Outbreak of Highly Pathogenic Avian Influenza in Commercial Poultry Farms, Kwahu-West Municipality, Ghana

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ABSTRACT

Introduction: Avian Influenza is a highly contagious viral infection that mainly affects domesticated birds. The Kwahu-West Municipal Veterinary Office was notified of sudden deaths of birds on a poultry farm in June-2018. Seven days later, a second farm sited 100 meters from the index case-farm also recorded bird deaths. We investigated to confirm the causative agent, identify its source, and implement control measures. **Methods:** We conducted a survey of six farms and human contacts in the area. A suspected case-farm of HPAI was any farm in Nkawkaw with sudden death of bird(s), with or without clinical signs of HPAI from June 1 to July 10, 2018. Six bird carcasses were collected on affected farms for laboratory confirmation of the causative agent. We interviewed owners of affected farms and assessed the farm environments, as well as clinical status of human contacts. **Results:** A total 2,280 birds were affected on two adjacent farms. HPAI-H5N1 was confirmed for all samples investigated. The index case-farm with 1,438 birds, reported 30 bird-deaths, 24 hours after receiving birds from an HPAI-H5N1 confirmed farm in Boankra in the Ashanti Region, 84 km away from the index case-farm. The second case-farm recorded bird deaths 7-days later. Both farms operated aluminum sheet-roofed metallic-mesh pens without fencing. All twenty-three human contacts were asymptomatic of flu, after 14-days follow-up. **Conclusion:** This HPAI-H5N1 outbreak was likely imported from the Ashanti Region of Ghana, due to lax livestock movement regulations and biosecurity measures. Disinfection and depopulation exercises effectively controlled the outbreak. We recommend strict implementation of biosafety measures on farms and at entry points in the district.

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Introduction

Avian influenza (AI) is a viral disease that infects bird species, with varying levels of severity, depending on the virus strain involved. The viruses with H5 or H7 surface proteins cause the most severe form of AI, and are described as the highly pathogenic avian influenza (HPAI) [1]. Wild birds are the common vectors of the AI viruses. The virus is however highly contagious among domesticated birds, including chickens, ducks, and turkeys. The infection occurs when a bird directly comes in contact with other infected birds, or indirectly through contact with contaminated water, feed, and surfaces. The HPAI may cause disease affecting multiple internal organs, with a 48-hour mortality rate ranging between 90-100% [2].

According to the World Health Organization (WHO) and International Organization for Animal Health (OIE), human infections with AI viruses have been sporadic globally, a majority of which originated from Asia [3,4]. However, person-to-person transmission of AI viruses is rarely reported. The risk of AI infection of humans remains high if biosafety measures are neglected, and is mainly related to outbreaks among poultry [5]. Most of the AI outbreaks in humans result from close contact with sick domesticated birds [6]. The ability of AI viruses to cross the species barrier is high because they are constantly evolving through mutation and genetic re-assortment leading to the emergence of new subtypes, thereby posing significant threat to both animal and human health [7].

The AI viruses that have crossed the species barrier to infect humans include A(H5N1), A(H5N6), A(H7N4), A(H7N9), and A(H9N2). Since its emergence in China in 2013, the outbreaks of A(H7N9) has infected more humans (excess of 1500 human cases) than any of the other types. However, A(H5N1) has caused the largest number of cases of severe disease and death in humans [2,4]. According to the WHO, more than 860 human cases of A(H5N1) virus were reported from 17 countries globally, with more than 50% case fatality rate between January 2003 and December 2019 [4].

In many African countries, outbreaks of HPAI A(H5N1), A(H5N2) and A(H5N8) in poultry have been confirmed [3]. The first of the infection in Africa occurred in Nigeria in 2006, which subsequently spread quickly to at least 17 other countries on the continent by 2017 [7,8]. Only three African countries including Djibouti, Nigeria, and Egypt reported confirmed human cases of HPAI A(H5N1) on the continent since its first confirmation in 2006 [7]. The first human case of HPAI A(H5N1) was confirmed on March 20, 2006 in Egypt. By November 2010, more than 100 human cases were confirmed in Egypt with majority of the confirmed cases linked to contact with poultry kept in backyard farms [9]. Globally, Egypt recorded the highest number of 346 confirmed human cases by the end of 2015 [7].

