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Insect Vectors as Bio-sentinels: Expanding the Frontiers of Xenosurveillance in Public Health for Pathogen Detection and Disease Monitoring

Hemant Kumar[1], Sagar D.[2*] and Suresh R. Jambagi[2]

[1] Department of Entomology, Agriculture College Garhwa, Birsa Agricultural University, Ranchi- 834006, Jharkhand, India
[2] Division of Genomic Resources, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru-560024, India

* garuda344@gmail.com

Abstract

Arthropod xenosurveillance is an emerging, non-invasive approach that leverages the blood-feeding behavior of insects to monitor circulating pathogens across human, livestock, and wildlife populations. Blood-fed arthropods such as mosquitoes, tsetse flies, and sand flies act as natural biosentinels, preserving genetic traces of viruses, bacteria, parasites, and host DNA within their blood meals. This review highlights the expanding frontiers of xenosurveillance in public health, emphasizing its application for early detection of arboviruses, retroviruses, and other zoonotic pathogens in complex ecological settings. By integrating metagenomic sequencing with targeted molecular assays, xenosurveillance overcomes limitations of conventional surveillance, including low sensitivity, invasiveness, and high logistical costs. Recent success stories demonstrate its effectiveness in detecting both known and unexpected pathogens at wildlife–livestock–human interfaces. Despite challenges such as nucleic acid degradation and data interpretation, xenosurveillance represents a scalable, cost-effective, and ethically sound One Health tool with strong potential for early warning systems and pandemic preparedness.

Introduction

What if the key to stopping the next big pathogen outbreak wasn't hidden in a lab or a high-tech surveillance system, but buzzing right in front of us? Around us, countless insects are constantly feeding, flying, and interacting with humans and animals unknowingly acting as tiny couriers of biological information. Every bite from a mosquito, tick, or fly is more than an irritating nuisance; it's a moment of sampling from the living library of their hosts' genetic and microbial worlds. Scientists are now realizing that these very tiny insects, often labeled as villains, could become our greatest allies in disease detection. This emerging idea, known as "*Arthropod Xenosurveillance*", turns blood-feeding insects into natural biosensors, flying syringe or bio-sentinels capable of revealing which viruses or pathogens like human bacteria, parasites, and viruses in mosquito blood meals are silently circulating in an ecosystem long before they reach humans. As we know that current methodologies for vector or parasite identification have limitations in comparison to arthropod xenosurveillance. Conventional surveillance methods tend to be limited by low sensitivity, especially during early and/or low-density infections, more so when sampling involves individual hosts or vectors directly. Such approaches can be labor-intensive and are more complex from a logistical aspect (McLure et al., 2025; Okorie and de Souza, 2016). Importantly, they mostly sample one pathogen at the time, which affects the cost requirement and throughput. Ethical concerns also arise from sampling from humans or other wildlife, and cryptic or elusive reservoir hosts are also likely to be missed.

The concept of arthropod xenosurveillance came to life in an experiment by researchers from Montpellier University, France, who explored how tsetse flies, notorious for spreading sleeping sickness, might instead help in tracking emerging diseases, which can utilize blood-feeding arthropods for natural sampling and has the capacity for broader, non-invasive, and more spatially and temporally integrated, multi-pathogen surveillance. The *Glossina fuscipes fuscipes* fed with rabbit blood spiked with six viruses such as Zika, Dengue, Chikungunya, African swine fever, Bluetongue, Peste des Petits Ruminants (PPR) and found that traces of these viral genetic material persisted in the flies' guts for up to six days. For dengue-endemic nations like Bangladesh, integrating Molecular Xenosurveillance (MX) or Molecular xenomonitoring into existing health systems could transform early outbreak detection and empower communities to act swiftly before diseases spread unchecked. These results were astonishing: the same insects once feared for transmitting illness could now act like "flying syringes or bio-sentinels," preserving snapshots of the invisible viral world around us. In a broader sense, this approach represents a revolution in how we monitor diseases, especially in regions with limited infrastructure.

This study aims to expand the scope of molecular xenosurveillance by evaluating the ability of blood-fed mosquitoes to detect a broad range of circulating viruses, including arboviruses and non-arbovirus retroviruses, within complex ecological settings. The objective is to determine whether mosquito-derived viral and host genetic material can reliably reflect pathogen presence, host exposure, and livestock–vector–environment interactions. By integrating metagenomic sequencing with targeted molecular assays, this work contributes to advancing non-invasive One Health surveillance approaches. The expected outcome is a more sensitive, scalable, and ecologically informative framework for early detection of emerging pathogens in high-risk interfaces.

