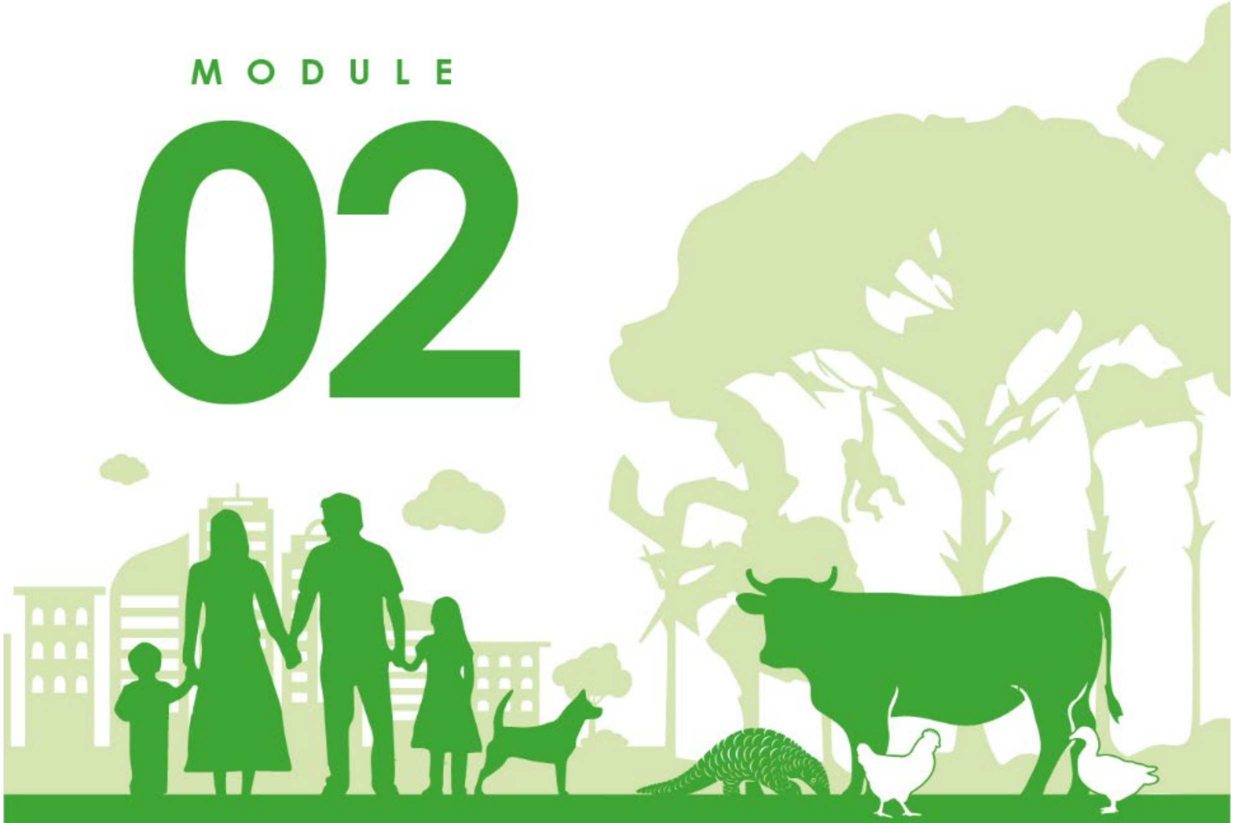
MODULE
02



HOW THE PAST INFORMS THE FUTURE

Opportunities in Southeast Asia to Prevent & Respond to Zoonotic Spillover

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MODULE 2: HOW THE PAST INFORMS THE FUTURE: OPPORTUNITIES IN SOUTHEAST ASIA TO PREVENT AND RESPOND TO ZOONOTIC SPILLOVER

Introduction

In this module, the authors address the pressing issue of zoonotic diseases in Southeast Asia and their overall impact on the public health systems. Given the prevalence of the COVID-19 pandemic, several countries have witnessed public health emergencies and emerging infectious diseases (EID) outbreaks, such as SARS, MERS-CoV, Nipah, avian influenza, and Nipah virus, highlighting the significance of understanding zoonotic spillover events. Multiple factors make Southeast Asia susceptible to EIDs, including its rich biodiversity, land-use changes, dense human settlements, extensive interface and interactions between humans and wildlife, high-density livestock and poultry, and the prevalence of traditional markets, along with both socioeconomic and ecological changes. Addressing these challenges requires a comprehensive and interdisciplinary approach involving scientists, governments, conservation organizations, local communities, and law enforcement agencies to name a few. In this module, we present a series of eight mechanistic steps that shed light on how pandemic pathogens can infiltrate and proliferate within the wildlife trade system, from reservoir hosts to wildlife trade to humans. Through a series of key interventions and theoretical and real-life case studies, we have identified strategies to minimize pathogen persistence in live animal supply chains, especially those involving wildlife, ways to reduce animal-human contact and protect individuals at risk, and the importance of effective monitoring and surveillance of zoonotic diseases. The goal of this module is to collectively develop comprehensive and mechanistic strategies to address the challenges posed by zoonotic spillover events in the region.

GLOBAL EPICENTER: WHY SOUTHEAST ASIA SERVES AS A HOTSPOT FOR EMERGING INFECTIOUS DISEASES

Over the last couple of decades, Southeast Asia and China have gained global attention regarding public health emergencies, food safety incidents, and emerging infectious disease (EIDs) outbreaks such as Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), H5N1 Influenza, Coronavirus Disease (COVID-19), and Nipah virus (Vourc'h et al., 2022). Over 60% of emerging infectious disease cases are caused by zoonosis, or infectious diseases shared between animals and people and over 70% of these originate in wildlife (Jones et al., 2008; Lee et al., 2023; Murray et al. 2015). These diseases are caused by pathogens, such as bacteria, viruses, and parasites, which naturally transfer between species in a process known as spillover.

It is important to note the difference between zoonotic disease cases, which oftentimes refer to instances where an individual or group of individuals have been diagnosed with a specific disease, and zoonotic disease events, which encompass a broader range of ecological, environmental, and epidemiological dynamics surrounding disease transmission. In general, the potential risk of zoonotic infectious diseases is increased by any situation that leads to increased contact between wildlife-to-humans, wildlife-to-livestock, or wildlife-to-wildlife, increased infection and shedding in wildlife, and increased behaviors of humans that lead to exposure (Plowright et al., 2017).

Nestled between the Indian and Pacific Oceans, the SEA region can be classified into two major ecological zones: the continental zone, which includes Thailand, Vietnam, and Myanmar, and the insular region, including Indonesia, Malaysia, and the Philippines (Hayami, 2001). Just south of China, Southeast Asia comprises eleven (11) countries: Lao PDR, Myanmar, Cambodia, Thailand, Indonesia, Darussalam, Malaysia, Singapore, Brunei, Vietnam, and Timor-Leste (Figure 2-1). The continental zone is characterized by major river basins while the insular zone is dominated by tropical rainforests (Figure 2-2) (Hayami, 2001). With a population of 690 million in 2023, Southeast Asia has experienced tremendous progress and development in recent years - from 2008 to 2017, its population underwent a substantial growth of 11.6%, with projections showing a population of 720 million by 2027. Its economic growth is also expected to rise as the gross domestic product (GDP) has grown to an average of nearly 5% per year from 2000 – 2016 (Lee and Hansen, 2019; OECD, 2017).

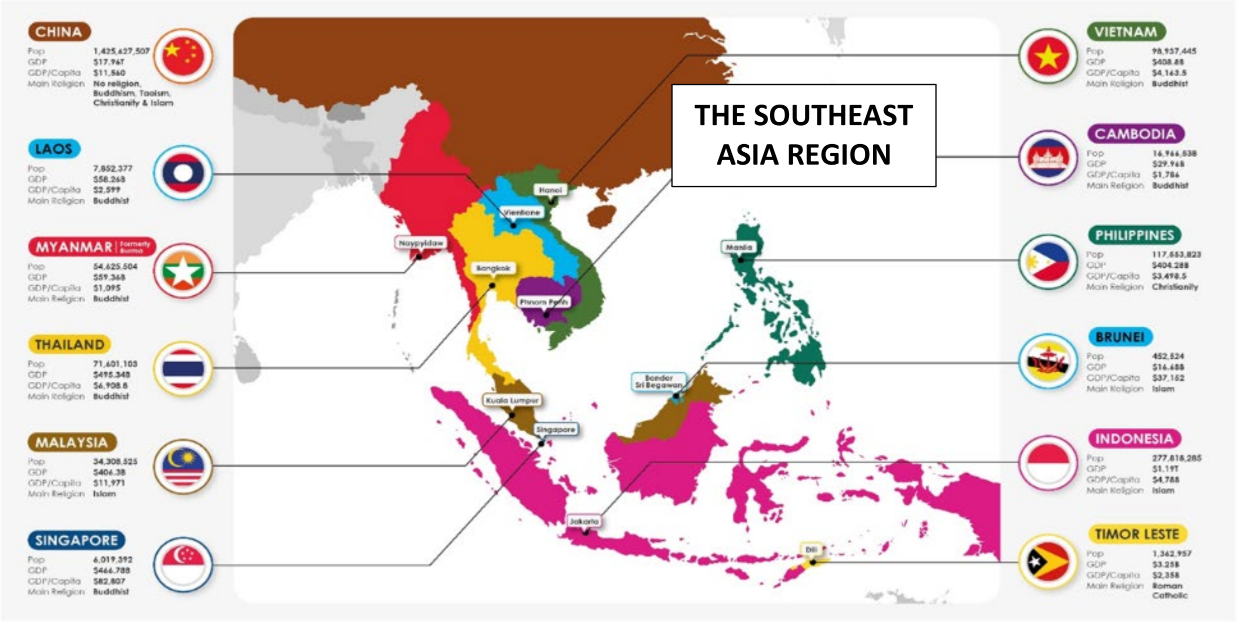


FIGURE 2-1. Map of the region (partial).

FIGURE 2-2. (Top) Mondulkuiri Province in Cambodia. Photo credit: Adam Roberts © WCS; (Middle) Tonle Sap Lake and a Floating Village in Cambodia. Photo credit: Cristian Samper © WCS; (Bottom) Nam Et-Phou Louey National Park protection area in northern Laos. Photo credit: Dominique le Roux © WCS.

In the last few decades, climate change and profound changes in human ecology, such as rising urbanization, deforestation, agricultural expansion, wildlife consumption and trade, increased global travel, and human encroachment on animal habitats, shifted land-use patterns leading to closer and more frequent human and animal interactions, have substantially increased the probability of future disease outbreaks (Saba Villarroel et al., 2023). Several crucial factors that significantly and jointly contribute to Southeast Asia's susceptibility to EIDs make up the reasons why this region serves as a zoonotic hotspot:

Biodiversity

FIGURE 2-3. Southeast Asia is home to a plethora of wild animals including the Malay Tapir *Tapirus indicus* (top-left), Sulawesi babirusa, *Babyrousa celebensis* (Bottom-left), sun bear *Helarctos malayanus* (middle), northern pig-tailed macaque Macaca leonine (top-right) and long-tailed macaque *Macaca fascicularis* (bottom-right). Photo credits: Julie Larsen © WCS; & Bill Meng © WCS. The macaques, Photo credit: K. Yoganand.

In the last few decades, there has been a rise of zoonotic diseases within the intertropical region. As of 2020, there are 364 billion pigs, 27 billion 223 million chickens, and one billion 587 million cattle (Morand, 2022). These numbers are important since emerging zoonoses can be transmitted from wildlife to an intermediary species, which are generally domestic or traded species. Transmission among intermediary hosts can lead to natural selection for viral strains that are more capable of infecting the human population.

