Literature review on Acoustic Detection and Tracking of Usutu Virus Outbreaks

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Literature Review: Acoustic Detection and Tracking of Usutu Virus Outbreaks

Overview

Acoustic detection and tracking of Usutu virus (USUV) outbreaks represents an emerging and largely unexplored intersection between **passive acoustic monitoring (PAM)** technology and **infectious disease epidemiology**. While bioacoustic methods have become established tools for biodiversity monitoring and disease surveillance in other zoonotic systems, their specific application to Usutu virus—a mosquito-borne flavivirus causing periodic avian mass mortality events—remains understudied. This review synthesizes current knowledge of USUV epidemiology, existing surveillance approaches, and acoustic monitoring frameworks to identify how sound-based monitoring could enhance early detection and tracking of outbreak dynamics.

Background: Usutu Virus Epidemiology and Current Surveillance Methods

Virus Biology and Geographic Spread

Usutu virus is a neurotropic Orthoflavivirus (genus *Flavivirus*, family *Flaviviridae*) maintained in an enzootic transmission cycle between mosquitoes and wild birds, with humans serving as incidental, dead-end hosts. The virus was first isolated in 1959 from *Culex neavei* mosquitoes in South Africa near the Usutu River. Since its initial detection in Italy in 1996, USUV has progressively spread across Europe, with multiple lineages identified (Europe 1, Europe 2/EU-2, Europe 3, Africa 2, Africa 3). As of 2024–2025, USUV is endemic in southern and central Europe, with documented circulation in Greece, France, Italy, Spain, Romania, Germany, Czech Republic, Belgium, Netherlands, and the United Kingdom.[1] [2] [3] [4]

Transmission and Avian Impact

The primary vector is *Culex pipiens* mosquitoes, though *Aedes albopictus*, *Culex modestus*, and other *Culex* species have been documented as secondary vectors. The virus follows a sylvatic bird–mosquito–bird cycle, with wild birds serving as amplifying hosts. Avian pathogenicity varies by lineage and species; European isolates cause more severe disease in birds than African isolates. Common blackbirds (*Turdus merula*) experience mass mortality during outbreaks, with some regional populations declining significantly. Other affected species include great grey owls (*Strix nebulosa*), kingfishers, and numerous passerines. Infected birds exhibit splenomegaly,

hepatomegaly, and multisystem inflammation, often dying within 5–7 days of infection.[5] [6] [7] [8] [9] [10]

Human and Other Mammalian Infection

Between 2012 and 2021, the EU/EEA reported 105 confirmed human cases, with 11 presenting neurological symptoms including encephalitis and meningoencephalitis. Most human infections are asymptomatic or cause mild febrile illness. USUV has also been detected in captive animals, zoo birds, bats, and rats, highlighting potential cross-species spillover risk.[2] [11] [3] [12] [13] [1]

Current Surveillance Methods

Existing USUV surveillance relies on multiple integrated approaches:

1. **Dead Bird Monitoring**: Passive (citizen reporting) and active (systematic necropsy) surveillance of wild birds from rescue centers and wild populations. Molecular detection via RT-PCR on brain, spleen, kidney, and other tissues; sensitivities vary by sample type (brain ~100%, feathers ~92%, throat/cloacal swabs ~88–92%).[14]

2. **Mosquito Surveillance**: Trapping and molecular screening of *Culex* spp. populations using RT-qPCR on whole mosquitoes or excreta (Molecular Xenomonitoring, MX). Detection of viral RNA in mosquito feces can indicate circulation 5–7 days before appearance in birds or humans.[15]

3. **Serological Monitoring**: Detection of antibodies (IgG/IgM) in wild birds (via micro-neutralization tests, ELISA) and sentinel species in zoological collections. Long-term serosurveys can identify cryptic exposure.[16] [13]

4. **Environmental Sampling**: Detection of USUV RNA in pigeon droppings, avian cloacal swabs, and environmental water samples (via digital PCR), demonstrating virus excretion pathways.[12]

5. **Vector Competence and Laboratory Studies**: Controlled experiments assessing temperature-dependent transmission in *Culex pipiens molestus* and other mosquito species, informing risk models.[17]

Limitations of Current Approaches

- Passive surveillance is biased toward conspicuous mortality; cryptic infections in asymptomatic birds are missed.

- Active bird sampling is labor-intensive and costly, limiting spatial and temporal coverage.

- Dead birds must be collected promptly; decomposition and scavenging reduce sample availability.

- Serological assays detect past exposure, not active transmission.

