



Outbreak Investigation and Response: Two Fatal Cases of Avian Influenza A(H5N1) Detected in Humans in October 2023, Cambodia

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Abstract

In October 2023, Cambodia reported two fatal human cases of influenza A(H5N1) from two provinces. The national and subnational rapid response teams investigated the outbreak to describe its characteristics, identify potential linkages between the cases, assess the outbreak magnitude, understand the local perspective and response. Medical records were reviewed, and contact tracing and active case finding were conducted in the affected areas. Laboratory investigation and environmental survey were performed. Local authorities were interviewed. The two cases, confirmed to have influenza A(H5N1) clade 2.3.2.1c, were a 50-year-old man from Svay Rieng Province and a 2-year-old girl from Prey Veng Province. Both cases had direct contact with sick and dead poultry at their house before developing respiratory symptoms. We identified 65 close contacts and 15 suspected cases who tested negative for influenza A, influenza B and SARS-CoV-2. Inappropriate practices of handling sick and dead poultry and allowing free-roaming backyard poultry within houses were identified in the affected villages. There was no linkage between the two cases and no additional cases were found during the investigation. During the response, there was a lack of awareness about avian influenza among communities and health workers. Appropriate medication (e.g., oseltamivir) should be stockpiled at the district level. A lack of coordination and communication among One Health sector was identified. We recommended promoting a joint multisectoral coordination for investigations, strengthening surveillance systems, increasing avian influenza awareness, allocating resources, and holding daily coordination meetings among stakeholders for effective planning and communication.

Keywords: outbreak investigation, avian influenza, influenza A(H5N1), Cambodia

Introduction

Avian influenza A(H5N1) viruses are associated with poultry outbreaks and transmission to humans, with a high case fatality rate (CFR) of 51% globally.^{1,2} In 1997, the first human A(H5N1) was associated with infected poultry in Hong Kong.³ Since 2003, A(H5N1) virus has emerged in East and Southeast Asia.⁴⁻⁷ Cambodia reported 56 human A(H5N1) cases with a CFR of 66%

between 2005 and 2014.^{8,9} No human cases of A(H5N1) were reported after 2014 until two cases were detected in February 2023 in Cambodia. In early October 2023, the rapid response team notified the Department of Communicable Disease Control, Ministry of Health (CDC-MoH) of two suspected avian influenza cases. A 50-year-old man from Svay Rieng Province (Case 1) and a 2-year-old girl from Prey Veng Province (Case 2)

both tested positive for A(H5N1) virus. The field epidemiologists from the national and subnational levels of the Ministry of Health (MoH) actively investigated from 9 to 23 Oct 2023. The investigation aimed to describe the characteristics of cases, identify potential linkage between the two cases, assess the outbreak magnitude, understand the local perspective and response to the outbreak.

Methods

Outbreak Setting

The investigation was conducted in two affected villages in Svay Rieng and Prey Veng provinces (Figure 1).



Case 1: Mrak Teap Village, Tros Commune, Romeas Haek District, Svay Rieng Province. Mrak Teap Village is approximately five kilometres from the nearest health center, three kilometres from the district referral hospital, and 40 kilometres from Svay Rieng Town.

Case 2: Veal Village, Smaong Khang Tboung Commune, Kamchay Meas District, Prey Veng Province. Veal Village is around five kilometres from the nearest health centre, about 10 kilometres from the district referral hospital, and about 40 kilometres from Prey Veng Town.

In the two affected villages, over 85% of households raise small-scale backyard poultry farming.

Figure 1. Location of avian influenza cases in Cambodia, 2023

Epidemiology Investigation

Medical records of both cases were reviewed by the investigation team using a case investigation form consisting of patient identifier information, clinical information, and signs and symptoms. We interviewed both family members and clinicians to collect information on the patient's medical history and exposures, such as contact with ill or dead poultry.

An active case finding was conducted at 1) the health facilities by reviewing medical records of cases with influenza-like illnesses (ILI) symptoms (i.e., having fever (≥ 38.0 °C) with either cough or sore throat) in the affected districts from 1 Sep 2023 to 9 Oct 2023, and 2) a community survey was conducted door-to-door in the affected villages. We sought information

on individuals with symptoms of ILI or who met the case definition. Those with symptoms were referred for sample collection.