What is Arthropod Xenosurveillance?

Xenosurveillance is an emerging technique that harnesses the blood-feeding (hematophagous) behavior of certain arthropods to monitor vertebrate hosts for infectious disease agents (Brinkmann *et al*., 2016). It derived from two Greek words xeno (foreign) and surveillance (watching); xenosurveillance involves using blood-feeding arthropods such as mosquitoes, tsetse flies, sand flies, leeches, and even carrion beetles as natural collectors of host DNA or RNA. Instead of bleeding animals or capturing wildlife, researchers can simply collect these insects, which already contain within them a snapshot of the genetic and microbial world of their hosts. Arthropod xenosurveillance evolved from classical mosquito surveillance during the 1950s–1980s, when entomologists like Rosen (1987) and Reeves et al. (1994) studied mosquitoes as indicators of arbovirus circulation. In the 2000s, molecular tools enabled the detection of vertebrate DNA in mosquito blood meals (Ngo and Kramer, 2003; Kent and Norris, 2005), revealing their potential as biological samplers. In 2013, Grubaugh and team introduced the concept of "xenosurveillance," formalizing the idea of using mosquitoes to monitor human pathogens non-invasively (Grubaugh *et al.,* 2015). In 2015, Grubaugh's study validated this concept, demonstrating that blood-fed mosquitoes can detect human viral, bacterial, and parasitic infections, revolutionizing biosurveillance approaches. In earlier studies, including those involving *Anopheles gambiae* mosquitoes, researchers have successfully detected viral genetic material from mosquito blood meals under both laboratory and field conditions.

A sound understanding of the biology and ecology of these arthropods along with pathogens they transmit is crucial for applying this method effectively. *Anopheles gambiae*, the main malaria vector in sub-Saharan Africa, is highly anthropophilic (prefers feeding on humans), endophilic (rests indoors), and endophagic (feeds indoors) that make it not only an efficient malaria vector but also a non-invasive and easily collected sampler of human blood for disease surveillance. Each blood-fed arthropod acts as a microscopic time capsule containing viral, bacterial, and host genetic material. This innovative approach marks a paradigm shift in epidemiology field. It is non-invasive, cost-effective, and highly informative which turn pest species into allies for detecting emerging pathogens. In essence, arthropod xenosurveillance transforms mosquitoes and other vectors into biological detectives, providing a simple yet powerful way to detect pathogens before disease outbreaks occur (Figure 1).

Workflow of Arthropod Xenosurveillance

The basic road map of arthropod Xenosurveillance (Ndione et al., 2025) as follows:

1. Field Collection of Arthropods

- Deploy CO_2-baited CDC light traps near villages, livestock enclosures, water bodies, and wildlife interfaces.
- Collect predominantly **blood-fed female mosquitoes** and identify them morphologically.

2. Pooling and Sample Preparation

- Group mosquitoes into pools based on species, location, or feeding status.
- Homogenize whole mosquitoes and extract **total nucleic acids** from each pool.

3. RNA Extraction & Enrichment

- Perform RNA extraction using viral/pathogen nucleic acid kits.
- Remove host ribosomal RNA to enrich viral and microbial sequences.

4. cDNA Synthesis & Library Preparation

- Convert enriched RNA into first-strand and then double-stranded cDNA.
- Prepare sequencing libraries (e.g., **Nextera XT**) for high-throughput sequencing.

5. Metagenomic Sequencing

- Sequence prepared libraries on Illumina platforms (e.g., **NextSeq550**) for unbiased detection of pathogens.

6. Bioinformatic Processing

- Conduct quality filtering, adapter trimming, and removal of host reads.
- Use pipelines such as **CZ-ID, Bowtie2, RAPSearch2, SPAdes, and BLAST** for classification and assembly.
- Identify viral, bacterial, or host-derived sequences present in the bloodmeal.

7. Molecular Confirmation (If Needed)

- Validate metagenomic detections using **RT-qPCR or hemi-nested PCR**, designed from detected viral fragments.

8. Phylogenetic Analysis

- Align sequences using **MAFFT** and infer evolutionary relationships with **IQ-TREE**.
- Confirm taxonomic identity and determine relatedness to known global strains.

9. Interpretation & Surveillance Output

- Integrate viral detections with ecological and host-interaction data.
- Use findings to infer pathogen circulation, host exposure, and spillover potential.
- Apply results to **early-warning systems** and One Health surveillance planning.