- Molecular xenomonitoring requires expertise and is not universally implemented.

Passive Acoustic Monitoring: Technology and Applications

Foundational Technology and Hardware

Passive acoustic monitoring (PAM) is a non-invasive method using autonomous recording units (ARUs) or handheld devices to capture ambient soundscapes for species identification and monitoring. Key hardware advances include:[18]

- **AudioMoth**: Open-source, microcomputer-based recording devices (~US$43) with programmable sampling rates (up to 384 kHz), sampling intervals (e.g., 15–30 s), and gain settings. Detection radius typically 50 m to 1,500 m depending on source frequency and environment.[19]

- **Raspberry Pi-based Systems**: Single-board computers (e.g., Raspberry Pi Zero 2 W, Raspberry Pi 4B) coupled with microphones, sound cards, and batteries. Bioaco-record prototype (cost ~€180): runs BirdNET-Pi for real-time species classification, transmits results via GSM to cloud platforms, supports 6.5-day battery life with 30-second sampling intervals.[20]

- **Alternative Platforms**: Wildlife Acoustics Song Meters, Cornell Swift recorders, Jetson Nano-based Bird@Edge systems; most operate locally with Wi-Fi connectivity or require post-hoc analysis.[20]

Autonomous Recording Units (ARUs) in Biodiversity Monitoring

ARUs are increasingly deployed for large-scale, long-term bird monitoring, offering advantages over human observers: reduced observer bias, standardized data collection, continuous recording capability, and minimal field personnel requirements. Studies comparing acoustic surveys to point-count methods in farmland and meadow habitats found acoustic recorders and human observers surveying within a 100 m radius produce similar biodiversity estimates, validating ARUs as surrogates.[21] [22] [18]

Machine Learning and Classification Algorithms

BirdNET: A deep neural network (ResNet-based) developed by Cornell Lab of Ornithology's Center for Conservation Bioacoustics, capable of recognizing 6,500+ bird species globally. BirdNET operates by:[23] [20]

- Splitting incoming audio into 3-second spectrograms (time–frequency–amplitude visualizations).
- Classifying each spectrogram and assigning confidence scores.
- Outputting species-level predictions with probability thresholds (typically 0.70–0.80).

Performance studies demonstrate high accuracy across diverse ecosystems: over 90% precision for 109 of 136 species across audio from Norway, Taiwan, Costa Rica, and Brazil (152,376 hours of data), though geographic and dataset-specific calibration is required.[23]

Extensions and Customization:

- **BirdNET-Pi**: Adapted for deployment on single-board computers, enabling real-time classification in field settings.

- **Faunanet**: Modular software framework supporting any TensorFlow-based classification algorithm, enabling extension beyond avian taxa to mammals, insects, and bats.[20]
- **Deep Learning (CNN/RNN)**: Convolutional and recurrent neural networks bypass feature extraction, performing well on noisy, complex soundscapes. Applied to detection of anthropogenic sounds (e.g., chainsaws, gunshots) and bioacoustic features (alarm calls, vocalizations).[19]

Data Analysis Workflows

Audio analysis typically follows a multi-step process:

1. **Signal Detection**: Thresholding or statistical methods isolate vocalizations from background noise.
2. **Spectrogram Conversion**: Waveforms transformed into time–frequency representations for visual/algorithmic inspection.
3. **Classification**: Signals compared against species templates or neural network models; outputs include species identity and confidence scores.
4. **Soundscape Analysis**: Beyond species identification, metrics summarizing entire acoustic environments:
   - **Acoustic Indices**: Acoustic Complexity Index (ACI), Acoustic Diversity Index (ADI), Bioacoustic Index (Bio), assess overall ecosystem acoustic activity and diversity.[24] [19]
   - **Species Richness/Composition**: Total species counts, abundance distributions, temporal/spatial patterns.
   - **Acoustic Space Use (ASU)**: Proportion of time-frequency acoustic space occupied, correlating with biodiversity in some ecosystems.[19]
5. **Statistical Modeling**: Occupancy models incorporating detection probability; Bayesian frameworks addressing detection biases, distance decay, and environmental noise.[19]

Cloud Platforms and Real-Time Systems

Modern acoustic monitoring integrates IoT (Internet of Things) connectivity:

- **ThingSpeak**: Cloud analytics platform enabling remote data aggregation, visualization, and real-time alerts.[20]
- **Arbimon Insights**: Web-based platform for acoustic data analysis, species detection mapping, and dashboard visualization (e.g., long-tailed macaque presence in *Plasmodium knowlesi*-endemic regions).[19]
- **PAMGUARD, Kaleidoscope, Raven Pro**: Software suites offering template matching, automated detection, and batch processing.[19]