Ghana reported its first confirmed HPAI A(H5N1) outbreak in poultry in 2007 in three regions including Greater Accra, Volta, and Brong Ahafo Regions. Until 2015, no outbreaks of HPAI were reported, with the Greater Accra Region first confirming cases of H5N1 in April 2015. Subsequently, five of the sixteen regions in Ghana, also recorded confirmed cases by June the same year, affecting both commercial and free-range poultry farms and leading to losses of approximately 100,000 birds [10]. Between 2017 and 2018, the A(H9N2) strain was identified in outbreaks in poultry in the Ashanti and Brong-Ahafo Regions of Ghana [11]. The spread of AI in Ghana has mainly been attributed to poor biosafety practices on farms and poor regulation of movement of live poultry [10]. No human cases of HPAI have been confirmed in Ghana since its introduction in 2007 [7].

On June 21, 2018, a farm owner notified the Veterinary Office in the Kwahu West Municipality of the death of birds on his small-scale commercial poultry farm at Nkawkaw. A different small-scale commercial farm located about 100 meters from the index-case farm also started recording deaths of birds 7 days later. Upon visiting the farms, the Municipal Veterinary Officers suspected a HPAI outbreak. Based on their notification, the Eastern Regional Health Directorate through the Municipal Health Management Team (MHMT), constituted a team to respond to the outbreak. We investigated the outbreak to confirm the causative agent, identify its source, assess risk factors for spread, and implement control measures.

Methods

Outbreak setting

The outbreak investigation was conducted from June 25 to 30, 2018 in Nkawkaw, capital of the Kwahu West Municipality in the Eastern Region of Ghana **Figure 1**. This was the first reported AI outbreak in the municipality. The municipality is one of the twenty-six (26) districts in the Eastern Region of Ghana [12]. The projected population for Kwahu West Municipality is about 115000 in 2018. About half of the population live in the rural areas [13]. Nearly 44% of households in the municipality are engaged in agricultural activities including crop farming, livestock rearing and fish farming.

Livestock rearing is the second most important agricultural activity accounting for about 25% of all agricultural activities. About 61% of livestock kept are birds with 95% being chicken [12]. The birds are mainly raised on a small-scale backyard basis with few commercial farms. Farm capacities range between 100 and 5,000 birds. About 47,000 commercial layers and 70,000 domestic or rural birds were raised in the district in 2018. There were three live bird markets located within the Nkawkaw Central Market near the roadside, where trading of birds and eggs is mainly done [14].

Only one veterinary clinic provides veterinary services to livestock farmers in the municipality. Fifty health facilities provide human healthcare services in the municipality. There is no routine surveillance for Influenza-like illnesses in the Kwahu West Municipality.

Study design

We conducted a survey involving interviews with poultry farmers, human contacts of affected farms and veterinary personnel. The interviews with the farmers were conducted using a structured questionnaire detailing farm demographic characteristics, flock history, farm biosecurity, farm management practices, contacts information and other risk factors to HPAI (S2 File). We actively searched for case farms within a 6 kilometer radius of the index-case farm and identified six farms for assessment. We defined a suspected HPAI case-farm as a poultry farm (domestic or commercial) in Nkawkaw with sudden death of birds, with or without clinical signs of HPAI on from June 1 to July 10, 2018. Six (6) farm owners/managers were interviewed. This includes the managers of the two affected and four other unaffected farms. All twenty-three contacts of the case farms were followed up daily for 14 days. The contacts were interviewed with a structured questionnaire to assess for any clinical manifestations of influenza. We defined a primary human contact as any person who has had physical contact with the case farms, and/or the birds or eggs of case farms from June 15 to 26 2018. A secondary human contact was defined as any person exposed by physical contact with a primary contact. We also conducted environmental assessment of the immediate vicinity of both farms to identify risk factors to the spread of the AI infection including the presence of wild birds and water bodies close to pens as well as the biosecurity measures practiced on the farms.

Laboratory investigations

Six whole bird carcasses were collected from the two affected farms. The fresh carcasses were transported in appropriate double-layered plastic bags inside a leak-proof plastic container to the Accra Veterinary laboratory within four hours of collection. In this Biosafety Level 3 laboratory, viral ribonucleic acid was extracted from cloacal and oropharyngeal swabs taken from the carcasses and diagnosed using Reverse Transcription Polymerase Chain Reaction (RT-PCR). The samples were tested for influenza viruses adhering to all standard protocols [15]. Appropriate personal protective equipment were donned in handling the specimen.