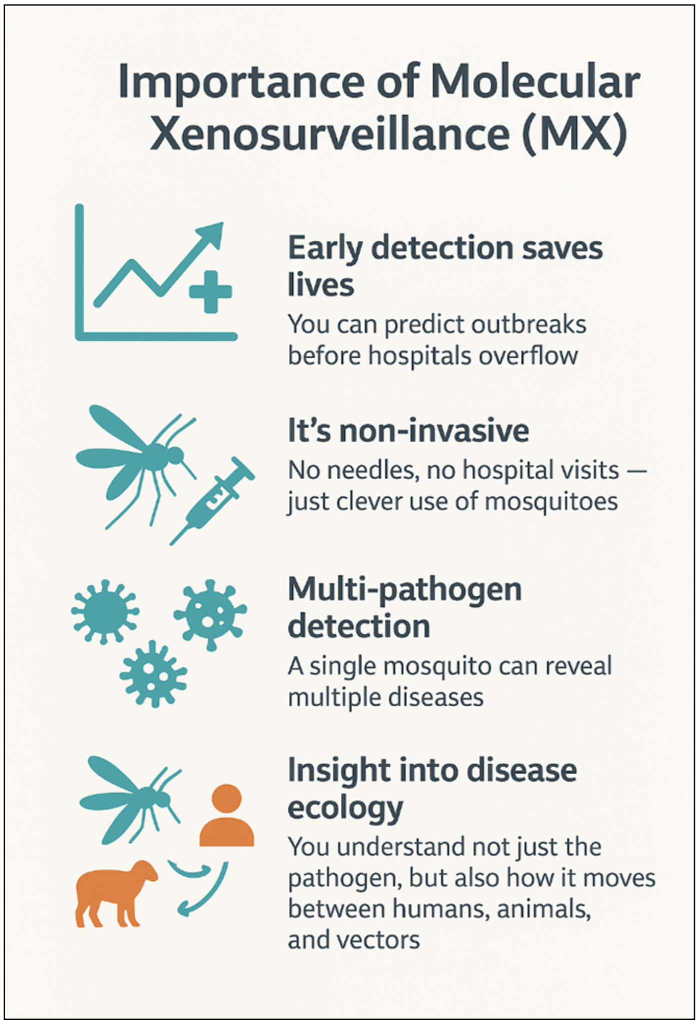
When should we go for it?

Timing is everything. If the goal is to catch pathogens early, we need to know both the vector's habits and the disease's seasonality.

1. Follow the mosquito calendar*:* Mosquitoes and other vectors are highly seasonal in nature. In tropical countries like India, their populations surge during and after the monsoon, when puddles and stagnant water create ideal breeding sites. Therefore, collecting mosquitoes before and during this period greatly enhances the chances of early detection of infections, including those present in asymptomatic individuals.

2. Catch them when they're full*:* Only blood-fed mosquitoes carry pathogens acquired from their human hosts. Since these tiny vampires primarily feed during dawn and dusk, collecting samples at these times yields the most effective results.

3. Keep an eye on pathogen activity*:* Viruses such as dengue, chikungunya, and Zika circulate in human blood for only a few days after infection. By targeting mosquitoes during this viremic window, scientists can identify these pathogens early, enabling the detection of diseases before they spread widely and cause outbreaks.



Importance of Molecular Xenosurveillance (MX)

Early detection saves lives
You can predict outbreaks before hospitals overflow

It's non-invasive
No needles, no hospital visits — just clever use of mosquitoes

Multi-pathogen detection
A single mosquito can reveal multiple diseases

Insight into disease ecology
You understand not just the pathogen, but also how it moves between humans, animals, and vectors

Figure 1: Significance of molecular Xenosurveillance (Valente et al., 2023)

4. Routine vs. Reactive surveillance*:* In endemic regions, xenosurveillance can be conducted regularly through weekly or biweekly collections, providing a real-time snapshot of circulating pathogens. During sudden increases in disease risk, it can also serve a reactive role, functioning as an early warning system for public health authorities.

The next time a mosquito lands on your arm, it should be viewed not merely as a nuisance but as a potential tool for disease surveillance. In the hands of scientists, mosquitoes act as miniature bio-samplers capable of providing valuable insights into circulating pathogens. Understanding the optimal timing and location for their collection is crucial, as it can determine whether an impending outbreak is detected early or allowed to spread undetected.

Success stories

Over the past decade, several successful studies have demonstrated the power and versatility of xenosurveillance (Table 1). From Ndione *et al.* (2025) identifying retroviruses and arboviruses in Senegalese mosquitoes to Valente et al. (2023) proving tsetse flies can retain viral RNA for enzootic virus screening, these works highlight the method's expanding scope. Likewise, Anvari *et al.* (2025) developed scalable techniques for detecting *Plasmodium falciparum* from mosquito blood meals, while McMinn *et al.* (2023) validated field-based vector surveillance in Guatemala. Together, these studies feature the xenosurveillance's growing promise for real-world disease monitoring across diverse ecological settings.