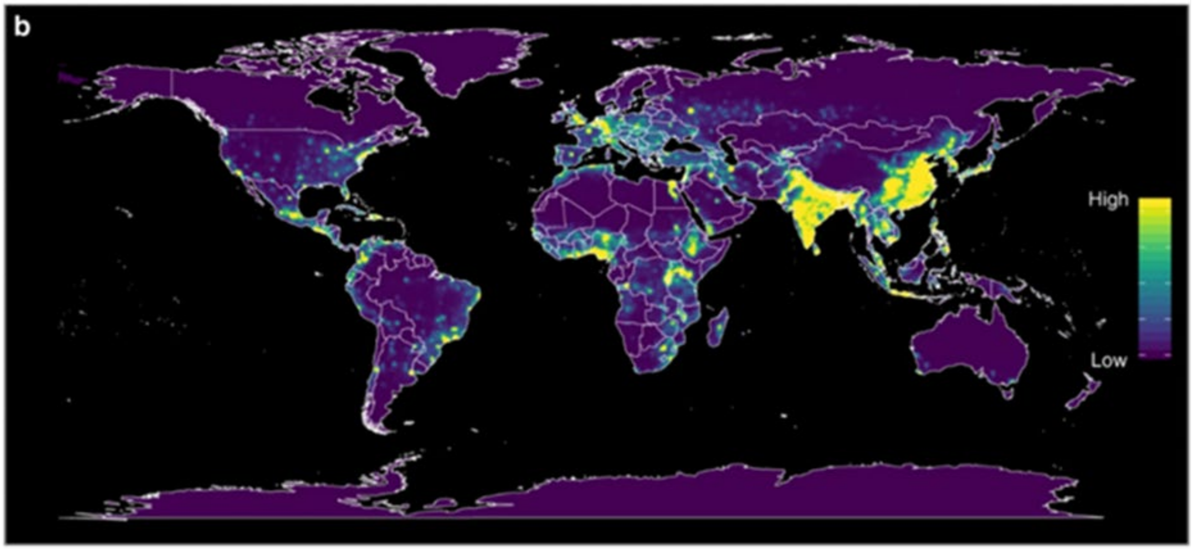
Traditional Markets, Livestock Farming, and Food Safety

Agriculture and livestock farming often serve as a vital source of economy, sustenance, and livelihood in Southeast Asia, adding over 10% to the region's total gross domestic product (GDP) (Ru et al., 2023). In countries like Indonesia, the Philippines, Thailand, and Vietnam, it employs roughly a third of the workforce (Alavi, 2011; SabaVillarroel et al., 2023). The reliance on livestock production and meat consumption in Southeast Asia is driven by rising incomes and urbanization.

FIGURE 2-4. Photos of traditional markets in Thailand (left) and Indonesia (right). Photo credits: Pixabay and Pexel.

Traditional markets, especially informal markets selling live animals, their products, and where live animals are sometimes housed and slaughtered on-site, are common in the region, and other low- and middle-income countries (Figures 2-4, 2-6) (Naguib et al., 2021). Together with farming and agriculture, these practices offer local communities many benefits including employment, improved household nutrition, food security, and economic well-being (Bardosh et al., 2023). The expansion rate of these sectors varies, with poultry farming growing substantially in Thailand and Malaysia compared to Vietnam, Indonesia, and Cambodia (Hassan 2014).

Human activities such as intensive farming and logging, linked to deforestation and road construction inadvertently bring vulnerable human populations into close contact with wildlife, some of which are competent reservoir hosts, increasing the risks of zoonotic spillover events (Figure 2-5). Deforestation has led to biodiversity loss, leaving competent hosts of zoonotic pathogens to thrive and dominate low-diversity environments (Gibb et al., 2020). Given the ongoing COVID-19 pandemic, the origin of the SARS-CoV-2 virus has brought attention to the potential role of traditional markets in disease emergence (Konda et al., 2020). This is often due to poorly regulated sourcing and transportation of animals, lack of adequate biosecurity measures, and the constant mixing and trading of animals (Engel and Ziegler, 2020; Greatorex et al., 2016; Pruvot et al., 2019).



Heat maps of predicted relative risk distribution of zoonotic EID events. estimated risk of event locations after factoring out reporting bias (weighted model output reweighted by population). Allen, T., Murray, K.A., Zambrana-Torrelio, C. *et al.* Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* **8,** 1124 (2017).

FIGURE 2-5. Global hotspot map of estimated risk in zoonotic disease emergence. Photo credit: (Allen et al., 2017).

Since the early 1990s, Southeast Asia and China have collectively contributed more than half the advancements in international livestock cultivation, and this growth is expected to continue (Jiao et al., 2021). Nevertheless, the increased proximity of humans and animals in these situations creates opportunities for spillover.

FIGURE 2-6. A large live animal market in Guangzhou, China, in April 2014. Note the mixture of multiple avian species in close proximity. Photo credit: Greg Gray.

Mitigating zoonotic disease risks in these environments necessitates collaborative efforts among policymakers, researchers, and local communities. Implementing rigorous biosecurity measures and hygiene protocols is crucial to prevent disease transmission while integrating modern surveillance systems aids in early detection and rapid response to potential outbreaks. Minimising the trade of wildlife taxa that are known to or potentially host high-consequence pathogens (HCP) should be included as a key prevention strategy. Educating market vendors and consumers about safe food handling practices is also vital, as is encouraging sustainable and responsible agricultural practices and wildlife trade. Therefore, a holistic approach - emphasizing evidence-based interventions, fostering community awareness, cross-disciplinary collaboration, and promoting responsible trade and farm/food safety practices from farm to fork – can help to ensure a more resilient and sustainable future for SEA, preserving its cultural practices and traditions while safeguarding public health and biodiversity. More information can be found on this in "Module 6: Strategies for Engaging with Diverse Stakeholders Across the Live Animal Value Chain."

Socioeconomic Factors

In Southeast Asia, the growing demand for animal protein, rapid population growth, rising disposable incomes, deforestation, and progressive urbanization have fueled an increased consumption of animal-based foods (Jiao et al., 2021). Between 2009 and 2018, Southeast Asia witnessed a large surge in meat production, particularly in countries like Vietnam and Thailand, where poultry farming has experienced a 56% increase, while pig farming increased by 23% (Jakobsen and Hansen, 2020). The escalating consumption of meat and seafood in SEA is projected to rise by 33% by 2030, with a 78% increase from 2017 to 2050 (Neo, 2018). This surge reflects a growing demand for meat consumption driven by rising disposable incomes and urbanization. At the same time, underlying social and economic factors, such as poverty and food insecurity, contribute to limited access to healthcare, lower vaccination rates, and heightened susceptibility to infectious diseases. Typically, areas with high levels of poverty and social inequalities have lower vaccination rates and tend to be least prepared to handle a pandemic. Poverty and social inequalities in affected areas can lead to differential morbidity despite improved housing, land drainage, mosquito repellents, nets, and electric fans (Farmer, 1996). Some of these life-saving items are out of reach of those most at risk of EIDs. Lastly, areas that tend to be most impacted by EIDs also have high gender inequality and low attendance of children in schools. These factors underscore the urgent need to address social and economic disparities to enhance resilience and preparedness against future pandemics.

Ecology

Urbanization changes the ecology of animal reservoirs, vectors, and pathogens, leading to declining biodiversity and increasing competitive dominant species that are also competent hosts of zoonoses (Blasdell et al., 2022; Gibb et al., 2020). Ecological systems are undergoing profound changes due to anthropogenic-caused urbanization, land use change, and climate change, causing major pathogen spillover from wildlife to human populations. For example, changes in land use, including deforestation, in subtropical Australia have disrupted natural bat habitats. This displacement, coupled with specific climate conditions, contributes to the spillover of Hendra virus from bats to horses and subsequently humans, as bats feed in human-dominated encroachments such as in agricultural areas where there are horses (Eby et al., 2023). Climate change plays an increasingly important role in spreading infectious diseases transmitted by vectors like ticks and mosquitoes (Franklinos et al., 2019). Other research indicates that resulting changing assemblages of wild mammals may increase viral sharing among species (Carlson et al. 2022). Climate change may also impact infectious disease spread by altering the frequency and intensity of extreme weather events, with which Southeast Asia is not unfamiliar, such as tropical storms, heavy rains, typhoons, monsoons, floods, and earthquakes being common (Syakbanah and Fuad, 2021; Torti, 2012). These natural events can disrupt healthcare infrastructure and its preparedness for managing emerging infectious diseases.

Wildlife Trade and Trafficking

Addressing the challenges posed by zoonotic spillover requires thorough examination of all aspects of the wildlife trade supply chain, as interventions must be strategically implemented at different points along the supply chain to manage potential risks and curb zoonotic disease transmission (FAO, 2011; Ludher & Kumar, 2018). Anthropogenic activities contributing to the spillover risk and emergence of zoonotic infectious diseases are international and domestic legal wildlife trade, illegal and unregulated wildlife trade and trafficking. Southeast Asia plays a major role in both legal and illegal wildlife and pet trade globally, contributing to nearly a quarter of the illegal wildlife trade worldwide. This includes the supply and demand dynamics for rare and exotic pets, as well as consumption practices (Rivera et al., 2021; Lin, 2021; Krishnasamy and Zavagli, 2020). The commercialization of wildlife trade has led to a greater supply of wild meat to urban consumers and international markets (Swamy and Pinedo-Vasquez, 2014; Milner-Gulland et al., 2003). Wildlife trade and trafficking increase the risk of spillover due to exchanges of bodily fluids and blood among humans and wildlife animals during various stages of the wildlife trade (Figures 2-7, 2-8, and 2-9). Thus, the risk of zoonotic diseases associated with live or dead animal movement, especially of mammalian species is real and significant, and can potentially elevate the probability of zoonotic pathogens being propagated through chains of transmission. Consequently, individuals working alongside the supply chain, including hunters, transporters, consumers, and local market sellers are exposed to a heightened risk of contracting zoonotic diseases.

FIGURE 2-7. (Left) A common palm civet *Paradoxurus hermaphroditus* left in a cage to be traded at a wildlife farm in Vietnam. Photo credit: WCS Vietnam. (Center) Large rat cage in Cambodia. Photo credit: Lucy Keatts at WCS. (Right) Monitor lizards being traded in Laos. Photo credit: Lucy Keatts at WCS.

The scope of the wildlife trade supply chain involves a vast network of animals, people, and activities. This involves everything from manufacturing and processing to the regulations and governance surrounding the hunting, capture, farming, transport, and distribution of wildlife and their associated products (FAO, 2011; Ludher & Kumar, 2018). Managing the risks associated with spillover demands an interdisciplinary and intersectoral collaborative approach that considers the interplay of factors along this complex trade network (Stephen 2021). Governments, conservation organizations, and law enforcement agencies must collaborate to enforce stringent oversight of both legal and illegal wildlife trading as well as pet ownership. Additionally, education and awareness campaigns are crucial in discouraging the demand for exotic pets and promoting responsible behavior among consumers (Verissimo and Wan, 2019). In the region, hunting is common for both trade and household consumption. These practices often display opportunistic and indiscriminate characteristics, with a variety of wildlife species, such as rodents, pangolins, carnivores and bats, being targeted (Engel & Ziegler, 2020). These wild animals are hunted at random and taken from their natural habitat. Subsequently, they are exploited for various purposes, such as collectibles, food items, servings in restaurants, merchandise for sale, pets, for medicinal purposes, and are found in open-air traditional markets, or marketed through online platforms and social media channels (Galindo-González, 2022; OECD/FAO, 2022).

Examples of Wildlife Supply Chain Structure: Various Points of Entry and Amplification

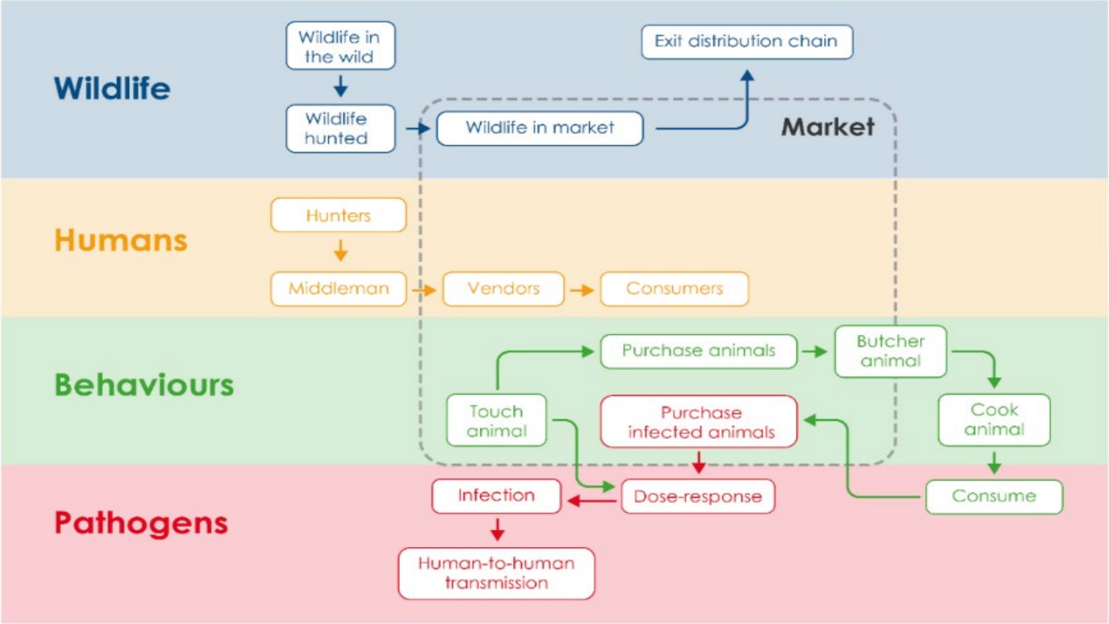


FIGURE 2-8. Conceptual model describing the potential for pathogen spillover and zoonotic disease transmission in the wild meat trade in Laos. Adapted from: Pruvot et al., 2019.