Data analysis

We performed descriptive analysis of the outbreak data by person, place and time. We calculated overall mortality rate and farm specific mortality rates. Continuous variables including age of human contacts were expressed with appropriate measures of central tendency and dispersion. The results were presented as frequencies and relative frequencies in tables and graphs. We drew a column graph to describe the magnitude and the course of the death of birds. We used quantum geographic information system tools [16] to map the poultry farms in the outbreak setting. Data was analyzed using Stata version 15.0. The interviews with the affected farm owners were transcribed and coded deductively,

noting the dates and key events that occurred during the outbreak. The results were presented as a narrative supported by a flow graph of dates of key events during the outbreak.

Coordination

The response to the outbreak was organized through multi-sectoral collaboration in a One Health approach. Field epidemiologists from the Ghana Field Epidemiology and Laboratory Training Programme supported the Municipal Health Management Team to respond to the outbreak. The team constituted by the Kwahu West Municipal Health Management Team included staff of the Ghana Health Service, Veterinary Services Department, and Municipal Assembly. The Municipal Fire Service Department, National Disaster Management Organization, and Ministry of Food and Agriculture supported the investigation team in the response to the outbreak.

Availability of data

All relevant data are within the manuscript and its Supplemental Materials files.

Supplemental Material

S1 File: Data collection tools used in outbreak investigation.

Ethical considerations

The investigation was considered a response to a public health emergency by the Ghana Health Service and Veterinary Services Department and therefore did not receive formal review by Ethical Review Committees. We sought and obtained permission from the Eastern Regional Health Directorate, Eastern Regional Veterinary Services Directorate, Kwahu West Municipal Assembly, Kwahu West Municipal Veterinary Department and Kwahu West Municipal Health Management Team before commencement of the investigation. All respondents provided consent and were assured of confidentiality. We anonymized all information collected on the affected farms and human contacts and stored them securely as confidential records. The use of a water-based foam in the depopulation exercise allowed for a humane and most effective way of euthanizing the birds on the affected farms.

Results

Outbreak characteristics

A total of 2,188 birds were present at the time of the outbreak on the two affected farms **Table 1**. The first Case farm had 1,438 birds (1400 chicken and 38 ducks) while Case farm 2 had 750 chickens at the time of the outbreak. In all, 189 dead birds were reported. The overall mortality rate of birds was 9 per 100 birds. The farm specific mortality rates were 13 per 100 birds for Case farm 1 and 2 per 100 birds for Case farm 2.

The interviews with affected farm owners revealed that the owner of Case farm 1 bought 650 live birds (layers) from a farm in Boankra in the Ashanti Region of Ghana in three consignments of 200, 250 and 200, and transported these birds without any inspection. The birds bought were kept in a separate pen from the farmer´s existing flock. After the owner of the first affected farm (Case farm 1) observed thirty (30) dead and ninety (90) sick birds in his farm 24 hours after receiving the last consignment of birds bought, he returned these birds back to the originating farm for replacement, also without any inspections. Although the birds were replaced, more deaths of birds were reported among the birds that were bought. The farmer sold some of the birds that looked healthy in the live bird market in the district. Subsequently, more deaths of birds were reported among his existing flock and, on a nearby farm. The farmers eventually reported the bird deaths to the Municipal Veterinary Office six (6) days later after they tried self-treating without success. **Figure 1** illustrates the key events in the outbreak.

The graph **Figure 2** below illustrates an incremental growth in the number of dead birds recorded on the affected farms, after the initial multiple deaths (30 birds) reported on Case farm 1, until the depopulation exercise conducted on June 26.

We found an intersection in the two case-farms within a 200m buffer area plotted around the case and non-case farms **Figure 3**. Figure 3 illustrates a 6 kilometer radius about the Case farm 1 and all other bird farms within the radius.

Characteristics of contacts

In all, 23 human contacts were identified including 20 primary contacts (87%) and 3 secondary (13%) contacts. About 53% (12/23) of the contacts were males. The median age of the contacts was 25 years (range = 5 to 64 years), with majority 65% (15/23) being resident at Amanfrom, an area close to the affected farms. All contacts were exposed between June 15 and 26, 2018 inclusive. None of the 23 contacts identified were symptomatic for influenza during the outbreak period and after follow up.

Laboratory results

RT-PCR results confirmed HPAI-H5N1 for all the six whole bird carcasses tested using universal primers for AI viruses H5 and H7.