Acoustic Epidemiology: Framework for Infectious Disease Surveillance

Conceptual Foundations

A paradigm shift is underway in disease epidemiology toward leveraging PAM technology for zoonotic and vector-borne disease surveillance. The framework addresses core epidemiological questions: [25] [26] [20] [19]

1. Which species are present in an area?

2. Where and how do key species move and behave across space?

3. When are species active?

4. Is there spatiotemporal overlap between host, vector, and human populations?

Traditional field methods (transects, trapping, questionnaires) provide fine-scale ecological data but are labor-intensive, expensive, and limited in spatial/temporal coverage. PAM offers a **scalable, non-invasive complement**, particularly suitable for **remote, densely vegetated, or logistically challenging environments.** [19]

Epidemiological Variables Derivable from Acoustic Data

Variable	Acoustic Metrics	Disease Application	Example
Occupancy (spatial/temporal presence)	Detection/non-detection; spatially explicit count data; acoustic localization	Identifying host/vector presence in outbreak zones	Fruit bats (*Rousettus aegyptiacus*) roosting near livestock (Nipah virus spillover risk); long-tailed macaques in human settlements (zoonotic malaria)
Behavioral Traits	Detection frequency of specific vocalizations; call pattern shifts	Detecting disease-driven behavior changes or breeding seasonality linked to spillover peaks	Egyptian fruit bat breeding season peaks corresponding to Marburg virus circulation in humans; host die-off indicated by acoustic silence
Ecosystem Health	Acoustic indices (ACI, ADI, Bio); soundscape fingerprints; species richness; community composition	Monitoring habitat disturbance linked to increased spillover risk	Habitat fragmentation assessed via soundscape metrics; species composition shifts indicating ecosystem stress
Human Activity	Detection of anthropogenic sounds (chainsaws, voices, machinery)	Mapping human–wildlife interface overlap, temporal risk of spillover	Detection of human chainsaw activity during evening hours overlapping with *Anopheles balabacensis* biting peaks (malaria receptivity)

Case Studies of PAM in Disease Ecology

1. **White-Nose Syndrome (WNS) in Bats**: Ecoacoustic surveys of hibernation sites detect behavioral changes associated with WNS infection, track seasonal disease progression, and assess climate-change impacts on disease dynamics. [19]

2. **Chytridiomycosis in Amphibians**: Call pattern changes in infected frogs detected acoustically, informing disease spread monitoring.[19]

3. **Pneumonia in Bighorn Sheep**: Coughing and respiratory distress detected acoustically, enabling early disease detection in populations.[19]

4. **Zoonotic Malaria (*Plasmodium knowlesi*)**: Acoustic monitoring of long-tailed macaque (*Macaca fascicularis*) presence and movement across land-use gradients (forests, oil palm plantations) in Malaysian landscapes, combined with mosquito and human activity data, to parameterize spillover risk models.[19]

Bird Vocalizations as Health Indicators

Physiological Basis

Bird vocalizations are generated via the syrinx, a specialized vocal organ relying on synchronized respiratory, laryngeal, and muscular control. Vocal output reflects individual condition: parasite load, body size, metabolic stress, and inflammation all influence call characteristics. **Respiratory infections** (e.g., infectious bronchitis, Newcastle disease) in poultry produce distinctive acoustic changes: elevated call rates, altered frequency profiles (spectral shift), and production of rales (abnormal breathing sounds).[27] [28] [29] [30] [31] [32]

Acoustic Features Associated with Disease

Studies on poultry disease detection identified key acoustic parameters distinguishing healthy from infected birds:

Feature	Computation	Disease Association	Performance
Mel-Frequency Cepstral Coefficients (MFCC)	Spectral envelope in log-frequency domain	Respiratory disease (bronchitis, Newcastle disease)	78–80% accuracy (Newcastle disease detection)
Wavelet Entropy (WET)	Entropy of wavelet coefficients across time-frequency	Infectious bronchitis	83% accuracy on day 3 post-inoculation
Spectral Centroid	Frequency center-of-mass of spectrum	Vaccine reactions, stress	Discriminates treated vs. untreated birds
Vocalization Rate	Number of calls per time interval	Stress, welfare	Decreases with elevated CO_2, increases with distress

Detection accuracies in controlled poultry studies reach **80–83% for early-stage respiratory disease** detection (days 3–4 post-inoculation), with type II error rates (false negatives) below 14%.[28] [30]

Limitations for Wild Bird Disease Detection

However, **wild bird disease acoustics remain understudied**:

- USUV infection in birds is not pathognomonic for any specific vocalization change (unlike distinctive respiratory disease rales).