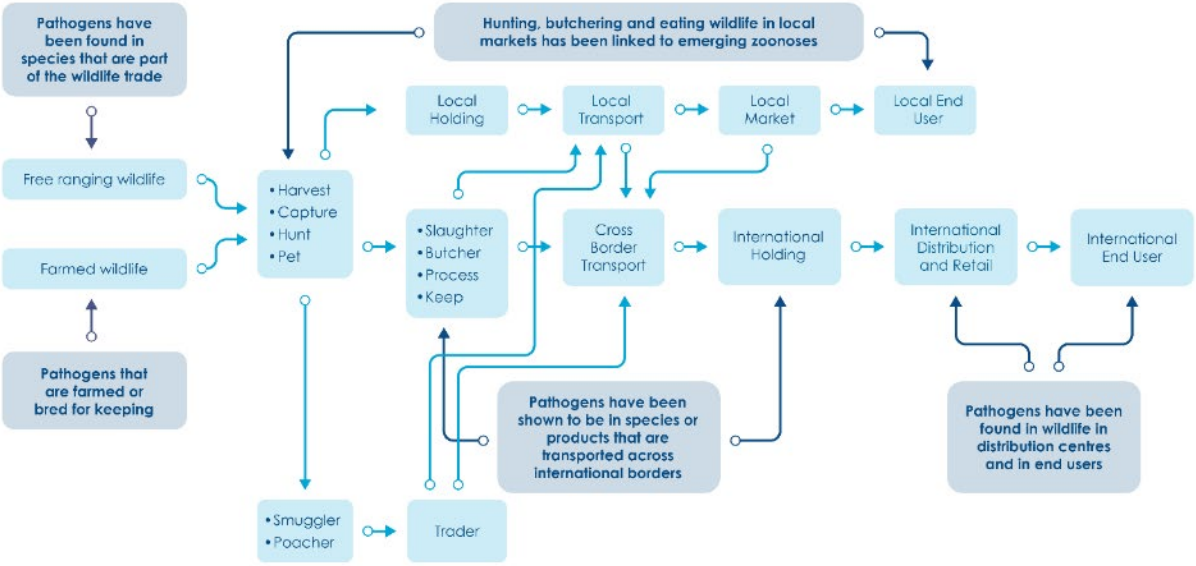


FIGURE 2-9. Pathogen detection and nodes for pathogen surveillance in the wildlife trade supply chain. Adapted from Stephen et al., 2021.

BOX 2-1 Avian Influenza in Southeast Asia

FIGURE 2-10. As part of a One Health study for avian influenza, veterinary workers collected an orotracheal swab specimen from a healthy chicken at Ha Vi live bird market in Vietnam, in July 2019. Vi Market is the largest live wholesale poultry market in Hanoi, Vietnam. It is estimated that 80,000-100,000 birds are slaughtered each day. The market includes 200 registered shops occupying a total of 20,000 square meters. Swab samples collected from healthy chickens indicate these birds have active influenza A virus infections. Photography by Greg Gray.

Avian influenza, or bird flu, is a viral disease of birds that has caused significant animal, public health, and economic consequences. While it is devastating to poultry, the concern has been that it may acquire the ability for human-to-human transmission. In 1996, China detected the first H5N1 outbreak in poultry. One year later in Hong Kong, the first human case was detected. Thereafter, an epidemic of Highly Pathogenic Avian Influenza (HPAI) H5N1 infections emerged in Southeast Asia in December 2003, affecting Cambodia, Indonesia, Laos, Thailand, and Vietnam. It is now considered endemic in poultry in many SEA countries and has caused sporadic zoonotic infections. Aside from public health impacts, it has also caused disruptions in poultry production and trade. Wild birds have likely contributed to its spread in SEA, but domestic poultry trade appears to have played a key role in the generation and maintenance of HPAI virus circulation (Gutierrez et al., 2009).

Unfortunately, high-risk poultry trading practices continue to persist, which impact the risk of infection and spread of HPAI within poultry. For example, a study of poultry trading behaviors in Vietnamese live bird markets demonstrated that middlemen, who are mobile, highly connected poultry traders that travel between farms and live bird markets to buy and sell birds, increase the likelihood of mixing poultry from different sources (Sealy et al., 2019). They are also more likely to be open for business for an extended period. This facilitates a network of live bird markets that can maintain circulation of influenza viruses. Rigorous quarantine, hygiene and sanitation protocols must be established for people who traffic or sell animals to avoid the transmission of pathogens.

STRENGTHENING BARRIERS: DEFINING ZOONOTIC SPILLOVER AND STRATEGIES FOR TRANSMISSION CONTROL

Zoonotic spillover is the passage of a microbe from a non-human vertebrate animal to a human (Temmam et al., 2019; Plowright et al., 2017). It occurs when a microbe that usually circulates in animal populations 'jumps' to humans. Most zoonotic spillovers are from endemic pathogens in domestic animals, impacting over 2 billion people and resulting in 2 million deaths worldwide each year(The World Bank, 2021). By contrast, spillover from wildlife caused most new and emerging human diseases and recent pandemics (Bernstein et al., 2022; Jones et al., 2008)., including COVID-19, which has resulted in at least 7.8 million deaths as of December 2023 (WHO COVID-19 dashboard).

Zoonotic spillover is a complex process that occurs along a pathway. This process happens in several steps, where an animal pathogen must overcome many barriers to infect a human. The movement of a pathogen through each barrier is facilitated by human drivers and influenced by factors like time, distance, changes in evolution and ecology, and random events. First, the pathogen circulates in its natural **reservoir host**, such as a mammal, or bird. Certain conditions can influence the prevalence and intensity of infection in the reservoir host population and the amount of pathogen excreted from the hosts. Next, a recipient host (for example, a human, domestic animal, or traded animal) needs to encounter the pathogen in the environment, known as **pathogen exposure**. Finally, the pathogen must be compatible with the recipient host, and the recipient host must receive a sufficient dose through the correct route of transmission for spillover to occur. It is important to note that only a small number of pathogens are compatible with humans. For example, most viruses cannot bind and enter human cells or evade our innate immune system, and an even smaller number of pathogens can spread from one person to another.

Pandemic Prevention

Pandemic prevention refers to a comprehensive set of proactive strategies and measures aimed at reducing the risk of infectious diseases on a global scale (Coccia, 2022). It involves several complementary approaches aimed at controlling and mitigating the impact of outbreaks including:

1. **Primary pandemic prevention:** These strategies focus on factors that reduce spillover risk factors by interrupting the transmission pathway of infectious diseases from animals to humans. These interventions target various points along this pathway to prevent the initial transmission (or spillover event) and the establishment of the pathogen in human populations (Vora et al., 2022; Markotter et al., 2023). Some examples of this include implementing regulations to reduce deforestation or habitat destruction, minimizing trade and consumption of certain taxa of wild animals, enforcing stricter biosecurity measures in animal markets, or promoting sustainable agricultural practices to reduce the frequency of spillover events. By understanding the factors contributing to spillover, such as the destruction of tropical forests, agricultural expansion and intensification, and wildlife trade and hunting, pandemic prevention can focus on mitigating these risk factors.
2. **Secondary pandemic prevention**: Secondary prevention focuses on detecting and containing the spread of pathogens once they have spilled over into humans (Vora and Varma, 2022). It involves early detection through surveillance, contact tracing, case

isolation, and diagnostics, along with treating infections, implementing targeted lockdowns or restrictions, ramping up healthcare policy and supplies to manage surges in cases, and recruiting and retaining healthcare workers.

In this module we explore primary pandemic prevention, focusing on evidence-based strategies aimed at controlling risk factors associated with disease transmission from animals to humans.

A One Health approach, which is a comprehensive approach that optimizes the health of the environment, animals, and people, will be needed to address both primary and secondary pandemic prevention and will involve identifying (Galindo-González 2023) and addressing anthropogenic drivers of spillover like land-use change, climate change, and wildlife trade (Adisasmito et al., 2022; One Health High-Level Expert et al., 2022; Keesing et al., 2010). This definition has been explored further in Module 1 of this guidebook. Barriers to zoonotic spillover are illustrated in Figure 2-11. Whole-of-government actions will need to be taken and at the same time, research should continue to develop greater biological insight into zoonotic spillover and drivers of different pathogens and systems. To support decision-makers on what they can do now to mitigate these drivers, this module highlights some key examples of zoonotic diseases in the Southeast Asia region.

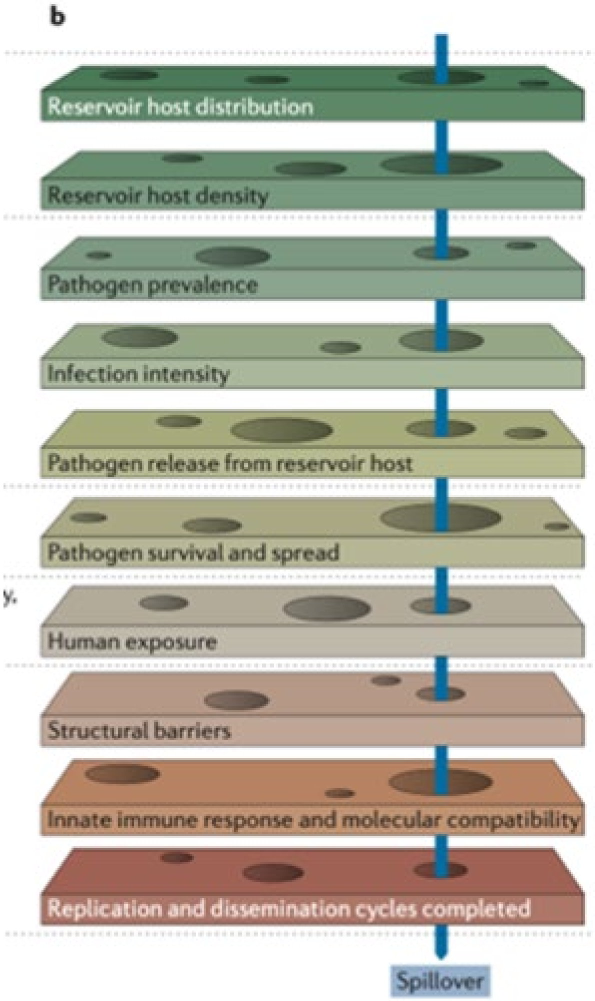


FIGURE 2-11. Barriers to Zoonotic Spillover. In many disciplines, determinants of spillovers are being studied. A pathogen needs to navigate through a variety of barriers to transmit from one species to another. If any of the barriers do not allow for the pathogen to pass through, then a spillover cannot occur. Spillover of some pathogens requires that gaps (depicted as holes) in all of the barriers align within a narrow window in space and time, indicated by the blue arrow. Adapted from Plowright et al., 2017.

FROM SOURCE TO SPILLOVER: UNDERSTANDING MECHANISMS OF ZOONOSES AND EVIDENCE-BASED GUIDELINES FOR INTERVENTION

Pathogens can make the physical jump from animals to humans in any number of ways. These include direct contact with infected animals or their bodily fluids, consumption of contaminated food sources, vector-borne disease transmission which occurs via mosquitoes, ticks, and fleas, environmental exposure, and occupational hazards for individuals working closely with animals. Understanding these different pathways is essential for preventing and managing zoonotic diseases and protecting human health.

