasion causing encephalitis, particularly severe in infants, young children, and the elderly (3–15% case fatality rate), often leaving neurological sequelae in survivors.

While a safe and effective multivalent veterinary vaccine has significantly reduced western equine encephalitis (WEE) in horses, there are no licensed human vaccines or specific antiviral treatments currently available. Current research explores various vaccine platforms, including inactivated viruses, virus-like particles (VLPs), and DNA vaccines. Preclinical and early-phase clinical trials have shown some promise, but further research and development are needed to bring a licensed human WEE vaccine to fruition. While there are no specific antiviral treatments for WEE in humans, control methods primarily focus on reducing the risk of transmission by targeting the mosquito vectors.

Critical Research Questions: The stark epidemiological contrast necessitates further research. Key questions include:

- What precise combination of ecological, host, and viral factors drove the North American decline versus the South American resurgence?
- What are the exact environmental triggers and thresholds for South American outbreaks, and how does WEEV persist between epidemics there?
- What are the specific molecular determinants linking receptor binding changes (PCDH10, VLDLR) to virulence and host tropism differences between continental strains? Can North American strains regain mammalian infectivity?
- What is the vector competence of key mosquito species (*Cx. tarsalis, Ae. albifasciatus*) for currently circulating strains? What are the definitive overwintering/persistence mechanisms?
- How effective are current equine vaccines against outbreak strains? What is the path forward for developing safe and effective human vaccines and specific antiviral therapies?
- How can surveillance (human, animal, vector) be optimized for early detection and response across the Americas?

Addressing these questions through integrated, multidisciplinary research is vital for predicting, preventing, and controlling this unpredictable and re-emerging arboviral threat.

Venezuelan Equine Encephalitis Complex

The genus alphavirus of the family Togaviridae encompasses eight antigenic complexes. Among those the VEE complex alphaviruses are categorized and recognized into six subtypes, named I to VI, and involving 13 species.[106] Of the six subtypes, the important from the veterinary and medical standpoint is the subtype I. The IAB and IC viruses are recognized as epidemic or epizootic considering that they have been isolated only during equine and human outbreaks resulting and affecting up to hundreds of thousands of equids and humans throughout the Americas. The two subtypes are different from enzootic strains (subtypes/varieties ID-*Mosso das Pedras virus*, II–VI) circulating in sylvatic or swamp habitats, and sporadically cause and manifest disease in humans or domestic animals.[134] Among enzootic/endemic strains subtypes ID and IE comprise such strains that circulate continuously in forests and swamps of northern South America, Central America and Mexico. They circulate between wild animals, rodents, and mosquitoes, particularly *Culex (Melanoconion)* spp. mosquito vectors, leading to a large burden of endemic disease from direct spillover.[134,135] Although many VEE complex viruses have not been implicated in human disease, those that are associated with human disease (principally VEEV) can cause acute, often severe febrile illness that may progress to encephalitis, causing severe human morbidity and mortality.[136] Clinically, human VEE is usually misdiagnosed as dengue or other arboviral diseases such as the ones associated with other flavivirus (West Nile, St Louis), other alphavirus (chikungunya), group C bunyavirus, etc. rendering it difficult to estimate the scope of its public health and economic impact. No differences in disease presentation have been observed in humans upon infection with epizootic versus enzootic VEEV strains. The most common signs and symptoms include fever, headache, retro-orbital pain, tremors, prostration, nausea and vomiting.[137] These nonspecific signs and symptoms that are characteristic of VEE cases that do not always progress to neurologic disease, overlap extensively with other arboviral etiologies such as dengue virus infection but with no hemorrhagic display. Acute VEE typically lasts 3–4 days. A small proportion of patients develop more serious neurological disease, characterized by convulsions, disorientation, drowsiness, mental depression and, in some cases, death.[138] Epizootic VEEV in equines particularly with subtypes IAB and IC is manifested by blindness, injury, depression, weakness, incoordination (walking in circles), ataxia, grinding teeth, convulsions, diarrhea, colic and sudden death.[139] In these scenarios, postulates have been proposed for VEEV emergence and closely associated with specific mutations in the VEEV envelope glycoprotein E2 gene of enzootic subtype ID or IE strains.[140] These mutations result in the accumulation of positively charged amino acid changes on the surface of the virion spikes bringing about increased virulence and viremia in equids and even boosting infection of epidemic vectors like *Ae. (Ochlerotatus) taeniorhynchus* and enhancing its ability to transmit the subtype IE VEEV. It was also demonstrated that *Ae. taeniorhynchus*, an abundant epizootic vector in coastal areas of Chiapas and Oaxaca, was much less susceptible to isolates obtained during the 1993 and 1996 epizootics compared to the epizootic IC strain of VEEV[140,141] Likewise, these approaches validate the hypothesis that the IAB and IC subtypes arise from mutations in enzootic ID strains highlighting the acquisition of epizootic/epidemic features.[140] In this picture and in the Mexican pacific coastal lowlands of Chiapas and Oaxaca in 1993 and 1996 respectively an equine epizootic was documented.[142] Studies demonstrated the VEEV Mexican outbreak as the first evidence of a VEE epizootic associated with subtype IE, suggesting that an equine-virulent strain emerged or was recently introduced into southern Mexico.[143] Experimental infections conducted with isolates from both outbreaks showed that the 1990s outbreaks probably did not spread beyond southern Mexico because the strains involved did not amplify efficiently in equids.[144,145] A mechanism of VEEV emergence was proposed by reverse genetic studies demonstrating that a single Ser → Asn amino acid substitution at position 218 of the E2 envelope glycoprotein was the major determinant escalating *Ae. taeniorhynchus* infectivity. Thus, viral adaptation to a vector that prefers to bite large mammals was suggested as the emergence mechanism in the 1990s outbreaks in southern Mexico.[136,141] The studies indicated long-term enzootic and endemic VEEV circulation in the Chiapas region and continued risk for disease in equines and humans. Data from serosurveys, sporadic equine cases and viral isolates implying that the coastal Pacific plains from Guatemala to the Oaxaca isthmus represents an enzootic and endemic zone of VEE. This zone also extends east from the Papaloapan basin to the Gulf coast, including lowland coastal regions of the States of Tamaulipas, Veracruz and Tabasco. To sum-up, VEEV subtype IE is endemic in large coastal strips, both in the Pacific and in the Gulf coasts of Mexico, and in the latter it was demonstrated the important role of *Culex (Mel.) taeniopus* plays in enzootic VEEV

