Quantitative evaluation of the sensitivity of a wildlife health surveillance network for the detection of highly pathogenic avian influenza H5N1 in Cambodia

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Summary
Early detection of highly pathogenic avian influenza (HPAI) H5N1 in wild bird populations requires surveillance networks that are not only operational but demonstrably sensitive. This study presents a quantitative evaluation of the Wildlife Health Surveillance Network (WHSN) in Cambodia, using a scenario tree approach applied to seven protected areas over the period October 2024 to March 2025. Node probabilities were estimated from network data and expert elicitation, with uncertainty propagated through Monte Carlo simulations. At a design prevalence of 0.1%, site-level sensitivity ranged from 0.005 at Prek Toal to 0.388 at Rohal Kes, and global sensitivity reached 0.869 at a design prevalence of 0.1%. Standardized regression and a Tornado diagram identified sampling as the dominant driver of system performance ($\beta = 0.896$), followed by discovery ($\beta = 0.374$) and notification ($\beta = 0.081$), while training coverage showed no direct effect. These results provide a practical basis for targeted reinforcement of the network, with priority given to sampling logistics, patrol coverage, and notification feedback loops.

Introduction
Wildlife plays a central role in the emergence and spread of zoonotic diseases. HPAI H5N1, in particular, has circulated in Southeast Asia for over two decades, with wild waterbirds — especially Anseriformes and Ciconiiformes — serving as reservoir hosts capable of disseminating the virus across long distances, often without visible clinical signs (1). Cambodia is a recognised biodiversity hotspot in the region, and the intensification of interactions between wildlife, domestic animals, and human populations — driven by deforestation, agricultural expansion, and wildlife trade — has increased the risk of spillover events with both animal health and public health consequences. Between February 2023 and August 2024, Cambodia reported 16 confirmed human H5N1 infections, and in 2021, a mass mortality event affected more than 2,000 wild birds in the southeast of the country, underlining the ongoing and active circulation of the virus.

To address this risk, the Wildlife Conservation Society (WCS) established in 2014 the Wildlife Health Surveillance Network (WHSN) in Cambodia, a passive surveillance system targeting mortality and morbidity events in wild birds across protected areas. The network relies on field actors — rangers, community forestry agents, conservation staff — trained to detect, report, and sample unusual events, following a Standard Operating Procedure (SOP) defined in 2023. In parallel, the BCOMING project (Biodiversity Conservation to Mitigate the Risks of Emerging Infectious Diseases), funded by the European Union, has been supporting the reinforcement of this network by integrating quantitative assessment approaches and connecting surveillance objectives to broader biodiversity conservation goals (2).

Despite the existence of the WHSN and its integration into national health surveillance frameworks, the actual sensitivity of the network for detecting HPAI H5N1 had not been formally quantified. In a context of limited resources and competing priorities, understanding where the system fails — and why — is a prerequisite for targeted and cost-effective improvement. This study applies the scenario tree method to estimate the sensitivity of the WHSN across seven protected areas and to identify the operational nodes that most constrain detection performance.

Materials and Methods
Study sites and target population
Seven wetland sites frequented by wild waterbirds were selected for inclusion: Boeung Snae (BS), Anlong Pring (AP), Boeung Prek Lpeou (BPL), Ang Trapeang Thmo (ATT), Rohal Kes (RK), Toul Prum (TP), and Prek Toal

(PT). These sites differ considerably in terms of avian diversity and density, surface area, geographic location, proximity to poultry farms, and the number and training status of field agents. The target population consisted of wild birds present at each site, classified into six taxonomic categories based on their order: Ciconiiformes, Anseriformes, Pelecaniformes, Charadriiformes, Raptors, and other species. Each category was assigned a relative infection risk derived from published literature and from WCS bird count data, reflecting differences in susceptibility to H5N1 and observed mortality rates across studies conducted in Asia and elsewhere.

Data sources and period of study
Data covered the period October 2024 to March 2025. Two main sources were used. First, the WHSN database provided information on species detected, collection dates and sites, carcass condition, sampling status, and the number of trained and untrained agents per site. Bird counts by protected area were provided by WCS. Second, a structured questionnaire was administered to key actors in the network: officers from provincial animal health offices estimated infection risk by site and by bird category, while field agents from the provincial environment departments provided information on detection and notification probabilities, as these are the steps they carry out directly on the ground. Questionnaires were translated into Khmer via KoBoToolbox, and responses were collected remotely by telephone or Telegram before being translated into English, exported into Excel, and analysed in R.

Scenario tree construction and parameter estimation
The scenario tree method decomposes the surveillance process into successive nodes, each associated with a probability or proportion, to estimate the overall probability that the system detects at least one infected animal if the pathogen is present at a given prevalence (3). Nodes were defined in chronological order and structured into three categories: category nodes (protected area, bird species group, agent training status), an infection node, and detection nodes (discovery, notification, sampling, PCR confirmation) (4).

The infection node was built by combining an ecological risk score for each site — based on proximity of poultry farms within a 5 km radius, history of avian influenza outbreaks, and presence of wetland habitats — with a biological risk component weighted by the proportion of each bird category present at the site. Expert elicitation responses were modelled using PERT distributions to account for parameter uncertainty. PCR test sensitivity was fixed at 0.95 based on laboratory data.
Monte Carlo simulations were then run to propagate uncertainty through the tree and estimate site-level and global sensitivity for four design prevalence values: 0.03%, 0.04%, 0.05%, and 0.1%.