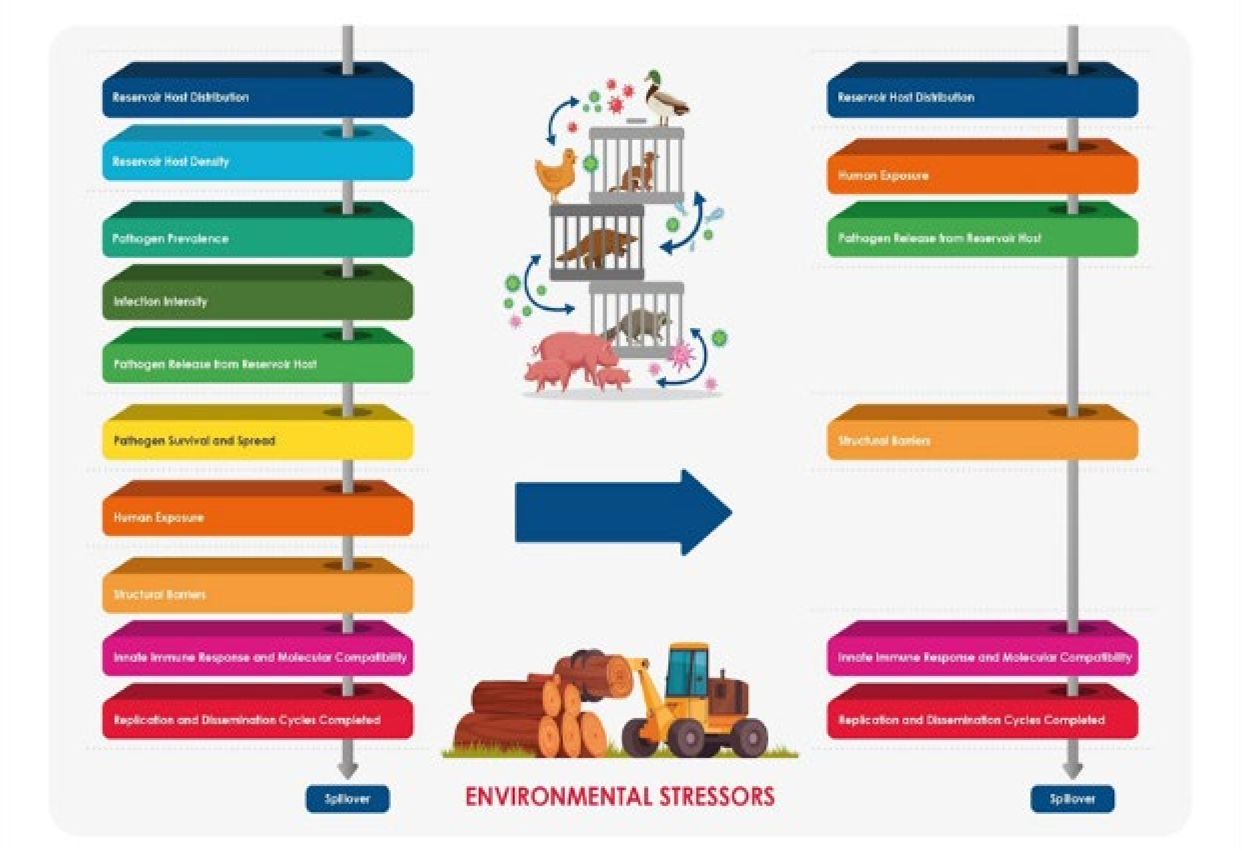


FIGURE 2-12. Environmental stressors and wildlife trade can rearrange natural barriers to spillover and increase their permeability. Adapted from Plowright et al., 2017.

We have developed a mechanistic and structured way of viewing the risk of zoonotic spillover through wildlife trade (see Figure 2-12). We summarize the components of the wildlife trade and where interventions could prevent the entry of new zoonotic pathogens into the trade or prevent the propagation and amplification of new zoonotic pathogens through populations of traded animals. We focus on points where the chain of transmission that leads to outbreaks in humans could be prevented.

Prevent movement of high-risk reservoir host species into farms and trade; and prevent contact of reservoir hosts with traded or farmed animals

In Southeast Asia, small-scale trade, hunting, and farming in wildlife coordinated by locals has transitioned to coordinated, domestic and international trade networks (IPBES, 2020). In 2016, wildlife farms and markets hired approximately 14 million people in China alone (Arranz & Huang, 2020). Global awareness surrounding the scale of legal and illegal domestic and international trade of animals has been heightened in the wake of the COVID-19 pandemic. The United States is the main market for wildlife pets, where millions of live animals come from countries identified as hot spots for emerging infectious diseases (EID). This global trade occurs without effective surveillance or regulatory oversight, posing potential risks to public health (Smith et al., 2009). Within the wildlife trade supply chain (Figures 2-8 and 2-9), it is important to consider the groups of individuals and activities from local hunters, transporters, and butchers to live animal market sellers, retailers, and pet shop owners, restaurant and culinary trade, and to international trade actors (Ludher & Kumar, 2018). Addressing the challenges and vulnerabilities within the components of these supply chains and across wildlife trade requires a comprehensive and holistic One Health approach to reduce overall risk.

Importing live animals, especially in the absence of regulations and biosecurity control measures brings the risk of importing pathogens that can infect native wildlife, livestock, and people. COVID-19 most likely developed from bat-origin coronaviruses (Rothan & Byrareddy, 2020). It has also been associated with a seafood market in Wuhan, China, where wild live animals were sold (Lam et al., 2020). However, to date, the origin of SARS-CoV-2 has not been established (NIH, 2022). Recognizing this interplay underscores the need to identify, understand and address the risks posed by the wildlife trade to effectively mitigate the spread of zoonotic diseases.

Strategies to Reduce the Risk of Zoonotic Diseases in Farming & Trade

The key issue is how to prevent these pathogens from entering the wildlife trade and the supply chain where they could have the opportunity for amplification, evolution, and exposure to humans. We suggest three strategies to reduce the probability of the introduction of these viruses into the animal trade:

1. Reduce the probability that free-ranging wild animals contact people or trade animals.
2. Reduce the probability that free-ranging wild animals are infected and shedding viral pathogens by improving landscape immunity.
3. Reduce the probability that high-risk host taxa (known to host pandemic potential viruses) enter the wildlife trade.

1) Reduce the probability that free-ranging wild animals contact people or traded animals

One primary driver of zoonotic disease outbreaks is land use change. Wild animals are less likely to use human-dominated areas if they have sufficient food and habitat in unmodified landscapes (Plowright et al. 2024). If resources are fleeting, animals need adequate habitat to locate places for roosting, resting, socializing, finding food and water, and engaging in migratory or nomadic movements. Deforestation and loss of habitat, particularly those providing resources during times of scarcity or high energetic demand, can force animals to seek alternative resources within human landscapes (Plowright et al. 2024; Eby et al., 2023). Additionally, it has been shown that landscape fragmentation along with certain smallholder farmers' behaviours along these patches of forests can increase the likelihood of human-animal contact events (Bloomfield, 2019). Consequently, ecological countermeasures like wildlife habitat conservation play a pivotal role in diminishing the occurrence of wild animal contact with traded animals, along with other targeted landscape-based interventions (Figure 2-13). For instance, in Australia, the spillover of the Hendra virus is thwarted by the blossoming of native trees, which lure bats away from areas with horses (refer to 'Case Study #1'). An effective intervention addressing the fundamental cause of spillover involves the restoration and preservation of winter flowering habitats for Pteropodidae bats in Australia (Eby et al., 2023). After the 1998-1999 Nipah virus outbreak in Malaysia, mango trees and other trees preferred by bats were removed from the vicinity of piggeries (refer to 'Case Study #2'), potentially explaining the absence of subsequent Nipah virus outbreaks in pigs in Malaysia.

FIGURE 2-13. Depiction of reforestation initiatives which help to redirect bat populations away from local and agricultural communities. This is crucial for preventing disease transmission.

To further amplify the spatial separation between reservoir hosts and potential spillover hosts, several recommendations can be considered:

- Enforcing stricter risk-based regulations on wildlife trade and ensuring that traded animals meet health conditions, including undergoing thorough disease monitoring can help prevent the introduction of potential pathogens into trading networks.
- Implementing comprehensive wildlife habitat conservation and restoration programs can enhance the availability of natural resources for wild animals, reducing their incentive to venture into human- and livestock-dominated areas.
- Promoting public awareness and education about the risks associated with wildlife trade and zoonotic disease transmission can encourage responsible behavior and informed decision-making among traders and consumers.

2) Reduce the probability that free-ranging wild animals are infected and shedding viral pathogens by improving landscape immunity

Viral pathogens circulate in their natural populations, rarely causing disease in their reservoir hosts. Moreover, many viruses are found at either low prevalence or are rarely detected at all in their natural hosts. For example, Nipah and Ebola viruses may circulate within a given population, causing local outbreaks in bats before moving to another susceptible population before dying out (Plowright et al., 2015). When animals are exposed to stressors, such as habitat loss, or they are unable to gain enough energy from available habitat, they can go into “allostatic overload” or stress, where energy is shifted away from energy-expensive processes such as reproduction and immunity (Plowright et al. 2024, in press; McEwen & Wingfield, 2003). Therefore, anthropogenic activities that disrupt ecological systems can trigger the infect-shed-spill-spread cascade, such as land use change, deforestation, and climate change (Plowright et al., 2021; Reaser et al., 2022). Stress increases the likelihood that wildlife will release (or shed) pathogens in ways and locations that lead to the infection of other animals of the same or different species (called spillover). One is more likely to see higher infection prevalence and shedding during those periods (Becker et al., 2023; Eby et al., 2023).

Landscape immunity is defined as ecological conditions that maintain and strengthen the immune function of wild species within a particular ecosystem (Reaser et al., 2022). Ensuring that wild animals have the habitat and resources they need for sufficient energy to sustain an immune response is fundamental to reducing the risk of pathogen infection and shedding from reservoir hosts (Plowright et al., 2021). To enhance landscape immunity, it is essential to focus on biodiversity conservation, habitat restoration, and minimizing habitat fragmentation. (ref. Case Example #2 on Nipah virus outbreak). Some real-life examples of ecological countermeasures to improve landscape immunity include agroforestry to provide food and shelter for bats while receiving the benefits of bat predation of agricultural insect pests (Voigt & Kingston, 2016), restoration of critical food plants along animal migratory routes (Reaser et al., 2022), and the creation of protective buffers around areas of aggregation such as caves where bats roost (Plowright et al., 2024, in press). These efforts can help maintain the balance of ecosystems and support the overall health and resilience of wild animal populations. By providing suitable conditions for wildlife, we can alleviate stressors and promote optimal immune responses in wild animals (McEwen & Wingfield, 2003).

3) Reduce the probability that high-risk host taxa enter the wildlife trade

The wildlife trade, particularly in high-risk host taxa known to harbor pandemic potential viruses, poses significant risks for zoonotic disease transmission to humans, discussed further in "Module 3: Efforts to prevent transboundary disease outbreaks in the Southeast Asia region". Certain host taxa have a higher likelihood of harboring viruses that are zoonotic and have pandemic potential. For example, bats not only harbor more viruses than rodents (Luis et al., 2013), but the viruses they harbor are more likely to be virulent in people (Guth et al., 2022). Eliminating bats from the wildlife trade would be a sensible step to ensure that undiscovered bat pathogens do not have the opportunity for amplification, evolution, and exposure to people (Osofsky et al., 2023). Primates, rodents, and birds are other taxa that are associated with pandemic potential viruses (Zhou et al., 2021). Other species that have been identified as susceptible bridging hosts for human pathogens could also be excluded from trade. For example, civets and raccoon dogs can be infected by SARS-CoV-1 and SARS-CoV-2, respectively, and may be able to maintain chains of infection in captivity under some circumstances (Korath et al., 2022; Mallapaty 2023). More information on this can be found in "Module 4: Priority Pathogens, Their Reservoirs, and How to Contain Them."

To combat the probability that high-risk hosts enter the wildlife trade, countries should implement strict regulations and policies based on scientific evidence and risk assessments. While the majority of Southeast Asian countries have indicated presence of provisions to regulate wildlife trade, especially CITES-listed and/or protected wildlife, there remains inconsistencies in the legal framework to tackle the different nodes of the supply chain (ASEAN Handbook on Legal Cooperation to Combat Illegal Wildlife Trade, 2021). Risk assessments on zoonotic disease risk introductions can take reference from lessons applied in the regulation of trade in domestic livestock or food safety regulation (Campbell et al., 2022). Additionally, raising public awareness about the risks associated with wildlife trade is crucial. Media campaigns, community outreach programs, and collaboration with local NGOs and wildlife conservation groups can be designed to educate consumers and stakeholders about the potential health risks of consuming high-risk species like civets and pangolins, which have been implicated in zoonotic outbreaks in the past (Mohapatra et al., 2016). More information on this can be found in "Module 8: Applying Participatory Methodologies to Countering Zoonotic Spillover." For additional information on alternative livelihood solutions, refer to "Module 6: Strategies to Engage Diverse Stakeholders Across the Live Animal Value Chain to Address Risk."

Another useful tactic is to enhance law enforcement and monitoring efforts to combat illegal wildlife trade by increasing resources and capacity. Malaysia, for example, has established wildlife crime units within its enforcement agencies to tackle wildlife trafficking (Ariffin, 2015). Similarly, Indonesia has implemented stricter penalties for wildlife smuggling (Shepherd et al., 2020). Singapore has introduced stiffer penalties for illegal trade in species protected under CITES, and stronger enforcement powers e.g., making clear that items used to deliberately conceal and/or convey wildlife products can be seized and forfeited (Republic of Singapore Veterinary Conditions and Declaration for Importation, 2021). In addition, a domestic trade ban on elephant ivory has also been implemented (Yeo HHT 2023). Collaborative transboundary efforts among Southeast Asian countries, such as intelligence sharing and joint operations, can help identify and dismantle wildlife trafficking networks. More information on this can be found in "Module 3: Efforts to Prevent Transboundary Disease Outbreaks in the Southeast Asia Region." Providing sustainable alternative livelihoods to communities engaged in wildlife trade is important as well. Initiatives like community-based ecotourism have been successful in reducing dependence on wildlife trade. For example, the Kinabatangan Wildlife Sanctuary in Borneo, Malaysia has supported community-based tourism and provided economic opportunities while protecting wildlife habitats.