Findings from environmental assessment

Both case farms were sited in a peri-urban area, but none of them was fenced. The two affected farms are about 100 meters apart. Case farm 1 had three pens used for poultry with sizes; 40 by 40 feet, 30 by 40 feet, and 25 by 20 feet. The farm also housed goats, sheep, and rabbits. The structure of the pen comprised concrete foundation with metallic mesh for walls and roofed with aluminum sheets. Each pen had feeding and water troughs. The water troughs were improvised from cooking oil gallons that were cut. Two of the pens lie directly on the side of a pathway that residents living behind the farm, use. There was a well dug on the compound from which water is drawn for domestic use and watering the animals. There was a small pen in-between the three pens where sick birds were kept for observation. There was a stream with a wooden bridge approximately 20 meters northwards from the Case farm 1. Wild birds were seen perching on the pens. The pens housing goats, sheep and rabbits were directly adjacent the pens with birds. The litter produced were stored in sacks by the side of the pens. The beddings were also not regularly changed.

Case farm 2 had two pens used for raising only poultry birds. The pens measure about 25 by 30 feet and 30 by 40 feet respectively. The pens had concrete floors and foundation. Metallic mesh was used to cover the open area from the foundation up to the roofing. The pens were roofed with aluminum sheets. Behind one of the pens was a manhole. No wild birds were seen on Case farm 2. The maintenance of the pens was similar to what was observed on Case farm 1. Human movements were reported between the two case farms.

Discussion

The highly pathogenic avian influenza (HPAI) virus generally causes severe outbreaks in poultry population. However, occasionally HPAI infect humans exposed to infected poultry. The HPAI-H5N1 outbreak confirmed in this study have been implicated in previous outbreaks in many African countries including Nigeria, Egypt, Côte d´Ivoire, Burkina Faso, Niger and Cameroun [10,17]. In Cameroun, two strains of HPAI; H5N1 and H5N8 were isolated in outbreaks that occurred on commercial farms in 2016 affecting birds with mortality rates ranging between 8% and 96%. In Ghana, previous outbreaks of HPAI (H5N1 and H9N2) have been reported in many regions since the first outbreak in 2007 [10,11]. The circulating H5N1 and H9N2 strains in Ghana have been reported to be homologous to that observed in Nigeria, Burkina Faso and other West African countries, suggesting a potential cross-border contamination of bird species [10,11]. The low mortality rate; 9% and 13%, observed on Case farms 2 and 1 respectively in the current study, are similar to those previously reported in the West Africa region [18]. This may be explained by the similarity in the strains identified in the region.

Our study revealed concerning findings of how birds were moved between farms without inspection by regulatory bodies at the entry points of the district. Even more concerning was how one of the affected farmers moved dead and sick birds back to the farm (source) in the neighboring region for replacement without detection. These lapses contributed to the importation and subsequent spread of the HPAI outbreak in the affected farms. Similar links with poor regulation, were shown in previous HPAI outbreaks [9,10,17,19]. The key sources of the transmissions of HPAI previously reported includes unregulated movements of poultry and eggs dealers from one farm, market, or town to another without appropriate preventive care as well as poor biosecurity measures on farms and live poultry markets [17]. In Nigeria, similar biosecurity non-compliances have been implicated in H5N1 and

H5N8 outbreaks occurring between 2014 and 2017 [7]. Similar lapses in biosecurity measures were implicated in HPAI outbreaks globally especially in Europe and Asia [20-23]. The poor regulation of movement of animals have a potential to affect trade negatively in the future.

Owing to the difficulties in restricting the movement of poultry in many settings, some control strategies implemented in previous HPAI outbreaks in Egypt include mass vaccination, surveillance and preemptive culling of infected birds [9]. In Ghana, control measures utilized in previous HPAI outbreaks involved depopulation of all birds on affected farms, disinfection of affected farms, and restricted movement of poultry and poultry products after which, active influenza surveillance is initiated among birds, domestic poultry, and the human population [10]. These measures were equally effective in the current outbreak response. The depopulation of all birds on affected farms, disinfection of farms and live bird market, and ban on sale and movement of live birds during the current outbreak response, contributed to a successful control of the outbreak. Similar control measures have been used in other settings with success [9,21]. The collaborative efforts of both human and animal health personnel through a One Health approach as well as the legislation and enforcement by the municipal assembly through the municipal coordinating council also contributed largely to containment and prevention of further spread of the infection in the present outbreak.