- Mortality from USUV can be acute (5–7 days), potentially allowing only brief detection window before death.

- Vocalizations from subclinically infected or asymptomatic birds are unknown.

- Individual variation in vocalization due to genetics, age, sex complicates disease signal detection.

Potential Integration: Acoustic Monitoring for Usutu Virus Detection

Conceptual Framework

Although no published studies have directly applied acoustic monitoring to USUV outbreak detection, the technical scaffolding and epidemiological rationale exist. Integration could operate on multiple levels:

Level 1: Population-Level Acoustic Monitoring

- **Baseline**: Establish acoustic profiles of bird communities in surveillance regions via long-term PAM deployment (6–12 months pre-outbreak).

- **Acoustic Indices**: Calculate temporal trends in acoustic diversity (ADI, ACI, Bio index) and species richness as proxies for avian community health.

- **Outbreak Signal**: Detect abrupt shifts in soundscape characteristics—decline in call rates, loss of species vocalizations, or reduced acoustic complexity—indicating mass mortality or population collapse.

- **Sensitivity**: Likely highest for species experiencing significant USUV-related mortality (e.g., common blackbirds), less sensitive for subclinical infections.

Advantages:

- Non-invasive, continuous monitoring across large areas.

- Real-time or near-real-time alerts via cloud-based platforms.

- Low operational cost once deployed.

- Captures population-level effects (mortality, behavioral disruption) without specimen collection.

Challenges:

- Requires baseline data; cannot retrospectively detect early outbreaks without prior acoustic baseline.

- Acoustic silence may be confounded with seasonal migration, breeding cycle suppression, or environmental factors (e.g., extreme heat reducing calling).

- Species-specificity: only vocalizing birds detected; USUV affecting silent species (e.g., raptors, waterbirds with limited call repertoires) would be missed.

Level 2: Health-Indicative Vocalizations

- **Hypothesis**: Early USUV infection induces subtle vocalization changes (frequency shifts, call rate alterations, respiratory sounds) due to viremia and inflammation.

- **Detection**: Train machine-learning models on vocalization spectrograms from acoustically-monitored USUV-infected birds (requires controlled laboratory or field studies) to identify disease-specific signatures.

- **Implementation**: Deploy models in field via IoT devices (Raspberry Pi + BirdNET-Pi) with real-time classification of health-indicative calls.

Advantages:

- Provides early warning (days before mortality).

- Could detect subclinical infections.

- Parallel approach to traditional virological surveillance.

Challenges:

- **Research gap**: No published acoustic signatures for USUV-infected wild birds exist; controlled studies needed.

- Limited temporal window (acute 5–7 day infection).

- Individual and species-level acoustic variability.

- Difficult to isolate USUV-specific vocalizations from stress responses (infection vs. environmental disturbance).

Level 3: Ecosystem and Habitat Integration

- **Landscape Context**: Combine acoustic data on bird community composition with mosquito vector presence (via acoustic trapping or molecular surveys) and environmental data (temperature, humidity, land use).

- **Risk Modeling**: Use acoustic-derived occupancy and diversity metrics as inputs to epidemiological models predicting spillover risk, informed by habitat fragmentation, human density, and wetland concentration (known risk factors from France 2018 outbreak).[9]

Level 4: Citizen Science and Distributed Monitoring Networks

- **Scalability**: Low-cost AudioMoth devices (~US$43) and open-source software enable distributed networks across regions.

- **Public Engagement**: Combine acoustic data collection by volunteers/landowners with public reporting of dead birds (e.g., Garden Wildlife Health, DWHC schemes).

- **Early Warning**: Real-time aggregation of acoustic and bird mortality reports via centralized platforms (web dashboards, alerts) facilitates rapid response.