Case Study 2-1: Ecological Interventions to Halt Spillover of Hendra Virus Within Bat Species in Australia

FIGURE 2-14. Flying foxes are known reservoir hosts of viruses of the family Paramyxoviridae, including Hendra and Nipah viruses. Some of the flying foxes are known to roost in colonies in the middle of urban settlements such as this Lyle's flying fox *Pteropus lyeli* colony in Phnom Penh city in Cambodia. Photo credit: K. Yoganand.

The black flying fox, a nomadic nectarous bat species, is a reservoir host of Hendra virus– a henipavirus (family Paramyxoviridae) that has a 57% fatality rate in humans and 75% fatality rate in horses (Figure 2-14). If infected bats feed in trees within horse paddocks, and contaminate the grass with urine, horses that consume or sniff the grass can become infected and serve as a bridging host for the virus to infect humans (Field et al., 2012; Plowright et al., 2015).

Like any other nectivore, Black flying foxes depend on having a constant supply of food, and as a result, historically, they moved nomadically from site-to-site to feed on nectar from ephemeral blossoming events in native forests. There are extensive feeding habitats that provide nectar for bats in the summer, but few trees provide nectar in the wintertime and these trees have been selectively cleared for agriculture and development. The lack of food during the cold seasons drives an ecological shift in bats' behavior: bats move into agricultural and urban areas to find food from weedy or ornamental trees planted by humans, including from trees in horse paddocks (Eby et al., 2023).

Viral shedding is more likely to occur from bats in these new overwintering habitats compared to bats in their historic winter habitats (Becker et al., 2023). However, after an El Niño-driven shortage, all bats are likely to be shedding the virus, especially the bats in novel overwintering habitats that are also feeding in proximity to horses. These bats are probably shedding more viruses because they are nutritionally stressed. However, if there is a native forest flowering event in winter, the bats will move away from areas with horses and no spillover event will occur (Eby et al., 2023).

The risk of spillover is the highest after long periods of food shortage when there's no flowering event during the winter seasons. If there's a flowering event during winter or there are no food shortages, there's very little risk of spillover. An ecological intervention to stop the spillover of Hendra (countermeasure) is replanting winter flowering trees (Eby et al., 2023).

General concepts that can be applied to other viruses:

- Two events aligning at the same time - the allocation of animals into novel habitats and the stress that drives pathogen shedding, are important for driving spillover.
- An easy countermeasure is to replant the habitats that provide food at periods of high energy demand or resource bottlenecks.

Case Example 2-2: Nipah Virus outbreak in Malaysia and Singapore (1998-1999)

Nipah virus (NiV) emerged in Malaysia in 1998 during an outbreak in commercially farmed pigs, most likely after viral spillover from the large flying foxes (*Pteropus vampyrus*; Figure 2-15) (Looi and Chua, 2007). The intensification of both the pig and mango industries in Malaysia likely enabled the conditions for spillover (Pulliam et al., 2012). At the time of spillover, the pig population on the index farm was ~ 30,000 animals. Moreover, mangoes, jackfruit, and durian were grown on the farm and several mango trees were close to pig enclosures (Pulliam et al., 2012).

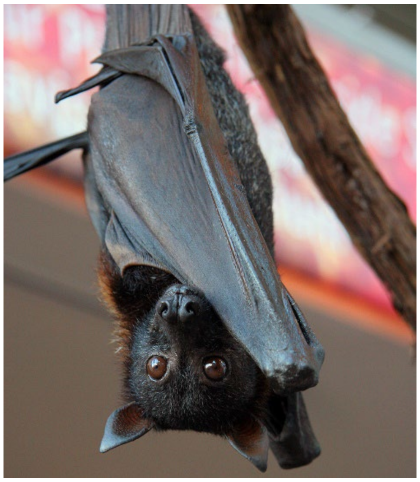


FIGURE 2-15. Flying fox (*Pteropus vampyrus*). Photo credit: Flickr

The virus was probably introduced into the pig population by infected flying foxes dropping partially consumed fruit into the pig enclosure. Bat saliva or urine containing Nipah virus may have been consumed by the pigs. Modeling studies suggest that the virus was introduced multiple times. At first, the virus may have caused an explosive but short epidemic in which it burned through the susceptible pig population and then went extinct. However, multiple reintroductions into the partially immune pig population allowed the virus to maintain slower chains of transmission that allowed viral persistence until the farm was depopulated in 1999 (Pulliam et al., 2012). Pigs were the source of infection for farmers and abattoir workers, and transportation of pigs across Malaysia and into Singapore resulted in a widespread outbreak of febrile encephalitis (with a reported >250 cases in Malaysia and 11 cases in Singapore) and a fatality rate close to 40% in humans in Malaysia and Singapore (Lo & Rota, 2008). When transporting animals from farms to abattoirs or slaughterhouses, a truck is used for large-scale transportation involving over 100 pigs at a given time at longer distances. Smaller-scale transportation is sometimes performed by individuals with a motorbike. Containment of excreta from the animals (i.e., saliva, feces, urine, etc.) could happen during the route of transportation from one place to another.

The beginning of the outbreak is correlated with the intensification of pig farming in Malaysia and the spatial overlap of mango and pig production. The bat reservoir hosts had probably been present, and at least periodically infected with the Nipah virus, for a long time before the pig farms were present. The spread of the virus from the index farm is a consequence of the transportation of animals and animal products, the absence of viral surveillance, and the absence of risk assessment control.

The outbreak caused widespread public fear in Malaysia and Singapore and nearly collapsed the billion-dollar pig farm industry. Singapore prohibited pig importation from Malaysia (Looi & Chua, 2007). In Malaysia, the outbreak ceased after the following responses:

- Allowing pig farming only in designated pig farming areas “PFAs”, which have the necessary infrastructure for biosecurity and waste management
- Performed national surveillance and testing of pig populations.
- Movement restrictions of pigs and pork (local, intrastate and interstate), with compensation for the loss of pigs

- Culling of pigs within infected areas, which can include infected or uninfected farms within a 10km radius of the outbreak
- Spatial separation of wildlife resources from livestock production facilities e.g. removal of fruit trees around farms.

Some additional solutions include:

- Training of farmers in early detection of disease and reporting, good hygiene practices, and management of sick animals (Looi & Chua, 2007).
- Enhance training and increased use of personal protective equipment by farm and abattoir workers.
- It was also found out that non-accredited farms sold Malaysian pigs to accredited farms, and the virus slipped through into Singapore. Thereafter, Singapore strengthened its accreditation process and implemented zoning and compartmentalization policies to improve the safety of the imported food supply.

Like the Nipah virus case study, poultry farms with intensive breeding for meat or eggs are often in open farming settings. A truck can carry 100 to 1,000 chickens and travel long distances. In some villages, the seller is carrying a few chickens via motorbike and traveling to nearby villages to sell. The excreta of the animal and the diseases they harbor can be carried from one place to another. Moreover, there is usually very little biosafety practice when processing the meat.

Reducing Persistence and Amplification of a Pathogen Once it enters the Wildlife Trade

Once a pathogen enters the wildlife trade, efforts need to be made to reduce the probability that the pathogen will persist and be amplified through animal-to-animal transmission. The persistence of a virus in any population is influenced by multiple variables related to the biology of the pathogen, including the route of transmission, survival outside of the host, infectious period, transmission rate, generation time, host range, and whether the virus causes lasting immunity in its host. Also critical to persistence are the attributes of the host population, including size, structure, and turnover. Essentially the viral characteristics interact with host characteristics to determine if the virus can maintain a chain of transmission through time and space, and therefore potentially spread throughout populations from facility to facility and even across borders. A pathogen with a short infectious period, high transmission rate, fast generation time, and generating lasting immunity in its hosts, might quickly burn through a population of susceptible animals before it can be passed onto another facility with naive animals (as probably occurred the first time Nipah virus was introduced into a pig farm; (Pulliam et al., 2012). A pathogen with a long infectious period but a low transmission rate might persist for a long time in a population if there is a turnover of susceptible hosts through periodic introductions into the population (Plowright et al., 2019). For example, feline enteric coronavirus in domestic cats was predicted to become persistent in catteries that contained over five animals, but smaller catteries would become reinfected by larger catteries (Foley et al., 1999). Many pathogens are expected to have a threshold population size which is basically determined by the ratio of the infectious and recovery rates (Drake et al., 2019).

Within the live animal supply chain, it may be possible to reduce the likelihood of a pathogen persisting by altering the population structure of animals within trade, reducing the population size of hosts, fragmenting the population into smaller sub-groups, and reducing connectivity among these groups to break chains of transmission. For example, routine depopulation of poultry (24-hour closure and disinfection) in several Chinese and Hong Kong markets broke the chains of transmission of avian influenza, reduced the prevalence of environmental detection of this virus and may have reduced the number of suspect or confirmed human cases (El-Zoghby, 2014; Offeddu et al., 2016). The frequency of such "rest days" may be important, particularly to reduce risk for human exposure, given that many studies identified re-contamination within days of market re-open, which suggests that the use of rest days monthly or less frequently is unlikely to have a strong impact (Offeddu et al., 2016). Another strategy is to eliminate residential animals, which can serve as reservoirs for exposure of new animals entering the market, e.g., through bans on overnight housing of animals (Offeddu et al., 2016). Lastly, husbandry practices could also help to mitigate captivity-induced stress and isolate species, populations, or individuals from each other (Lin et al., 2021). "Module 5: How to Design and Conduct Risk-based Surveillance for Zoonotic Diseases at the Human-Animal Interface" will delve deeper into the critical role of animal surveillance and veterinary services in monitoring known zoonotic pathogens.

Case Study 2-3: Transmission Risk Increases Along Wildlife Supply Chains in Vietnam

FIGURE 2-16. Rat slaughter at a large market (left) and a rat vendor stall displaying live rats in cages in a large market (right) in Dong Thap province, Vietnam, in October 2013. Source: Huong et al., 2020 © WCS Viet Nam.

In the early 2000s, the live rat trade in the Mekong Delta region was valued at about $2 million, producing up to 3,600 tons of live rats per year for human consumption (Nguyen et al., 2008; Huong et al., 2020). In 2013-2014 researchers sampled and PCR tested rats at different points in the value chain for the presence of coronaviruses in three southern provinces in Vietnam. Coronavirus detection increased along the supply chain from those sold by traders (~20%) to those sold in large markets (32%) to those sold and being served in restaurants (~55%). This indicates a 10-fold increase in virus levels when compared to free-ranging rodents and further dramatic amplification of viral load in the environment that is consistent with other classic models of amplification. The authors explain that high viral levels and amplification are likely a result of admixing five rat species, overcrowding and subsequent stress, close contact, and low biosafety (Figures 2-16 and 2-17). The high viral load also increases opportunities for viral recombination, when separate viruses infect one cell and mix and match genetic components (Huong et al., 2020). Additional information can be found in the case study in "Module 3: Efforts to Prevent Transboundary Disease Outbreaks in the Southeast Asia Region."

FIGURE 2-17. Rodents are being traded at the Cambodia and Vietnam border, Kandal province in Cambodia. Source: Virology unit, IPC (Predict II project). Photo credit: Vibol Hul.

Control the pathogen contaminating the environment (reduce pathogen load and indirect transmission routes)

The role and relative importance of the environment as a route of transmission or reservoir for pathogens will vary by pathogen, environmental, and host factors. Specifically, pathogen factors include the ability to persist in the environment, and certain pathogens (e.g., parasite eggs, Gram-positive bacteria, and anthrax spores) have physical barriers that potentiate longer-term survival. Nonetheless, even enveloped viruses and Gram-negative bacteria can survive for days, and sometimes longer, given suitable conditions of moisture (including humidity) and protection from inactivation (e.g., from UV light and chemicals) by organic material or other natural or built environment factors. For example, some pathogens might remain viable in a dark, moist cave environment than they would in a sunny environment or where they would be subjected to desiccation by air movement. Host factors include behaviors that influence the route of exposure, and physiologic factors and co-morbidities that influence both susceptibility (how likely the host is to become infected) and pathogen shedding (how much and for how long the host sheds infectious pathogens).