Why it matters?

The COVID-19 pandemic revealed how swiftly animal viruses can cross species barriers and trigger global crisis. Around 60% of emerging infectious diseases are zoonotic, with most originating in tropical regions that lack robust surveillance systems. Traditional wildlife monitoring requires the capture and bleeding of animals, which is laborious, costly, ethically complex, and sometimes risky. Xenosurveillance provides a more practical and humane alternative. Blood-feeding insects naturally sample a wide range of hosts daily including humans and livestock. Strategically placed traps in villages or forests can generate vital data about circulating viruses. With portable sequencing technologies, field teams could soon analyze these insect-derived samples on-site and in real time. Additionally, the potential of using this technique near bat caves or bat-dwelling areas is particularly significant, as bats serve as intermediate hosts for many zoonotic viruses. Although bats commonly feed on mosquitoes, some studies have detected bat blood in mosquitoes, indicating natural blood exchange between these species. Additionally, applying xenosurveillance approaches using ectoparasitic bat flies or blood-fed mosquitoes around bat roosts located close to human settlements can provide an early warning system for emerging pathogens. This strategy enhances early detection of vector-borne microorganisms and may help identify future pandemic threats before they spill over into human populations (Szentiványi et al., 2020; Fagre and Kading, 2019).

Challenges on the horizon

Xenosurveillance is a novel method for passive monitoring of circulating pathogens from blood-fed arthropods using nucleic acid analyses, but complex biological, technical, logistical, and interpretational challenges limit its adoption. Biologically, the process of digestion can quickly degrade viral nucleic acids within the insect gut, restricting the window of detection to a few days after the blood-meal and possibly missing low-titer infections. Furthermore, viral fragments do not necessarily confirm ongoing active infections in the vertebrate host, nor do they indicate viable virus infection in the vertebrate host. Environmental contamination (in housing or sampling vessels), mixed blood-meals, and inconsistency in host availability for blood consumption can further pose logistical and ecological challenges and caused complexity in interpretation.

Technically, identifying an insect-associated virus from a vertebrate-derived virus will require complete metagenomics, taking care to remove background sequences, and confirming the presences of viral RNA from the insect vector host using RT-qPCR or similar assays. Maintaining high-quality preservation of nucleic acids in the field continues to be difficult, but it appears that use of FTA cards and RNAlater may offer better alternatives. Logistically, regular blood-feeding insect capture is difficult, especially at remote wildlife interfaces where no routine trapping may take place, and where personnel and resources may limit repeat sampling and transport of field samples. Policy-related barriers include limited integration into national surveillance systems, lack of standard operating procedures, biosafety concerns, and unclear frameworks for sharing genetic data, especially those linked to wildlife or livestock. Despite these challenges, xenosurveillance offers significant advantages as reported by Ndione et al. (2025) that even partial viral sequences provide valuable ecological insights into host exposure, viral diversity, and regional circulation patterns. High-throughput metagenomics enhances this potential by enabling unbiased detection of known and novel viruses. Moreover, expanding surveillance beyond

Table 1. Historical timeline highlighting major milestones in the development of arthropod Xenosurveillance

Category	Insect Vector	Associated Pathogen(s)	Disease / Host Application	Key Reference
Lab-fed	Mosquito, *Anopheles gambiae*	*Trypanosoma brucei gambiense*, *Bacillus anthracis*, MERS-CoV, Zika virus	Demonstrated multi-taxa detection: trypanosomiasis, anthrax, MERS, Zika	Fauver et al., 2017
	Glossina spp. (tsetse)	Zika, DENV-2, Chikungunya, ASFV, Bluetongue, PPRV	Wildlife/livestock viral surveillance "flying syringe" concept	Valente et al., 2023
Field-collected	Mosquito, *Aedes aegypti*	Dengue virus serotype-2	Dengue fever: first MX report from Bangladesh	Ghosh et al., 2025
	Mosquito, *Culex* / *Aedes* sp.	West Nile, Usutu, Bagaza, Sindbis, Barkedji viruses	Arbovirus surveillance in humans and birds	Ndione et al., 2025
	Mosquitoes (blood-fed)	Retroviruses: JSRV, ENTV-2	Livestock surveillance on sheep/goats	Ndione et al., 2025
	Mosquito, *Anopheles* / *Culex*	*Plasmodium falciparum* and other *Plasmodium* spp.	Malaria surveillance on parasite detection + drug-resistance markers	Anvari et al., 2023
	Sand flies (*Phlebotomine* spp.)	*Leishmania* spp.	Cutaneous/visceral leishmaniasis vector surveillance	Nikookar et al., 2024
	Mosquitoes (bloodmeal analysis)	*Toxoplasma gondii*; SARS-CoV-2 antibodies	Xeno-serosurveillance on protozoa & host immune exposure	Štefanić et al., 2022
	House / biting flies (*Musca*, *Stomoxys*)	Ebola virus (exposure models)	Tested mechanical detection potential (low)	Haddow et al., 2017
	Mosquitoes (rural habitats)	Broad pathogen panel: arboviruses, veterinary viruses, parasite DNA	Validated practical feasibility in rural public health settings	McMinn et al., 2023; Grubaugh et al., 2019