Although human cases of HPAI have been reported in 15 different countries following outbreaks in birds including in Hong Kong, Thailand, Turkey, Egypt and Cameroon [17,21,24-26], none of the human contacts in our study developed flu-like symptoms after two maximum incubation periods follow-up. Almost all of the human cases reported in previous AI outbreaks were caused by zoonotic transmission from poultry and disproportionately affects women and their children. This is because women and children were observed to be more exposed when nursing birds raised in backyard farms compared to adult males who are less involved on the backyard farms [9]. Transmission generally depends on every country´s own dynamics associated with raising poultry and other intrinsic immunologic susceptibility to the infection. Nevertheless, transmission of HPAI from poultry to humans continues to be rare, despite frequent and widespread close contact between humans and affected poultry [27]. However, our study could not assess how and why the human contacts were asymptomatic after follow-up.

The effects of AI outbreaks are often devastating for farmers. Generally, the economic consequences of these outbreaks are severe, since it results in culling of all affected birds and their eggs [28]. To alleviate the impact of losses suffered on farmers and encourage farmers to report bird deaths, payment of financial compensation to farmers is common in some settings including in Egypt [9] and the United States of America [29]. Although compensation was not paid to the affected farmers in the present outbreak, the farmers were educated and counselled to prevent future outbreaks.

Public Health Actions

The affected farmers were informed of laboratory confirmation of HPAI-H5 on Saturday June, 23 2018. The farmers were educated on the essential biosecurity measures to adopt to mitigate future occurrences. They were sensitized on the need for regular hand washing with soap and running water after attending to the poultry. The health facilities within the municipality were also placed on high alert for influenza-like illnesses and any unusual events.

The Municipal Assembly initiated a ban on the sale and movement of birds from affected farms. Similarly, a ban on the sale of live birds at the Nkawkaw market was also instituted. A depopulation exercise was conducted on June 26, 2018. A pit of about 3 meters high was dug with an excavator and the birds transported in a container to the pit together with all the eggs found on the farms. Carbon dioxide foam concentrate was sprayed on the birds in the pit before they were covered with a soil layer of about 2 meters. Disinfection of the live bird market was conducted on June 27, while the infected farms were disinfected on June 30, 2018. The farms were cleared of remaining litters and beddings and the decontamination of the pens done using Izal solution.

Conclusion

An outbreak of HPAI subtype H5N1 occurred on two adjacent farms located in Nkawkaw in the Kwahu West Municipality between June 16 and 26 2018. The source of the outbreak was probably birds purchased from a commercial farm in Boankra in the Ashanti Region of Ghana by the owner of Case farm 1 on June 15 2018. The outbreak was confined to the two farms in the affected municipality. Rapid response to the outbreak involving depopulation and disinfection of affected farms and live bird market, ban on sale of live birds and eggs in the markets and health education to the community helped in controlling the outbreak and preventing more cases. The implementation of strict monitoring and certification of livestock before, during and after movement in the municipality, sensitization of the security services on biosafety measures at various entry and exit points in the municipality, and regular and routine inspection of poultry farms by veterinary personnel are justified measures to prevent future occurrences.

What is known about this topic

- Previous research has highlighted the devastative effect of avian influenza as pathogen causing substantial losses to farmers and its zoonotic potential;
- The focus of control strategies includes depopulation of all birds on affected farms, disinfection of affected farms, and restricted movement of poultry and poultry products after which, active influenza surveillance is initiated among birds, domestic poultry, and the human population.

What this study adds

- This study has shown reinforced the essence of routine movement control of livestock in any country;
- It has highlighted some of the weaknesses in veterinary surveillance which permitted the transportation of birds from a farm with an outbreak, across regional boarders resulting in an importation of infection to an area previously without infected birds;
- This study applied the previous knowledge on controlling of avian influenza outbreaks in poultry;
- In addition to this, our study has shown the value a multisectoral collaboration in a One Health approach, involving local authorities, human and animal health providers and livestock owners, in controlling an outbreak. The collaboration between sectors helped in the timely response and control of this outbreak thereby limiting its spread to nearby farms and human contacts.

Competing interests

The authors declare no competing interests.

Authors' contributions

Conception and design: FSN BBK ED PKM ED TW DKA EK. Administrative support: JA BBK DKA EK. Provision of study materials: BBK DKA EK. Collection and assembly of data: FSN BBK ED PKM ED TW JA. Data analysis and interpretation: FSN BBK ED PKM ED. Manuscript writing: FSN BBK ED PKM ED TW JA DKA EK. All the authors read and approved the Final manuscript version.