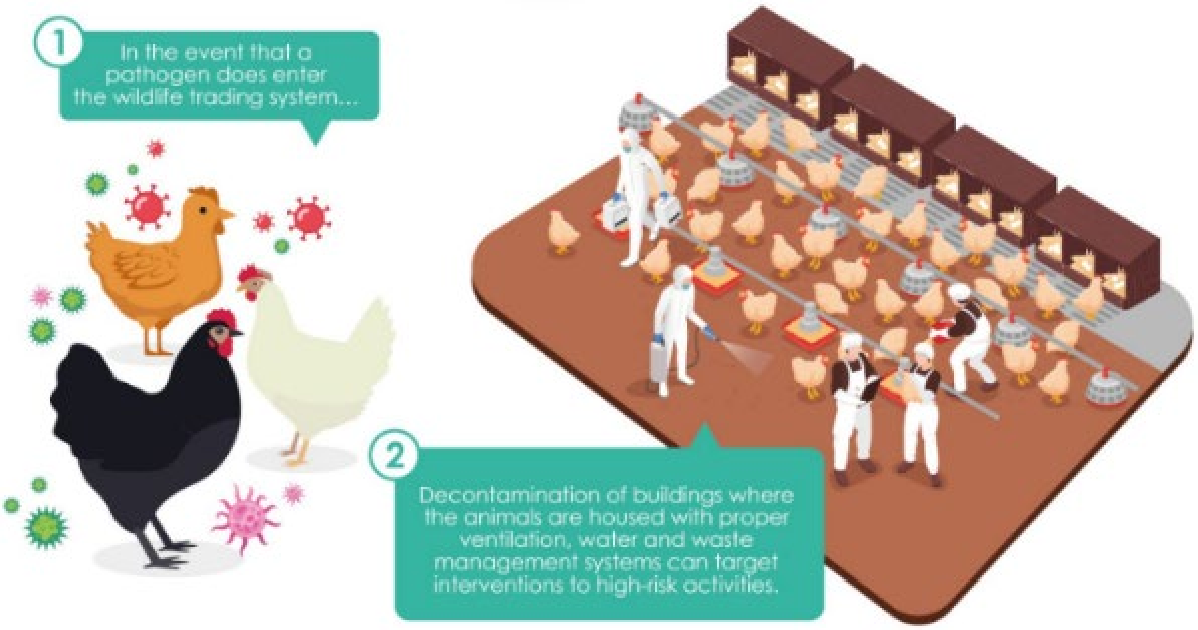


FIGURE 2-18. In the event of pathogen entry into animal trading systems, decontamination of buildings and animal housing is crucial.

Therefore, control strategies to address indirect transmission routes through the environment or limit the persistence of environmental reservoirs of pathogens need to be informed by the specific pathogen characteristics that would enhance their survival and can address environmental and host factors suitable for intervention. These should address all environmental media: water, air, food or feed, and soil, dust, and surfaces. There are standard and codified practices to minimize pathogen loads in animal production systems. Minimum practices should foster biosecurity and hygiene standards, along the entire value chain and follow standard food safety regulations, as outlined in FAO's Biosecurity and Agricultural Management Act of 2007. Depopulation, vaccination, and market-rest days are additional interventions used to reduce pathogen load in livestock and trade-related facilities (Figure 2-18). Attention to sources of feed for the animals also is important, given the potential for live animals or animal products used to feed market animals to be a source of introduction of zoonotic pathogens, or for pest animals to contaminate grains and other feed products. Risk factors e.g., increased interspecies mixing of live animals, with increased animal densities can heighten risks (Woo et al., 2006; Lin et al., 2021). Conditions in the trade should minimize stress and contact (Figure 2-19). Additionally, animal slaughtering locations for livestock and wildlife within markets should be kept separate. While these efforts may reduce some risks, shifting national and global food systems away from livestock-sourced foods is another strategy to reduce global pathogen loads with potential climate, biodiversity, and health co-benefits (Convention on Migratory Species, 2022; Ramey et al., 2021; Wegner et al., 2022).

FIGURE 2-19. Nov 2016 photo from a pig farm in China, demonstrating the ease at which passerine birds, rodents, and other mammals can mix with the pigs and share viruses. Also, insects can move swine secretions between barns. Modern swine husbandry calls for better isolation of the pigs. Photo credit: Gregory Gray.

Decontamination

The first principle of decontamination is the removal of fecal and other organic material that might serve to protect pathogens from chemical or physical agents meant to inhibit or inactivate them. Water safety, sanitation, and hygiene best practices provide the foundation for this work—attention to clean water sources, water treatment, management of human and animal feces through latrines and waste/wastewater treatment facilities, and routine cleaning of surfaces. Treatment by chemical (e.g., disinfectant) or physical (e.g., UV light) processes may be indicated, depending on the pathogen, and attention to the potential for some pathogen strains to be resistant to disinfectants and chemotherapeutics may also need to be considered. The frequency of cleaning and treatment of the environment is a final factor, related both to the speed and degree of re-contamination and to the frequency of use of the environment by susceptible hosts. In this, some understanding of the inoculation dose needed to infect a host—literally the number of bacteria, virions, or parasite eggs, which will vary by pathogen—can help drive decisions regarding the frequency of decontamination that is needed. Even simple provision of a sufficient number of hand-washing stations in suitable areas can be an important part of decontamination strategies.

Administrative controls

Buildings, where animals are housed, transported, slaughtered, or their products processed, may benefit from the use of administrative controls, which are policies or procedures for when, how, and where to perform certain tasks. These controls were originally designed for occupational health uses to reduce worker exposures to hazards and can be used to reduce both human and animal exposures and to limit environmental contamination by zoonotic pathogens. Decontamination activities may be part of administrative controls that also include isolation of certain activities or segregation of personnel according to job task, workflow procedures (doing task A before task B), and movement controls (single direction rather than bidirectional movement of people or animals through a space or building). Administrative controls often must be tailored to the pathogen(s) in question, to the task, and to the facility. For example, this could include restricting high-contamination activities to a part of a building with better ventilation or sewage. Locations for animal slaughtering within markets can be kept separate. Another example of an administrative control focused on animal populations would be all in – all out strategies, whether these are applied at the food animal production level or the animal market level, allowing the environment to be fully cleaned and decontaminated prior to re-population or the next market event (rest days and overnight bans). Allowing even a few resident animals (including pests like rats and mice) or failure to decontaminate between cohorts or market days may allow for some pathogens to persist over time, and even provide selective pressure to drive survival of pathogens of concern that can infect multiple species of hosts or survive better in environmental conditions.

FIGURE 2-20. Photo illustrating wastewater management system in a traditional market in Bangkok, Thailand in 2022. Note the presence of standing water, which contributes moisture that can enhance virus and Gram-negative bacterial survival, and ad hoc modification of water discharge systems, which could create further opportunities for the persistence of pathogens in engineered spaces that would not be in contact with surface disinfectant. Also note the chair, which could signal the potential for human-environment interaction with this location. Photo credit: Meghan Davis.

Engineering controls

Where resources allow, modification of buildings or other facilities can be an effective strategy to reduce exposure and prevent environmental persistence of pathogens. For example, laboratories with biosafety level 2 or 3 designation (BSL 2, BSL 3) have varying requirements for ventilation, including negative pressure cabinets or spaces, air, and water filtration systems to prevent pathogen escape, non-porous surfaces well suited to chemical and physical decontamination, and other engineering measures, such as built-in UV lights, to allow for routine or periodic surface treatment. While modifying animal markets or processing facilities to comply with BSL specifications is likely to be resource-intensive to be feasible or practical, a combination of targeted engineering controls—ventilation, water systems, wastewater, and waste management systems—with administrative controls can be a more cost-effective way to target interventions to high-risk activities or environments (Figure 2-20). For example, if there is a procedure that tends to produce droplets or aerosols where there is a concern for airborne pathogens, installation, or upgrade of ventilation systems in that location, or movement of that activity to an area with better ventilation, would be important to consider. Facility installation or upgrade should also consider principles of biosecurity and biocontainment.

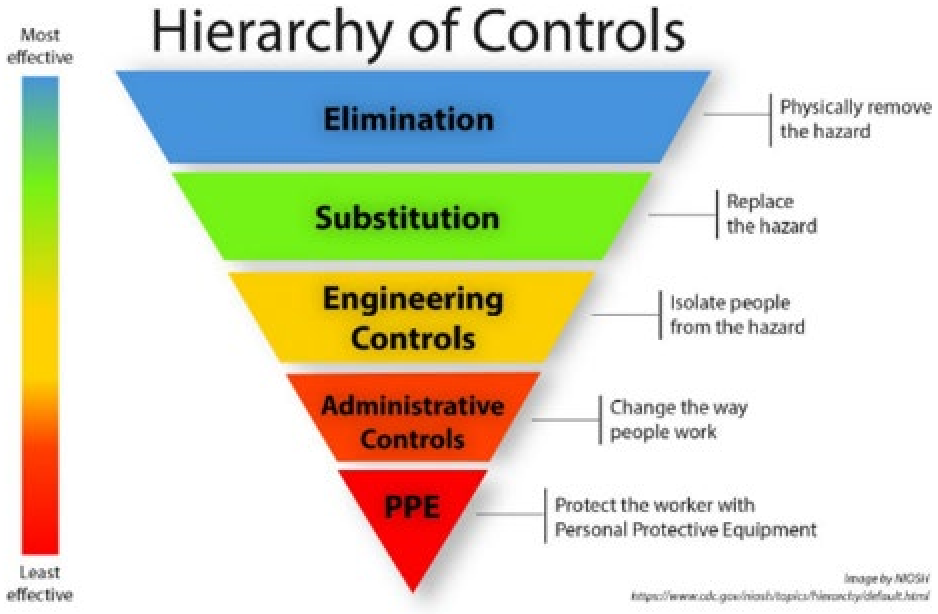


Image by NIOSH
https://www.cdc.gov/niosh/topics/hierarchy/default.html

FIGURE 2-21. Hierarchy of Controls for controlling pathogen contamination in the environment (U.S. National Institute of Occupational Safety and Health), demonstrating that measures to eliminate, substitute, or provide engineering controls for a hazard are more effective than administrative controls and use of personal protective equipment, e.g., masks, gloves, and boots.

In occupational health, engineering and administrative controls are considered more effective than use of personal protective equipment (PPE) by workers to limit exposures, but typically, organizational policies and decisions often include control measures at all three of these levels in the Hierarchy of Controls (Figure 2-21). Consultation with occupational safety and health professionals, including occupational medicine or nursing practitioners and industrial hygienists, can be useful during facility design and during the development of policies and procedures in this context.

Inter-Species Interactions: Ways to Reduce Animal and Human Behaviors that Increase Exposure Throughout the Supply Chain

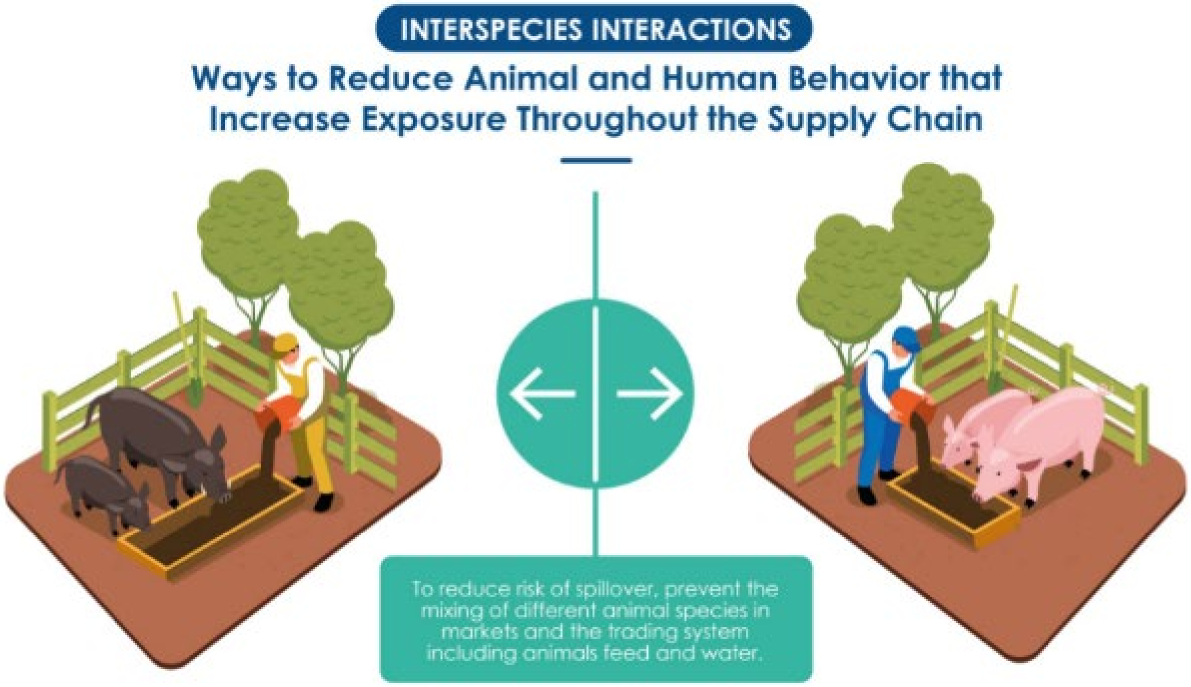


FIGURE 2-22. To reduce the risk of spillover, it is imperative to prevent the mixing of different animal species in markets and trading systems, including during water and feed.