Sensitivity analysis
To identify which nodes most influenced system performance, a standardized multiple regression was conducted using the node probabilities as predictors and site-level sensitivity as the outcome. A Tornado diagram was also constructed by varying each parameter independently between its 10th and 90th percentiles and measuring the resulting range of predicted global sensitivity, which provided a visual and quantitative measure of each node's influence.

Results
Node parameter estimates
Parameter estimates varied substantially across sites. The proportion of trained agents ranged from 26% at Boeung Snae to 100% at Anlong Pring. Discovery probabilities ranged from 0.075 at Prek Toal to 0.577 at Rohal Kes, and notification probabilities from 0.344 to 0.680. The sampling probability was uniform across sites at 0.807, derived directly from the WHSN database.

Site-level sensitivity
At a design prevalence of 0.1%, site-level sensitivity showed strong variation. The lowest values were observed at Prek Toal (0.005; 95% CI: 0.002–0.010), Boeung Prek Lpeou (0.033; 0.018–0.058), and Boeung Snae (0.054; 0.033–0.084). Higher sensitivities were estimated at Toul Prum (0.231; 0.147–0.333), Ang Trapeang Thmo (0.268; 0.175–0.379), Anlong Pring (0.278; 0.169–0.417), and particularly Rohal Kes (0.388; 0.237–0.555). These differences reflect the combined effect of local infection risk, patrol coverage, and detection chain performance rather than any single factor.

Global sensitivity
Global sensitivity — the probability that the system detects at least one case across all sites — increased with design prevalence: 0.456 (95% CI: 0.253–0.479) at D = 0.03%, 0.556 (0.322–0.581) at 0.04%, 0.638 (0.385–0.663) at 0.05%, and 0.869 (0.622–0.886) at 0.1%. This reflects the mechanical relationship between prevalence and detection probability: as more animals are potentially infected, the chance of encountering at least one becomes greater.
Sensitivity analysis

The standardized regression identified sampling as the dominant driver of system sensitivity ($\beta = 0.896$, $p < 2\times10^{-16}$), followed by discovery ($\beta = 0.374$, $p < 2\times10^{-16}$) and notification ($\beta = 0.081$, $p < 2\times10^{-16}$). Training coverage had no direct effect ($\beta = 0.0003$, $p = 0.891$), though this likely reflects the fact that training acts indirectly through its influence on discovery and notification rather than as an independent predictor. The model explained 95.5% of variance in sensitivity (adjusted $R^2 = 0.9545$).

The Tornado diagram confirmed this hierarchy. Around a median global sensitivity of 0.770, sampling produced the widest range of predicted values (0.680 to 0.862; range 0.182), followed by discovery (0.735 to 0.806; range 0.071), notification (0.762 to 0.778; range 0.015), and training (range 0.00006).

Notification barriers

Among the 66 field agents who responded to the questionnaire, all (100%) identified not knowing who to contact as a barrier to reporting, and an equal proportion felt that notification was not part of their formal responsibilities. Almost all (99%) cited the absence of feedback following previous reports as a discouraging factor. Despite these declared obstacles, 92% stated that none of these factors would actually prevent them from reporting a sick or dead bird — suggesting that motivation is present, but the structural conditions for notification are not.

Discussion

The scenario tree approach proved well suited to this context. It allows explicit modelling of each step of the surveillance chain, integrates expert knowledge where formal data are absent, and propagates uncertainty through PERT distributions rather than relying on point estimates. It is also more appropriate than capture-recapture methods here, as the latter require independent data sources and a sufficient volume of confirmed cases with independent detection pathways — conditions that are rarely met in wildlife surveillance (5).

The most immediate operational implication of our results is the priority of the sampling node. Even where discovery and notification function reasonably well, the proportion of reported cases that are actually sampled and sent to the laboratory remains the strongest determinant of whether the system can confirm an event. This suggests that improvements to sampling logistics — kit availability, cold chain, transport to Institut Pasteur du Cambodge for PCR confirmation — would have a greater impact on sensitivity than expanding training coverage in its current form.

Discovery is the second priority, and the results suggest that patrol coverage and the ability of field agents to recognise mortality events are the relevant dimensions. Standardised alert signs and simplified field guides, rather than comprehensive training programmes, may be more effective at this stage. The low discovery probability at Prek Toal is a particular concern given the site's large area and density of waterbirds.

On notification, the questionnaire results are instructive. Field agents are not reluctant to report — the vast majority say they would report regardless of barriers. The problem is structural: unclear roles, no designated contact, and no feedback loop after reporting. These are fixable problems that do not require significant new resources.

Training coverage, as measured, had no direct effect. This probably reflects the fact that training was captured as a binary proportion of staff who had received any training, rather than a measure of operational competence. Transitioning to competency-based indicators, with standardised practical assessments, would likely reveal a different and more actionable picture.

Finally, our results differ from a previous 2022 evaluation of the same network, which covered fewer sites and did not incorporate expert elicitation. Both methodological differences and possible changes in surveillance effort between the two periods may explain these discrepancies.

Keywords: avian influenza, wildlife surveillance, scenario tree, sensitivity, Cambodia.

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