Two A(H5N1) events occurred in two different provinces. A suspected case was a person who lived in Romeas Haek District, Svay Rieng Province between 26 Sep 2023 and 7 Oct 2023, or Kamchay Meas District, Prey Veng Province, between 25 Sep 2023 and 6 Oct 2023 and had a history of unprotected exposure to any infected birds, or to an environment where infected birds or visited live bird markets within seven days before the onset of symptom with a fever or history of fever and at least one of the following symptoms: cough, sore throat, dyspnea, runny nose, difficulty breathing, or headache. A confirmed case was a person who had a nasopharyngeal (NP) and oropharyngeal (OP) swab specimen that tested positive for A(H5N1) by molecular technique. A close contact was a person who had direct (distance less than two meters and duration more than five minutes) and unprotected (no personal protective equipment) contact, in a room or other enclosed space (e.g., vehicle) with a confirmed case between one day before and 14 days after the onset of symptoms of the case.

Laboratory Investigation

All suspected cases had NP and OP swabs tested for influenza A, influenza B and SARS-CoV-2 by reverse transcription-polymerase chain reaction technique. Close contacts and suspected cases were tested for antibodies for A(H5N1) virus.

Environmental Study

Five individuals were interviewed: two village chiefs, one poultry dealer from Mrak Teap Village, and two village animal health volunteers from Mrak Teap Village and Veal Village. Interviews focused on their perspectives on poultry deaths, movement, and feeding practices by using an unstructured questionnaire.

We inspected index houses (observed poultry and their surrounding environment) and households in the villages to determine if there were any ill or dead poultry. A total of 58% (67/116) of households in Mrak Teap Village and 31% (40/130) of households in Veal Village were visited. We used an unstructured questionnaire to interview households in the affected villages about ill or dead poultry.

Data Analysis

Descriptive analysis was conducted by calculating the median (1st quartile (Q1), 3rd quartile (Q3)) values for the age of close contacts and suspected cases, treated as continuous data. Frequencies and percentages were

calculated for categorical variables. Data analysis was performed using Microsoft Excel version 14. Households in villages with ill or dead poultry were mapped using QGIS software version 3.28. Qualitative data analysis was used to examine key informants' ideas and opinions, which were transformed into themes and then categorised based on different elements of the responses.

Ethics

The outbreak is a part of the public health emergency response conducted by the Cambodia Ministry of Health. The data collected went through a process of anonymization, guaranteeing that the authors had no access to any identifiable information.

Results

Description of the Two Confirmed A(H5N1) Cases

Case 1

A 50-year-old male with diabetes (reported by his son) living in Mrak Teap Village (Figure 1). The village is located in a rural area that shares a border with Vietnam. Most residents were farmers with raised small-scale poultry free-roaming in their backyards. The case prepared an ill chicken a few days before symptoms onset. On 2 October, patients developed fever, vomiting, and trembling. He was self-treated with 500 mg of paracetamol and 500 mL of intravenous fluid. On 4 October, he visited a private clinic and was referred to the district referral hospital because symptoms did not improve. The referral hospital physician referred the patient to the national hospital in Phnom Penh. The family opted for a private clinic in Vietnam, which is closer to their home. The patient was admitted to the clinic in Vietnam and diagnosed with severe pneumonia from 5–6 October. The patient's vital signs included blood pressure of 175/100 mmHg, pulse of 130 beats per minute, respiratory rate of 28 breaths per minute, SpO₂ of 92%, and body temperature of 37.7 °C. On 7 October, his condition

worsened, and he was referred to Calmette Hospital in Phnom Penh, where he died. Tests on 8 October confirmed A(H5N1) virus clade 2.3.2.1c.

Case 2

A 2-year-old girl who did not have underlying disease and lived with her grandmother at Veal Village (Figure 1). Most of the people were farmers and had free-roaming poultry around their homes. The girl played in the yard. The grandmother reported that their chickens died off every day for a week before the girl's onset of symptoms. Ill chickens that died were buried behind the house, while the ill or dead adult chickens in good condition were prepared for meals. However, ill or dead chickens in the villages were not reported to the local authorities. On 1 October, the girl developed a fever and vomiting and was treated at home by a local nurse. The patient's symptoms did not improve. The girl was transferred to a private clinic in the district. On 3 October, the girl developed coughing and difficulty breathing. On 4 October, despite the girl's deteriorating condition, her family was relocated to the National Pediatric Hospital in Phnom Penh on 5 October. On 6 October, NP and OP swab samples were sent to the NIPH laboratory and tested positive for A(H5N1) virus clade 2.3.2.1c on 9 October. The patient died with a diagnosis of septic shock and severe bronchopneumonia.