Comparative Strength and Limitations of Acoustic vs. Traditional Surveillance

Aspect	Acoustic Monitoring	Traditional Surveillance (Dead Birds, Mosquitoes)
Timeliness	Real-time or near-real-time detection possible	Delayed: dependent on dead bird collection, lab processing
Scalability	High: automated, low-cost hardware; minimal field personnel	Limited: labor-intensive, expensive, requires field expertise
Spatial Coverage	Broad coverage with network of ARUs; suitable for remote areas	Opportunistic or targeted sampling; biased toward accessible sites
Specificity	Low: detects population effects, not USUV directly	High: molecular confirmation of viral presence
Sensitivity for Subclinical Infection	Potentially high if vocalization signatures identified	Low: detects only sick/dead individuals
Environmental Constraints	Affected by rain, wind, anthropogenic noise; seasonal call activity	Less sensitive to weather; independent of vocalization behavior
Cost per Unit Area	€180–300/device; minimal maintenance after deployment	€500+/sample; ongoing personnel, lab costs
Data Type	Continuous audio (large data volumes); requires computational processing	Discrete samples; straightforward molecular analysis
Complementarity	Best as early warning system; validates via traditional methods	Gold standard; necessary for confirmation and public health response

Technical and Logistical Requirements for Implementation

Hardware Deployment

1. **Device Selection**:

   - **Pilot Scale**: AudioMoth units (~US$43) with post-hoc BirdNET analysis on personal computers or cloud (e.g., BirdCLEF, Arbimon free tier).

   - **Operational Scale**: Raspberry Pi Zero 2W-based devices (€180/unit) with real-time classification, GSM connectivity, and solar power for long-term off-grid deployment.

2. **Microphone Specifications**:

   - Budget microphones (e.g., AGPTEK, ~US$10): adequate for environmental soundscape monitoring; effective detection range ~100 m.

   - Mid-range microphones (e.g., Rode Lavalier, ~US$50): improved frequency response, clearer spectrograms, reduced noise sensitivity.

   - Trade-off between cost and sensitivity; high-quality microphones increase feasibility of scaled networks.

3. **Positioning and Maintenance**:

   - Deploy in locations distant from dominant noise sources (e.g., nests of highly vocal species, human settlements) to minimize acoustic masking.

- Position above ground level and protect from weather/animals (Figure from Treskova et al.: waterproof casings, protective cages).
- Vertical placement optimization (height above ground) should be site-specific, informed by target species and vegetation structure.
- Battery life: lead-acid batteries provide 6.5–10 days with optimized sampling intervals; solar panels extend indefinitely.

Sampling Design

1. **Temporal Resolution**:
   - Sampling intervals: 15–30 seconds (balance between classification frequency and power consumption).
   - Continuous recording vs. scheduled intervals: design depends on research question and field logistics.
   - Align with bird calling phenology: intensive recording during breeding/territory season (peak vocal activity) when USUV mortality expected.

2. **Spatial Sampling**:
   - Grid deployment: 100–150 m spacing (in line with microphone detection range) enables spatial heatmaps and multipoint localization.
   - Stratified by habitat/land use (forests, wetlands, urban areas) to capture ecological diversity affecting spillover risk.
   - Cover known USUV outbreak epicenters and predicted spillover zones.

3. **Sample Size and Duration**:
   - Baseline: minimum 6–12 months pre-outbreak to establish acoustic community baselines.
   - Operational: continuous year-round monitoring; intensive sampling during summer (peak USUV circulation and mosquito activity).

Data Management and Analysis

1. **Storage**:
   - AudioMoth devices: on-board microSD cards (32 GB provides ~15 days continuous 48 kHz mono).
   - Raspberry Pi systems: local storage + cloud backup via GSM/WiFi.
   - Data loads: continuous monitoring generates ~10–50 GB/device/month; requires robust data infrastructure.

2. **Processing**:
   - Local on-device classification (real-time alerts) via BirdNET-Pi or faunanet.
   - Batch processing: upload raw audio to cloud platforms (Arbimon, PAMGUARD with GUI) for quality control and re-analysis.
   - Machine learning: pre-trained BirdNET or custom models (fine-tuned to regional birds, local acoustic environment).

3. **Statistical Analysis**:
   - **Occupancy modeling**: estimate detection probability and true presence/absence accounting for imperfect detections.
   - **Acoustic indices**: compute temporal trends in ADI, ACI, Bio Index; test for abrupt shifts (e.g., changepoint detection).
   - **Species composition**: cluster analysis or NMDS ordination to detect community shifts.
   - **Integration**: combine acoustic data with concurrent mosquito trap data, ambient temperature, and human activity metrics for multivariable risk models.