Reducing interactions between animals and humans throughout the live animal supply chain is crucial in minimizing the potential for zoonotic disease transmission. Factors that are prominent in Southeast Asia that bring together naive hosts with pathogens include deforestation, fragmentation of natural habitats, agricultural intensification, crowding, urbanization, globalization, and rising human populations. The growing population also has increased demand for animal-sourced food, which is driven by increasing population, urbanization, and wealth (Hatab et al., 2019). There are challenges associated with meeting this demand, especially in countries that lack the proper infrastructure. In areas where people prefer live animals and fresh meat, oftentimes the animals are moved from rural to urban areas or from urban to peri-urban farms. And yet, despite the increasing affordability of refrigeration, the demand for live animals or fresh meat in traditional markets persists. Many people believe fresh meat is safer, and tastier (Roesel & Grace, 2015). Risks posed by traditional markets to human health, in the context of emerging infectious diseases, include the presence of high disease-risk taxa and live animals (both domesticated and wild), unsanitary conditions, large and dense markets, increased interspecies mixing and animal densities, and multinational animal sourcing as well as lengthy supply chains (Galindo-González, 2022) (Figure 2-22). There are various strategies that can be implemented to address behaviors that increase exposure, and they should target the different stages of the supply chain, from wildlife collection to transportation, market handling, and consumption (Figures 2-8 and 2-9). For more information, refer to “Module 6: Strategies for Engaging with Diverse Stakeholders Across Key Points in the Live Animal Value Chain.”

Implementing Regulations and Enforcement Measures to Restrict Wild Animal Sales in Live Animal Markets

Certain animal taxa like rodents, bats, primates, carnivores, and ungulates might have elevated zoonotic spillover potential. More information on this can be found in "Module 4: Priority Pathogens, their reservoirs and how to contain them." Additionally, the presence and sale of live animals in traditional markets creates an elevated risk of viral pathogen transmission; mixing of live animals (interspecies and intraspecies) in both storage and slaughter areas can facilitate pathogen shedding and viral recombination in new hosts (Galindo-González, 2023; Woo et al., 2006). For example, new strains of influenza A virus can emerge when 2 circulating subtypes infect one cell in an organism; this is the case when a circulating human subtype encounters a circulating poultry subtype (Figure 2-23)(Koçer et al. 2014).

FIGURE 2-23. Live animal markets with multiple species in close proximity. Photo credit: Gregory Gray.

Therefore, a major risk factor for infectious diseases includes visiting or mixing animals in live poultry markets (Aguirre et al., 2020). This is true also for swine influenza variant virus, while infrequent in humans, infections occur due to exposure to infected pigs in live markets, as indicated in two cases in the US (Galindo-González, 2023). For instance, housing domestic pigs within or in close proximity to wild boar habitats; using different feed sources for domestic pigs than those foraged by wild boars; separating slaughter areas; and preventing the cross-breeding between wild boars and free-ranging domestic animals can help to lessen the occurrence of both direct and indirect interactions between wild and domestic pigs, which could facilitate viral spillover of African Swine Fever (ASF) (Denstedt et al., 2021). These mechanisms are discussed in further detail in "Module 3: Efforts to Prevent Transboundary Disease Outbreaks in the Southeast Asia Region." Additional factors can also impact immune system responses and raise the transmission of disease: heightened stress levels of captive animals due to high-density confinement, new environments, and unfamiliar interspecies contact; caged animals are commonly injured and have open wounds, which facilitates the transmission of pathogens (Galindo-González, 2023). One major risk factor in the 1997 H5N1 outbreak in Hong Kong was exposure to live poultry in the marketplace. (Wan et al. 2011).

Improve Hygiene Standards and Sanitation in Live Traditional Markets to Reduce the Risk of Zoonotic Disease Transmission

Lack of good hygiene is a major risk factor for human health in traditional markets, both through limited or unenforced biosecurity controls in markets themselves and through hygiene risks that magnify along supply chains. Lapses in hygienic practices in traditional markets without live animals, have been linked to bacterial and parasitic infections, through various means including improper handling or storage of carcasses, polluted water, or proximity to other contaminants, improper waste disposal and inadequate sanitation measures (Lin et al., 2021; Woo et al. 2006; Lo et al., 2019). To mitigate these risks, proper sanitation practices, vendor handwashing, routine cleaning practices, separation of different species, and quarantine protocols can help contain potential pathogens and reduce the risk of cross-species transmission. Enhancing the traceability and labeling of wildlife products also plays a vital role. Implementing tracking systems that can certify the origin of traded species can promote transparency, while ensuring the legality of the supply chain, and inform consumers about potential risks associated with certain products (Campbell et al. 2022).

Market Infrastructure and Layout that Increase Disease Transmission: High Population-Dense Structures, Stacked Cages, and Mixing of Different Animal Species

Many traditional markets are characterized by high population density, with vendors, buyers, and animals in proximity. Such crowded conditions create an environment conducive to disease transmission, as respiratory droplets, animal feces, and other bodily fluids from animals can easily spread. Additionally, stacked cages, commonly used in some traditional markets, can facilitate the proximity of different animal species, increasing risk (Figures 2-24 and 2-25). When animals are housed in vertically stacked cages, their bodily fluids or waste can easily encounter animals housed in neighboring ages, allowing pathogens to easily spread (Galindo-González, 2022).

To mitigate these risks, consider redesigning the infrastructure and layout of wet markets to ensure adequate spacing between animal stalls, adopt cage-free or open-air enclosures, or implement physical barriers to prevent direct contact between animals.

Additional recommendations include:

- Implementing crowd management protocols to promote physical distancing among visitors, vendors, and animals. Examples include controlling the number of customers allowed in the market at a given time,
- Provide adequate ventilation and proper airflow within market structures to help disperse and dilute the buildup of airborne pathogens. Additional information on this can be found in “Module 3: Efforts to Combat Transboundary Disease Outbreaks in the Southeast Asia Region.”
- Within live market settings, physically separate areas for slaughter and carcass processing from retail areas where customers interact.

Additionally, the consumption of meat from wild animals facilitates spillover, by putting humans in close contact with fresh meat, offal and animal blood that can host different pathogens. Although there are trade-offs where reduced consumption of wild meat could lead to enhanced risk from intensively raised domestic animals, promoting and stimulating sustainable and legal alternatives, such as captive breeding programs or domesticated species, can reduce the pressure on wildlife populations, demand for wild-caught animals and minimize interactions between humans and wildlife animals. This could subsequently be shared as best practices.

FIGURE 2-24. (Left) Examples of stacked cages in a rat market in Dong Thap Province, Vietnam. Photo credit: WCS Vietnam. (Right) Examples of stacked cages with various wild and domestic bird and mammal species being sold in a pet market in Vientiane, the capital city of Laos. Photo credit: K. Yoganand.

FIGURE 2-25. Hunted wild animals, including carnivores and rodents being sold for meat in a traditional fresh market in Laos. Photo source: WCS Lao PDR.

Other complex factors such as awareness of disease risk, direct imposition of national animal health regulations, and societal expectations can impact these processes as well (Hidano et al. 2018; Verelst et al., 2016). To manage diseases shared with wildlife requires strategic development that reduces pathogen transmission between wildlife, domestic animals, and humans. In each country and region where outbreaks occur, the control measures should be adjusted to the specific local setting, while considering the population size, farming practices, disease risk factors, and religious and social traditions and beliefs. These locally specific factors will ultimately determine which measures are feasible and can be realistically implemented. Additional information can be found in "Module 7: Strategies to Overcome Barriers, Fill Gaps, and Address Systemic Issues."

BOX 2-2: Emerging Threat: *Streptococcus suis* Transmission from Pigs to Humans

Streptococcus suis is a pathogen commonly found in pigs that causes severe systemic illness in people if consumed, causing certain clinical conditions such as meningitis, septicemia, and arthritis. The number of reported cases in humans have significantly increased in Southeast Asia, and it is typically seen as an occupational hazard involved in pig handling amongst farmers, abattoir workers, and carcass cutters in Western countries, Japan, China, and Hong Kong. Its significance as a foodborne illness in Southeast Asia is important, considering the consumption of meals containing raw pork, blood, and other related products.

Socio-behavioral factors play a role in controlling the disease. For example, a food safety campaign was implemented in the Phayao province in Thailand in 2011-2013, which led to a decreased incidence of human cases(Segura et al., 2020). This includes educational lectures for residents and dissemination of print materials explaining the pathogen, risk behaviors such as the consumption of raw pork products, and major symptoms of the disease, including appropriate culturally sensitive messages targeting at-risk populations like alcohol drinkers and adult men. As changing consumption practices may be difficult due to strong traditions, practices, and financial implications, risk communication approaches that respect these traditions and engage with community, religious, and social leaders can be effective.

Reduce Probability of Animals in Allostatic Overload Throughout the Supply Chain

1. Reduce susceptibility (i.e., stress, reduce overcrowding, provide adequate nutrition, coinfections)
2. Reduce opportunities for continued exposure.

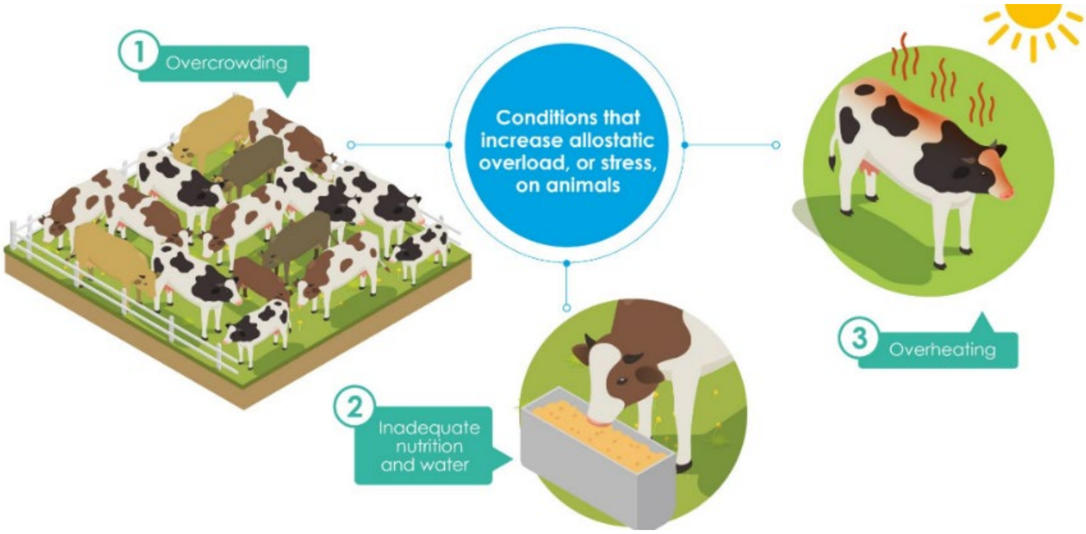


FIGURE 2-26. Conditions that cause allostatic overload, or stress, on animals.

Allostatic load is a measure of the cumulative stress and energy budget of an individual. When energy outputs are higher than energy inputs, or animals are exposed to repeated stressors, then animals can enter allostatic overload (McEwen & Wingfield, 2003). Animals within the wildlife trade are at high risk of allostatic overload through the stress of overcrowding, overheating, noise and other disturbances, and inadequate nutrition or water (Figure 2-26). When animals are in allostatic overload, they shift resources away from immune function and towards life sustaining functions and can become more susceptible to circulating viruses and more likely to excrete high loads of these viruses (Hing et al. 2016; Plowright et al., 2024, in press).

One intervention to reduce the probability of pathogen infection and shedding, is to reduce the allostatic overload of animals within the wildlife trade. This could be achieved by reducing overcrowding, providing comfortable environmental conditions, providing adequate calories and nutrients and plentiful water. Such measures not only address the risk of spread of pandemic pathogens but also the basic welfare of the animals in wildlife trade.

Protect Humans at Risk of Infection

FIGURE 2-27. Protective measures to prevent transmission.

The impact of the COVID-19 pandemic on global production and manufacturing demonstrated the need for constant supply of personal protection equipment (PPE), particularly for individuals most at-risk (i.e., individuals working alongside the animal market and supply chain). Health and market workers are the first responders to infectious disease outbreaks (Grasselli et al., 2020) and they need PPE products such as face masks, gloves, and protective gowns to prevent direct contact with bodily fluids and contaminated surfaces (Verbeek et al., 2020). These individuals. Concurrently, other people need PPE to protect themselves from contracting or spreading these diseases via face masks and shields. The constant stock of life-saving medical devices like ventilators and PPE become precious commodities during moments of intense health crisis. In severe cases of COVID-19, patients cannot breathe without the use of a ventilator due to fluid-filled lungs that no longer deliver adequate oxygen (Buheji et al., 2020). Surgical masks and PPE should be selected or based on the route of disease transmission identified for the infectious disease, for example, airborne, contact, or droplet (Figure 2-27). Designated healthcare personnel should first identify the transmission pathway of the disease and work to anticipate the exposures that will occur during patient care to select the proper PPE. The National Institute for Occupational Safety and Health (NIOSH) has created the PPE-Info database, which includes descriptions of test methods, regulations, and consensus standards for PPE across various workplace hazards (Sieber et al. 1996).