Active Case Finding and Contacts from Svay Rieng and Prey Veng Provinces

In Svay Rieng Province, the active case finding identified 51 individuals (44 close contacts and 7 suspected cases), of which 38 (70%) were male. The median age was 34 years (Q1=26, Q3=51). Most (84%) were aged ≥15 years. Twenty-two (43%) were reported as farmers. In Prey Veng Province, there were 21 close contacts. Out of 29 individuals, 15 (52%) were male; the median age was 26 years (Q1=14, Q3=39), and 21 (72%) were aged ≥15 years. Almost half were farmers (Table 1).

Table 1. Characteristics of the close contacts and suspected cases from the avian influenza events in Cambodia, 2023

Characteristics	Svay Rieng Province (n=51)		Prey Veng Province (n=29)	
	No.	%	No.	%
Gender				
Male	38	75	14	48
Female	13	25	15	52
Age in years				
Median in years (Q1–Q3)	34 (26–51)		26 (14–39)	
≤6	3	6	4	14
7–14	5	10	4	14
15–40	23	45	14	48
>40	20	39	7	24

Table 1. Characteristics of the close contacts and suspected cases from the avian influenza events in Cambodia, 2023 (cont.)

Characteristics	Svay Rieng Province (n=51)		Prey Veng Province (n=29)	
	No.	%	No.	%
Occupations				
Farmers	22	43	13	45
Healthcare providers	9	18	5	17
Students	7	14	8	28
Ivory priest	5	10	-	
Childs	3	6	3	10
Driver	2	4	-	
Animal/health volunteer	2	4	-	
Government staff	1	2	-	
Types				
Suspected cases	7	14	8	28
Close contacts	44	86	21	72
Laboratory results for SARS-CoV-2 and A(H5N1)				
PCR tests negatives	51	100	29	100
Serology testing for A(H5N1)				
Tested negative for acute serum (n=59)	44	100	15	100
Tested negative for convalescent serum after 21 days (n=48)	37	100	11	100

Laboratory Detection for Close Contacts and Suspected Cases

A total of 15 suspected cases (7 from Svay Rieng Province and 8 from Prey Veng Province) had symptoms, including fever (100%), coughing (87%), runny nose (47%), and sore throat (27%). Laboratory test results for all 15 cases showed absence of SARS-CoV-2 and influenza A and B. Regarding the antibodies of A(H5N1), a total of 59 acute serum and 48 convalescent serum samples were collected and tested negative antibodies (Table 1).

Environmental Study

Animal health volunteers living in the villages have unspecified roles; however, they assist the community by supporting animal husbandry, particularly by providing basic care to families with ill or dead poultry when needed. The animal health volunteers from Mrak Teap and Veal villages reported that poultry deaths occurred in early September 2023, including at the house of cases 1 and 2. This pattern of poultry death spread from one household to another in their village. The villagers were unaware of avian flu. They cooked and consumed the ill or dead chickens for daily meals and even shared them with neighbouring households. An animal health volunteer reported his chickens tested positive for A(H5N1) virus during the outbreak. He stated that the villagers reported, "Poultry deaths occurred every year, and people typically prepared them as daily meals instead of buying food from the market". The villagers became

aware of eating ill or dead chickens after the death of Case 1.

Most households in the affected villages who had dead poultry reported that poultry started dying in early September 2023 until cases were detected. The ill or dead chickens were cooked for meals. As noted by the villagers, "Every year chicken died, and people usually cooked them for meals". If the poultry died overnight, the household in the village typically buried it, as they considered it poor-quality meat. However, if the bird was ill or had recently died, the majority were cooked for food.

According to the poultry dealer in Svay Rieng Province, the dealer bought chickens from the village and surrounding areas and sold them to market in Phnom Penh. He emphasized that all the chickens were healthy and had never been removed from their cages. He reported, similarly to other responders, that chicken deaths were a normal occurrence, which happened every year, and that ill or dead chickens in good condition were often prepared for meals.