mosquitoes to include vectors such as tsetse flies, as proposed by Valente et al. (2023), extends monitoring deeper into wildlife systems that are otherwise difficult to sample. Together, these advancements strengthen One Health surveillance across human, livestock, and wildlife interfaces.

Future directions of Xenosurveillance

Envision networks of "insect observatories" spread across Africa's national parks and rural landscapes solar-powered smart traps passively capturing mosquitoes, midges, and tsetse flies, with weekly screening for viral signatures. Uploaded to global databases, these data streams could generate early outbreak alerts well before symptoms appear in livestock or humans. This scenario is increasingly realistic: recent studies demonstrate that blood-feeding insects function as natural biosentinels, their bodies preserving molecular snapshots of ecosystems that reveal hidden host–pathogen interactions and the silent movement of viruses. From Senegal's savannas to Gabon's forests, scientists are learning to interpret these subtle "insect whispers" embedded in blood meals.

Portable Field Diagnostics:

Develop compact, battery-operated molecular tools for rapid on-site detection of viral fragments in insect blood meals to minimize nucleic-acid degradation and strengthen sensitivity.

AI-Assisted Monitoring:

Incorporate machine-learning pipelines to enhance detection of low-abundance viral sequences, distinguish true signals from background noise, identify novel pathogens, automate host-blood profiling, and forecast spillover hotspots.

Standardized Protocols:

Establish harmonized guidelines for vector collection, blood-meal preservation (e.g., FTA cards vs. RNAlater), optimal detection windows, sequencing thresholds, and data-reporting standards to ensure comparability across studies and regions.

Expanded Wildlife-Interface Surveillance:

Deploy xenosurveillance near bat caves, livestock corridors, forest-edge habitats, and peri-urban settlements to intercept viral circulation at high-risk wildlife–livestock–human interfaces without disturbing host animals.

Global Data-Sharing Networks:

Create interoperable genomic databases and real-time One-Health information pipelines to share viral signatures, host profiles, and ecological metadata, enabling coordinated, cross-border early-warning systems for emerging zoonotic threats.

Implications for one health

Xenosurveillance embodies the principles of the One Health framework by integrating human, animal, and environmental health monitoring. This approach enables the early detection of zoonotic spillover events before they develop into widespread outbreaks. It also provides a cost-effective and non-invasive method for monitoring wildlife in remote or inaccessible regions, while offering deeper insights into the complex interactions among hosts, pathogens, and ecosystems.

Conclusion

Blood-feeding insects long viewed simply as pests, but now they are increasingly recognized as powerful sentinels of pathogen circulation. Their blood meals preserve genetic traces of the often-hidden viral world that surrounds humans, livestock, and wildlife. Xenosurveillance, strengthened by metagenomic sequencing, shows that even partial viral fragments recovered from mosquitoes, sand flies, or tsetse flies can yield valuable insights into host exposure, viral diversity, and regional transmission patterns. Findings of past researches demonstrate how mosquito-derived sequences can reveal both arboviruses and unexpected retroviruses and highlight tsetse flies as reliable "flying syringes" capable of detecting multiple medically important viruses several days after feeding. Despite challenges such as nucleic acid degradation, short detection windows, environmental contamination, and the need for robust analytical pipelines, xenosurveillance remains a promising One Health tool. These limitations outline clear priorities for future improvement, including standardized protocols, better field preservation, and stronger integration into public and veterinary health surveillance systems. Ultimately, insect sentinels offer a remarkable, noninvasive pathway for early detection of enzootic and emerging viruses. By harnessing the natural feeding habits of these small yet influential organisms, we move closer to anticipating spillover events and strengthening global health security proving that even the tiniest creatures can play a transformative role in preventing future pandemics.

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