More recommendations for identifying the proper PPE: 1) job hazard analysis, 2) infectious disease hazard analysis, 3) PPE selection, and 4) PPE evaluation (Jones et al. 2020). The use of PPE and adoption of standard hygienic practices among health workers should be encouraged. Supply of protective materials and equipment should be improved. Another important strategy is vaccination, which can protect individuals from specific infectious diseases and reduce disease spread. Additionally, measures to reduce aerosol exposure, such as improving ventilation and installing air filtration systems, can help prevent airborne disease transmission (Horwood et al., 2023). Education and awareness campaigns are also crucial in promoting good hygiene practices, such as handwashing. Overall, a comprehensive, One Health approach involving both individuals and community-level interventions is necessary to protect individuals at risk of emerging infectious diseases.

Quick Detection of Spillovers Upon Entering Human Populations: Monitoring & Surveillance of People at Risk

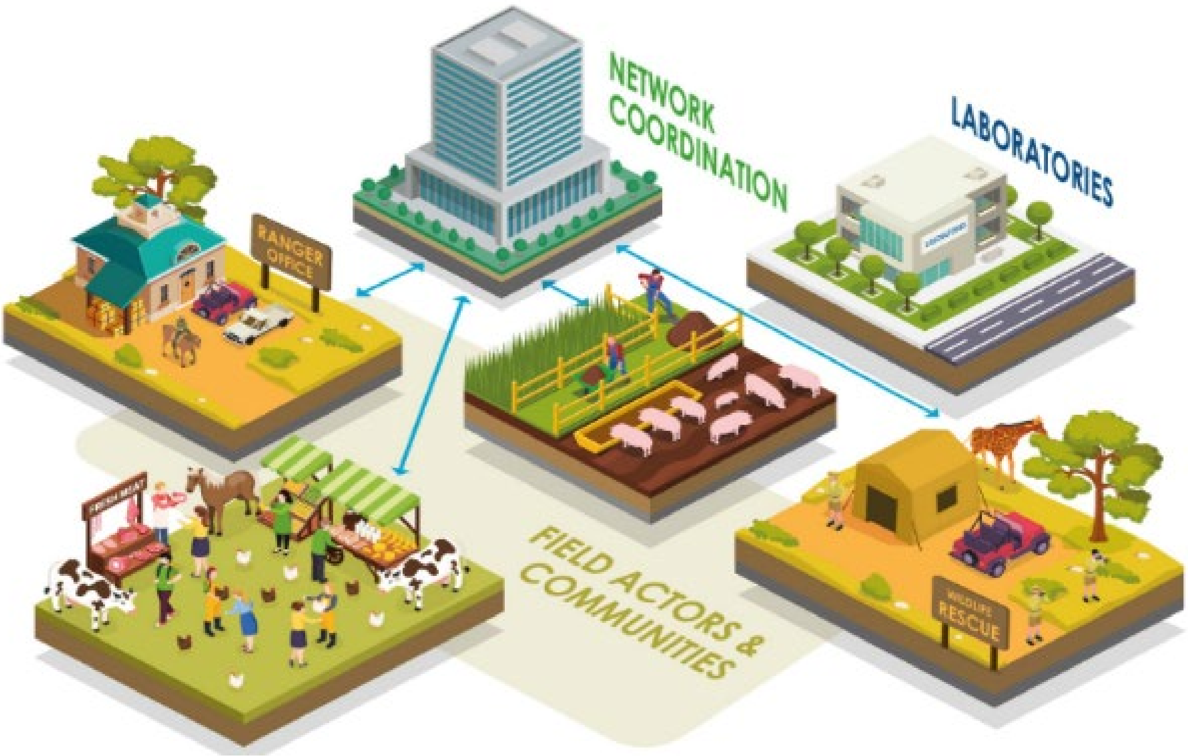


FIGURE 2-28. Detecting microbe spillover when entering human populations: monitoring and surveillance of people at risk.

Two of the greatest barriers in establishing effective and sustainable microbe spillover surveillance are cost and effort. There is just not enough money or detection capability available to conduct surveillance for the millions of animal microbe spillovers in all sites where spillovers could infect humans. There are too many animal species and unique animal microbes in diverse geographical areas. Additionally, field workers and laboratories capable of conducting such spillover surveillance are relatively few. To illustrate, it has been estimated that the earth currently has 6,399 unique species of mammals which, in total, harbor ~40,000 unique species of viruses (Carlson et al., 2019). A large portion of animal microbes have yet to be characterized so tools to detect them do not exist. In a 2018 study, Carroll et al. estimate, that there were 1.67 million not-previously-discovered viruses in mammal and bird hosts (Carroll et al., 2018).

Hence, a data reduction strategy is needed to make such spillover surveillance possible and sustainable. If we embrace the statistic that 60% of human infections have an animal origin, and of these 75% are zoonotic in nature, it seems logical to conduct spillover surveillance where large populations of animals come in close and frequent contact with humans (Jones et al., 2008). Several scientists are embracing the notion that the most efficient approach is to conduct spillover surveillance at specific human-animal interfaces (Gray et al., 2021; Wille et al., 2021).

If spillover surveillance cannot be conducted at the human-animal interface as described above, a secondary strategy might be to conduct surveillance for disease syndromes of most concern in geographical areas known to have previously been the site of novel human pathogen emergence. For instance, if one is concerned about pneumonias caused by novel zoonotic respiratory viruses, one might perform periodic novel viral pathogen detection studies among patients hospitalized with pneumonia in geographic emerging infection disease hotspots (Gray et al., 2021; Xiu et al., 2020). Another strategy is to focus surveillance on high-risk interfaces, where wildlife is coming into frequent and recurrent contact with people or their livestock, such as live mammals and bird markets, extractive industries, and areas of land use change (Huong et al., 2020). More information on this can be found in Module 5: How to Design and Conduct Risk-Based Surveillance for Zoonotic Diseases at the Human-Animal Interface.

One can further reduce the scope of spillover surveillance by focusing on pathogen families known to have high spillover potential, particularly when these spillovers have caused epidemics. One might additionally reduce the scope of spillover surveillance by focusing on species of animals that are prone to novel virus generation within those families. For instance, for multiple reasons, pigs (*Sus domesticus*) have previously been prone to novel influenza A virus and novel coronavirus generation so focusing a portion of spillover surveillance on large pig farms seems appropriate if one is concerned about these microbes (Morse et al., 2012; Wardeh et al., 2021; Webster et al., 1997). Additionally, some animal species seem to have lower species barriers for sharing specific microbes with humans. This is true again for pigs and influenza A (Borkenhagen et al., 2019). Finally, one may further reduce the effort required for spillover pathogen surveillance by employing relatively cost-effective, pan-species diagnostics. Serological assays are useful as they can detect host exposure to a pathogen within a period of months to years, whereas active infections may only be detectable over days or weeks. Other pan-species assays can detect different species within a microbial family and are particularly effective in detecting and characterizing new microbial variants (Vlasova et al., 2022; Xiu et al., 2020). These pan-species assays might be supplemented with periodic, unbiased, next-generation sequencing assays and novel pathogen discovery software pipelines, when targeting molecular assays might miss spillover of an unexpected microbial group (Ramesh et al., 2021). More information on this can be found in "Module 4: Priority Pathogens, Their Reservoirs, and How to Contain Them."

System-Level / Governance Changes (e.g., Regional and National Strategic Plans)

The Association of Southeast Asian Nations (ASEAN) recognizes the obstacles facing the veterinary and animal health community in Southeast Asia. The ASEAN Strategy for Exotic, Emerging, Re-emerging Diseases and Animal Health Emergencies was agreed in May 2021 (ASEAN, 2021), which sought to provide a regional framework for animal health and zoonosis. Since 2012, the ASEAN Coordinating Centre for Animal Health and Zoonoses (ACCAHZ) has been initiated to facilitate and provide a framework for cooperation and coordination amongst ASEAN member states in response to an increasing risk of introduction and spread of emerging and re-emerging zoonotic diseases. This was formally agreed by the ASEAN Member Countries in 2016, and the agreement entered into force in September 2021, reinforcing the region's commitment to combat the threat of zoonotic diseases. Once established, the center is expected to provide policy and technical advisory support to the relevant ASEAN sectoral working groups e.g., ASEAN Sectoral Working Group for Livestock (ASWGL). This sets the stage for the discussion and development of regional implementation mechanisms for animal health emergencies, and regional strategies for the prevention, control, and eradication of transboundary animal diseases and zoonoses.

Other than livestock, at the Special ASEAN Ministerial Meeting on Illegal Wildlife Trade in 2019, the ASEAN Ministers responsible for CITES and Wildlife Enforcement on Illegal Wildlife Trade have committed to strengthen cooperation to address wildlife trade in ASEAN (USAID, 2018). The ASEAN Centre for Biodiversity, which focuses on the conservation and sustainable use of biological diversity and coordinated efforts amongst ASEAN Member States, has also placed emphasis on this topic amongst its key stakeholders, illuminating the issues between animal and human health, wildlife habitat protection, wildlife trade, using a One Health approach. Multiple global partners have also committed to work with ASEAN Senior Officials. The U.S. Agency for International Development (USAID) supported the convening of the first multi-sectoral meeting to develop a regional strategy for preventing transmission of zoonotic diseases from wildlife trade. This is led by the ASEAN Working Group on the CITES and Wildlife Enforcement (USAID, 2022). The ASEAN Strategy for Preventing Transmission of Zoonotic Diseases from Wildlife Trade was adopted in 2022 by the ASEAN Senior Officials on Forestry. Organizations, including WOAH, FAO, and U.S. Centers for Disease Control and Prevention (CDC) have supported various Field Epidemiology Training Programs (FETPs) for veterinarians and animal health officials. The Emergency Centre for Transboundary Animal Diseases (ECTAD) in FAO Regional Office for Asia and the Pacific has been providing support in developing and implementing the Regional Field Epidemiology Training Program for Veterinarians hosted by Thailand (Pinto et al. 2023) that trains new cohorts annually from across the region as well as applied epidemiology training programs for veterinarians in the countries. Field Training Program for Wildlife, Ecosystems, Biodiversity, and Environment (FTP-WEBE) is a training program developed by FAO and provides country-adapted training based on the results from the Environment Sector Stakeholder Mapping and Needs Assessment Tool. It is intended to complement FETPs and FETPVs for the wildlife and environment sectors.

Southeast Asia has been a beneficiary of several international partner funding. For example, with funding from the Australian Government, the FAO is helping ASEAN to strengthen regional mechanisms to address animal and zoonotic diseases with pandemic potential and support in development of the ASEAN Coordinating Centre for Animal Health and Zoonoses (ACCAHZ). This is done through an ASEAN-Australia-FAO regional technical assistance project called

"Strengthening Mechanism in Animal-health for a Resilient ASEAN or (SMART-ASEAN)" project.

At the national level, countries in Southeast Asia have undertaken unique measures to prevent zoonotic spillover. For example, Singapore's approach recognizes that people's well-being and health are interconnected with that of animals and wildlife in our community. The country implements several measures to protect animals which in turn safeguards public health, they include bio surveillance initiatives to bolster its pre-border, border, and post-border defenses against zoonotic diseases and utilization of complementary animal biosecurity/quarantine and wildlife rehabilitation centers for early disease monitoring and surveillance. Thailand's approach recognizing the endemic, imported, and emerging infectious diseases that threaten its country. They implemented a National Strategic Plan for Emerging Infectious Diseases. It aims to operationalize One Health policies from integrated human-animal-wildlife surveillance systems to enforcement of Communicable Diseases Law, with coverage of biosecurity and infection control measures in hospitals and zoological parks. Vietnam has developed a One Health Strategic Plan for Zoonotic Diseases, co-developed by the Ministry of Agriculture and Rural Development, together with the Ministry of Health and related ministries and agencies. This approach not only sets out the initiatives to combat disease spillover but also identifies core One Health competencies and gaps in funding or donor support to drive participation or involvement from government and non-government actors.

Conclusion

In this module, we presented eight crucial mechanistic steps that shed light on how pandemic pathogens can infiltrate and proliferate within the wildlife trade system. Through a series of key interventions, some theoretical and others drawn from real-life case studies, we have identified potential strategies to disrupt each of these mechanisms, aiming to limit pathogen circulation in the wildlife trading system. Given the challenges posed by global risk drivers such as climate change and urbanization, and the current trends of population and economic growth in Southeast Asia and identified risks of emerging and re-emerging zoonoses, it is vital not to underestimate the importance of preventing zoonotic spillover.

Many of the strategies in this guidebook will be aimed at underscoring and implementing the "One Health" concept, recognizing the intricate link between human, animal, and environmental health. By adopting a One Health approach, we can design programs, policies, and initiatives in an interdisciplinary and intersectoral manner, given the complexity of animal supply chains and movements in Southeast Asia, which are directly tied to the livelihoods and aspirations of people. This way, we can effectively minimize the risks of incursion, transmission, and spread of zoonotic pathogens. As we move forward, the fight against zoonotic spillover demands sustained commitment and collaboration from all interested parties.