Village chiefs from Mrak Teap and Veal villages reported that most villagers had moved to other provinces for work, and only children and the elderly resided in the villages. A villager reported that poultry had died in early September 2023. These poultry were raised as free-roaming chickens in the backyards of households. The poultry dealer comes from Romeas Haek District in Svay Rieng Province, although the exact village of origin of the chickens was uncertain. A lack of awareness among local authorities and the

community regarding A(H5N1) virus transmission between poultry and humans was evident. Poor practices in the community related to the handling of ill or dead poultry were identified. From our investigation, there was no epidemiological linkage between Case 1 and Case 2. There was uncertain information about the poultry transmission chain between both affected villages.

We conducted direct observation in Mrak Teap and Veal villages from 9–10 Oct 2024. In Mrak Teap Village, 19 (28%) of 67 households reported ill or dead poultry. Villagers in this area allowed their poultry to roam freely for feeding. Similarly, in Veal Village, 18 (45%) of 40 households reported ill or dead poultry, with free-roaming feeding practices observed (Figure 2).

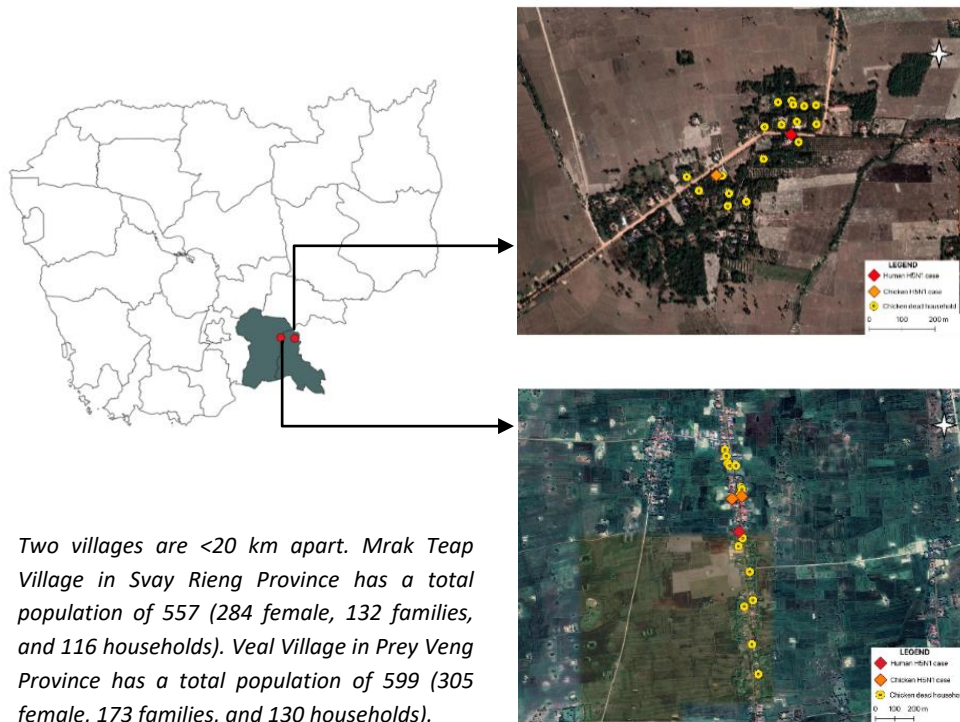


Figure 2. Geographical distribution of the two avian influenza cases in Cambodia, 2023

Action Taken for Control Measures and Case Management

The active case finding identified 15 suspected cases with ILI symptoms during the follow-up period in both villages; however, none of the samples tested positive for pathogen (Table 1). Patients with mild ILI symptoms who did not meet the case definition were recommended for home quarantine and provided basic care and/or treatment with oseltamivir. We conducted active surveillance to find additional cases at health facilities and in the community. We set up a mobile health post in both affected villages, starting from the first detected case, and if new confirmed cases were identified, we began a new 14-day follow-up following the case definition. If a person met the case definition, a specimen was collected at the health facility level and sent to a laboratory for testing. Awareness about A(H5N1) infections, including the risk of transmission and signs and symptoms, was raised among healthcare workers and the community during the response.