Ethical and Data Privacy Considerations

Deployment of audio surveillance in natural environments or near human populations raises privacy concerns, particularly regarding:

- **Incidental Human Speech**: BirdNET-Pi includes privacy threshold parameters to omit classifications with confidence in human vocalizations, preventing raw audio transmission or analysis of human speech.
- **Local Processing**: Onboard classification ensures no raw audio leaves devices; only species-level detections are transmitted.
- **Metadata Anonymization**: GPS coordinates, timestamps recorded but linked to sites (not individuals).
- **Regulatory Compliance**: Deployment should adhere to data protection guidelines (GDPR in EU) and seek ethical clearance from relevant authorities.

Research Gaps and Future Directions

1. **Acoustic Signatures of USUV Infection**:
   - Controlled laboratory studies exposing wild bird species to USUV and characterizing vocalization changes (frequency, call rate, spectral entropy, presence of respiratory sounds).
   - Field studies correlating acoustic phenotypes with qRT-PCR viremia or serology status in naturally infected birds.
2. **Detection Algorithm Development**:
   - Train species-specific and disease-specific machine-learning models on USUV-infected bird vocalizations.
   - Evaluate performance across ecological settings (habitat types, seasons, background noise conditions).
   - Address geographic generalization: how do models trained in one region transfer to others?
3. **Operational Surveillance Pilots**:
   - Deploy acoustic networks in known USUV endemic zones (France, Italy, Germany, UK) and compare detection timing/sensitivity to traditional surveillance.

- Quantify early warning lead time: days between acoustic signal and first confirmed human case or peak bird mortality.

4. **Integration with Multisensor Systems**:

    - Combine acoustic monitoring with:

        - Molecular xenomonitoring of mosquitoes (MX on trapped excreta).

        - Environmental eDNA sampling (water filtration for USUV genetic material).

        - Real-time temperature/humidity sensors (inform thermal-dependent transmission models).

    - Develop unified data platform enabling cross-data inference.

5. **Cost-Benefit Analysis**:

    - Compare operational costs (equipment, deployment, data processing, personnel) of acoustic surveillance vs. traditional methods.

    - Quantify public health benefit (lead time to outbreak confirmation, averted human cases).

6. **Non-Avian Taxa Extension**:

    - Develop faunanet-compatible models for bats and rodents (potential USUV amplifying hosts or spillover risks).

    - Evaluate whether acoustic occupancy of mammalian reservoirs predicts viral circulation.

Limitations and Caveats

1. **Soniferous Bias**: Acoustic monitoring detects only vocalizing species; USUV-infected silent birds (some waterfowl, raptors) remain invisible.

2. **Acoustic Confounds**:

    - Seasonal call suppression (molt, non-breeding season) may mimic disease-induced silence.

    - Weather (rain, high wind) reduces detection and increases false negatives.

    - Anthropogenic noise masks bird vocalizations, particularly in urban/industrial areas.

3. **Sensitivity Specificity Tradeoff**:

    - Acoustic indices are sensitive to community changes but non-specific; cannot distinguish USUV mortality from other causes (predation, food shortage, pesticide effects).

    - Health-indicative models require validation; false positives from stress responses (territorial conflicts, storms) likely.

4. **Early Detection Window**:

    - USUV infection is acute (5–7 days to mortality); window for detecting vocalization changes is narrow, unless asymptomatic vocalizing individuals exist.

5. **Data Infrastructure Barriers**:

- Cloud computing, GSM connectivity, and IT support for data processing may be unavailable in low-resource regions where USUV is emerging.
- Requires interdisciplinary expertise (ornithology, machine learning, epidemiology, IT).

Conclusion

Acoustic detection and tracking of Usutu virus outbreaks is a **promising but currently underdeveloped frontier** in infectious disease surveillance. The foundational technologies—low-cost autonomous recording units, machine learning-based species classification, and real-time IoT connectivity—are mature and increasingly accessible. Epidemiological frameworks for applying PAM to zoonotic disease surveillance are established, with successful proof-of-concept studies in malaria, rabies, and other wildlife diseases.

However, **direct application to USUV surveillance requires significant research investment**. Key priorities include:

1. Characterizing acoustic signatures of USUV infection in wild and captive birds.
2. Developing and validating disease-detection models.
3. Conducting operational pilots comparing acoustic surveillance to traditional methods.
4. Integrating acoustic data with molecular, serological, and environmental sampling for holistic early warning systems.

When realized, acoustic monitoring could provide **early, scalable, non-invasive detection** of USUV outbreaks, complementing traditional laboratory-based surveillance and enabling rapid public health and veterinary responses. The combination of acoustic biodiversity monitoring with targeted health-indicative vocalization detection offers a novel toolkit for tracking emergence of this expanding European flavivirus.

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