cycles.[136,143,145] Yet, the extent of human VEE in most regions of Mexico remains unknown due to the lack of surveillance and laboratory diagnostics. Further, the picture gets complicated for the presence of several arboviral etiologies including dengue and other alphavirus such as chikungunya overlapping the diagnostic scenario and confounding a definite assessment.

Chikungunya

Chikungunya virus (CHIKV) of the Togaviridae family is a pathogen that phylogenetically has been linked to the Semliki Forest complex of the genus *Alphavirus*.[146] CHIKV has been associated to an approximate 16.9 million cases annually in over 104 countries in Africa, Asia, the Americas, Europe and Oceania since 2004.[147] CHIKV was first recognized in 1952 during an outbreak in Tanzania and named based on the kimakonde words for "that which bends up" meaning and describing a distorted posture of infected people with harsh joint pain.[148] Outbreaks and epidemics are characterized by their explosive and unpredictable nature, with attack rates ranging from 35% to 86% in impacted regions.[147] Herd immunity appears to limit the efficiency of CHIKV transmission because preceding infections likely induce lifelong immunity, protecting against re-infection[147,148] Phylogenetically, four major genetic lineages of CHIKV have been described including West African, East/Central/South African (ECSA), Asian, and the Indian Ocean[149–151] CHIKV is an arthropod-borne virus transmitted in peridomestic settings by *Ae. (Stegomyia) aegypti* and *Ae. (Stegomyia) albopictus* mosquitoes[151] In the Americas, the first reported outbreak of CHIKF involving autochthonous transmission was reported in December of 2013 on the Caribbean Island of St. Martin.[152] Between 2013 and 2023, PAHO/WHO estimated 3,684,554 cases in 50 countries in the Americas.[153] The first wave was documented in most regions the Caribbean, Central America, Mexico and northern region of South America between 2014 and 2015. A second wave in 2023 occurred among native populations in South America, with a new epicenter in Brazil that began expanding northeastward toward Central America and the Caribbean.[151] Many of these countries experienced large CHIKF outbreaks (Nicaragua, El Salvador, Honduras, Guatemala, and Haiti)[152] In 2022, 273,685 CHIKF cases were documented, and in 2023, 410,754 cases were reported from 17 countries of the Americas, including 419 deaths, representing a 43% increase. In 2024, 186,274 cases with 60 deaths were recorded.[153] The escalation in cases was mainly in South American, Central American and Caribbean countries with high human migratory trends towards the north of the continent and coming throughout the Mexican Republic.[153,154] Also, between 2022 and 2024, Mexico alone recorded 1.5 million people migrating across its territory. A large percentage, more than 600,000 people in 2023, the time of the highest number of registered cases of CHIKF came from Central and South American regions of the Continent. Such countries recorded a significant input to the migration caravans into the Mexican Republic.[154] Despite such facts, the numbers of CHIKF cases in Mexico remain at its lowest (only 21 cases) between 2020 and 2024[155] The reasons of such figures remain an enigma.