Discussion

Two fatal cases were confirmed positive for A(H5N1) virus with clade 2.3.2.1c; a 50-year-old man and a 2-year-old girl. Two cases had direct contact with ill or dead poultry a few days before the start of symptoms. Both cases were primarily treated by local nurses, resulting in delayed admission to the hospital. A high CFR is associated with delayed hospital admissions.¹⁰ There was a lack of oseltamivir stock at the district level for response during the investigation. The A(H5N1) virus has been sporadic in humans in Cambodia since 2005.¹¹ There was no epidemiological linkage between the two cases evidenced from the investigation. In both households, there were poultry die-offs in their village, and cases had direct contact with ill or dead chickens in their respective houses. Delayed treatment in both cases may have played an important role in the outcome. Early treatment with oseltamivir is advised, as starting it more than 48 hours after symptoms appear can lead to poorer outcomes.^{12,13} The 50-year-old man died seven days

after his onset date. This indicates he was diagnosed and admitted late, leading to delays in receiving proper treatment at the hospital. The 2-year-old girl died nine days after the onset. It might be appropriate treatment was not given during the onset period, and there was a delay in diagnosis and admission. A reported in Indonesia, delays in providing proper care to human cases of A(H5N1) are associated with delays in diagnosis.¹⁴ Case 1 butchered dead chickens a few days before symptoms onset. Case 2 had a history of exposure to ill or dead chickens at her house, and chickens were also cooked for meals, similar to Case 1, but it is uncertain whether the girl consumed the chicken meat. Both cases had direct contact with dead chickens, similar to two cases with confirmed A(H5N1) in the Prey Veng in February 2023.¹⁵ All specimens collected from close contacts and suspected cases living in the affected villages tested negative for influenza A and B and SARS-CoV-2. In 2005, Thailand reported 12 people died from A(H5N1) infection; two of the deaths had no direct contact with birds, indicating they may have contracted the virus from another person while transmission between poultry to humans.^{16,17} In rural Cambodia, people raise small-scale backyard poultry farms where chickens forage for their own feed or are provided with grains of rice once or twice a day. Our study reveals that in both affected villages, the human cases of A(H5N1) infection were linked to ill or dead birds carrying the virus or environments contaminated with A(H5N1) virus as evidenced by samples from ill poultry with A(H5N1) viruses detected. Since 2005, cases of human infection due to poultry-to-human transmission have been sporadically reported in Cambodia. A study among women showed that half lacked sufficient knowledge and failed to follow the right practices for dealing with the high-pathogenic avian influenza virus.¹⁸ Another study in Vietnam compared a group at high environmental risk for A(H5N1) with a non-exposed group, revealing that less than 40% of participants did not seek medical attention either due to flu-like symptoms or out of fear.¹⁹ However, there was a lack of information regarding the movement of poultry in the villages, particularly concerning ill or dead poultry. The report from the local key informants highlights uncertain information about the reasons behind chicken ill and deaths in the villages.

Limitations

Several limitations were identified during the investigation. There was a lack of manpower to respond to the outbreak at the national level due to two overlapping events that occurred over the weekend. Additionally, there was no information available

regarding poultry movement or exchange from Vietnam, which borders the affected provinces. There was also a lack of information on wild bird movement and its potential relationship with domestic birds, making it difficult to identify the source of A(H5N1) virus infection in the villages.

Conclusion

Two fatalities have been confirmed as a result of A(H5N1) infection, with the 2.3.2.1c. Both cases presented with severe pneumonia and had delayed treatment. Both cases were exposed to ill or dead chickens at their homes at the same time in late September. No epidemiological linkage between the two cases and no secondary cases were identified. We identified a lack of A(H5N1) awareness in the two communities for both human and animal health practices. Control measures included active case detection, enhanced surveillance, and medical management of persons with ILI were implemented.

Recommendations

Our findings suggest that joint multisectoral collaboration, with coordination and communication among human, animal and environmental health sectors using the One Health approach, is necessary for responding to H5N1 outbreaks. Furthermore, we recommend conducting daily coordination meetings among stakeholders during investigations to facilitate planning, coordination, and the exchange of information. Strengthening the surveillance system at both health facilities and community levels is crucial to capture any unusual events which trigger an early response to the H5N1 outbreak in the country. The MoH should allocate resources for antiviral medication and antiviral oseltamivir and ensure its availability at the provincial level. Additionally, risk communication and community engagement can play an important role in preventing further bird flu outbreaks by increasing awareness of the A(H5N1) virus among healthcare professionals and the community.

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Conflicts of Interests

The authors declare that there is no conflict of interest.

Suggested Citation

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