Conclusion

The symposium and workshop conducted in Chiapas, Mexico, in September 2024 focused on and intended to encompass an approach that falls within the framework conferred by the FAO, United Nations Environment Program, WHO, and World Animal Health Organization of the six interdependent action tracks proposed by the One Health joint plan of action (2022–2026).[45] Such tracks jointly provide to complete health and food systems, ease global health threats, and advance ecosystem management. Among the six tracks, two tracks include lowering "the risks from emerging and re-emerging zoonotic epidemics and pandemics" and another one "controlling and eliminating endemic zoonotic, neglected tropical and vector-borne diseases."[26,46] The two tracks undoubtedly are drivers that highlight and converge important zoonotic and vector-borne diseases that our commentary and workshop in the Mexico-Chiapas and Mesoamerican region focused on.[26]

In such scenarios, viruses including the hemorrhagic fever viruses, the coronaviruses, the highly pathogenic avian influenza viruses, and viruses causing zoonotic diseases in rodents and chiropterans such as Guanarito, Machupo and Junin are connected with high global health threats causing millions of cases with high mortality and morbidity both in humans and animals with corresponding socioeconomic impact. Complementing the picture, the arthropod-borne pathogens are responsible for over 200,000,000 cases of illness and about 700,000 deaths every year. In addition,

arthropod-borne pathogens are responsible for many severe domestic animal diseases.[26] Diseases in humans include those caused by protozoa, such as malaria, leishmaniasis, and *Trypanosoma* infections; filarial nematodes such as *O. volvulus* connected with river blindness disease; bacteria, such as bubonic plague, Lyme disease and various rickettsial diseases; arthropod-linked viral diseases such as Dengue, yellow fever, Zika, Chikungunya, Venezuelan equine encephalitis, and many others.[52,156–162] While the diseases mentioned above have been known for many years, other diseases, such as those caused by Mayaro, Oropouche, and Madariaga viruses,[98,126,163] viruses are becoming more recognized and parasitic infestations of live animals by fly larvae maturing and feeding inside the body of a host and known as myiasis are re-emerging. The vectors of these diseases include a wide variety of arthropods including mosquitoes, sandflies, flies, fleas, lice, kissing bugs, and ticks.[26]

Different methods are used to control different vector species, and we need to know which species are likely to be able to transmit a particular pathogen and under what conditions transmission is likely to occur so that appropriate control measures can be instituted to prevent these diseases.[46]

To ensure more efficient collaboration among various public health workers and spread the state-of-the-art knowledge on arboviruses and zoonotic emerging diseases in the Americas, we developed a multi-country symposium including attendees from Mexico, Cuba, Angola, Argentina, and the United States. The approach was a interdisciplinary lecture providing a scope of scientific and public and animal health policies designed to communicate and grasp the one health concept provided by the United Nations focusing in health diseases prevention strategies. The classroom-based work, on lectures were presented by keynote speakers and expert scientific specialists providing attendees with the scientific background on the basics of the epidemiology of important diseases in their region of origin including life cycle of pathogens, disease symptoms, diagnostics methods, arthropod/rodent trapping techniques, etc. This was subsequently followed by field-based work during which the attendees collected rodents, mosquitoes, ticks, birds, etc. in wild settings. Collected specimens were processed under the guidance of subject matter experts. Attendees of the workshop in Tuxtla Gutierrez, Chiapas, Mexico, included public health workers, veterinarians, physicians, and policy makers, and each came away with a better understanding of their and each other's role in preventing arboviruses and other zoonotic emerging diseases, which is the key topic of the present commentary.

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