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Tables and figures

Table 1: Distribution of exposed poultry birds by farms in Nkwakaw, Kwahu West Municipality

Figure 1: Flow diagram of key events that occurred between June 15 and 26 2018 during the Highly Pathogenic Avian Influenza Outbreak, Nkwakaw, Kwahu West Municipality, June 2018

Figure 2: Number of bird deaths by day of death during the Highly Pathogenic Avian Influenza outbreak in Nkwakaw, Kwahu West Municipality, June 2018

Figure 3: Distribution of Highly Pathogenic Avian Influenza infected and non-infected farms about the index case farm

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Table 1: Distribution of exposed poultry birds by farms in Nkwakaw, Kwahu West Municipality		
Bird type	**Case farm 1**	**Case farm 2**
Chicken	1400	750
Ducks	38	0
Total	1438	750

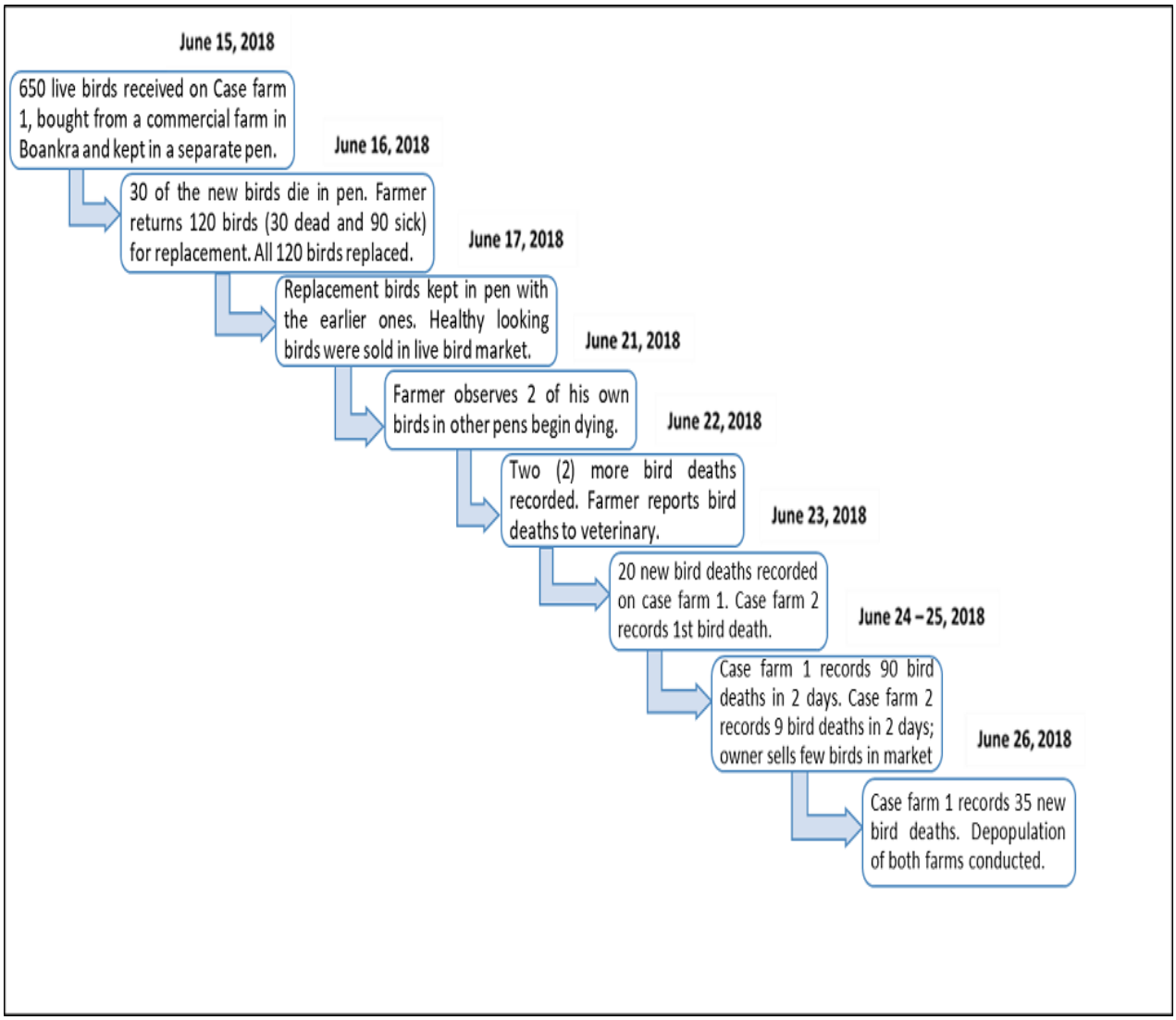

Figure 1: Flow diagram of key events that occurred between June 15 and 26 2018 during the Highly Pathogenic Avian Influenza Outbreak, Nkwakaw, Kwahu West Municipality, June 2018

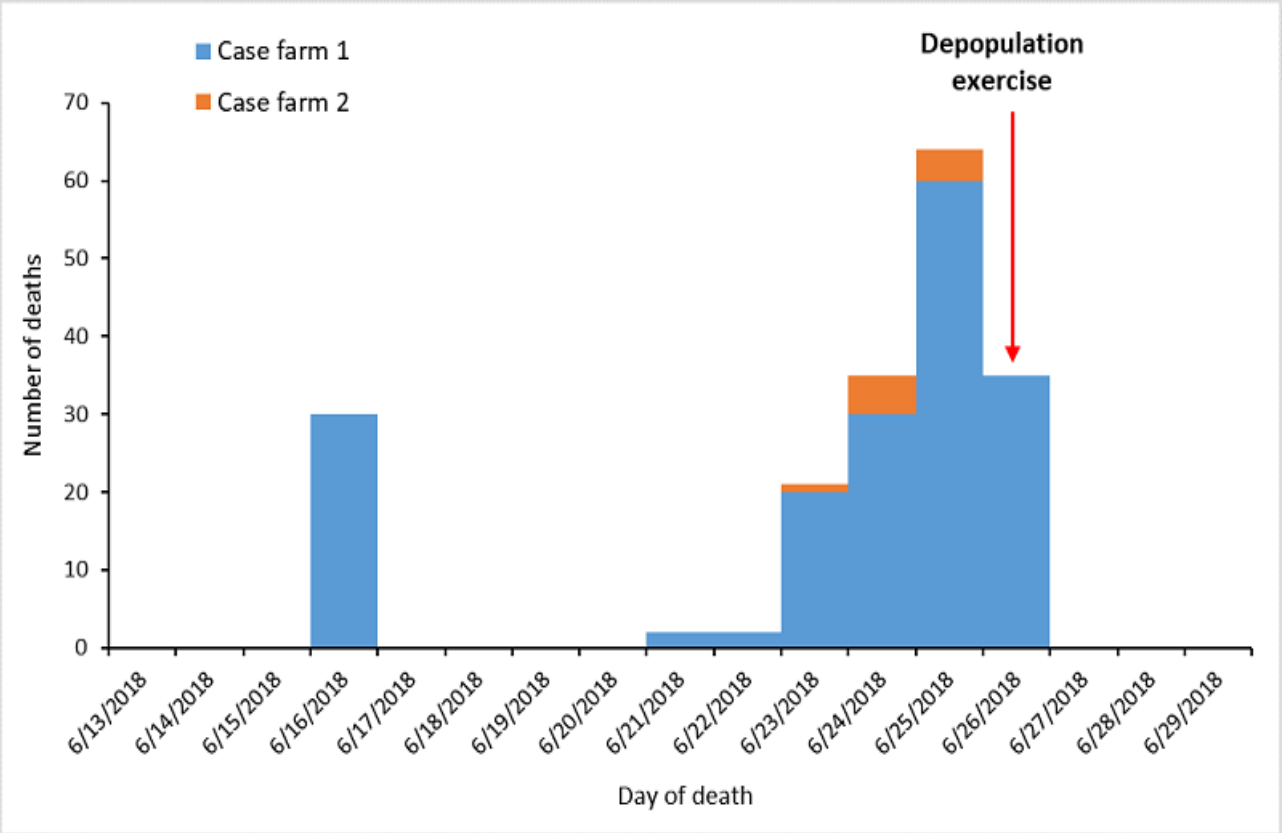

Figure 2: Number of bird deaths by day of death during the Highly Pathogenic Avian Influenza outbreak in Nkwakaw, Kwahu West Municipality, June 2018

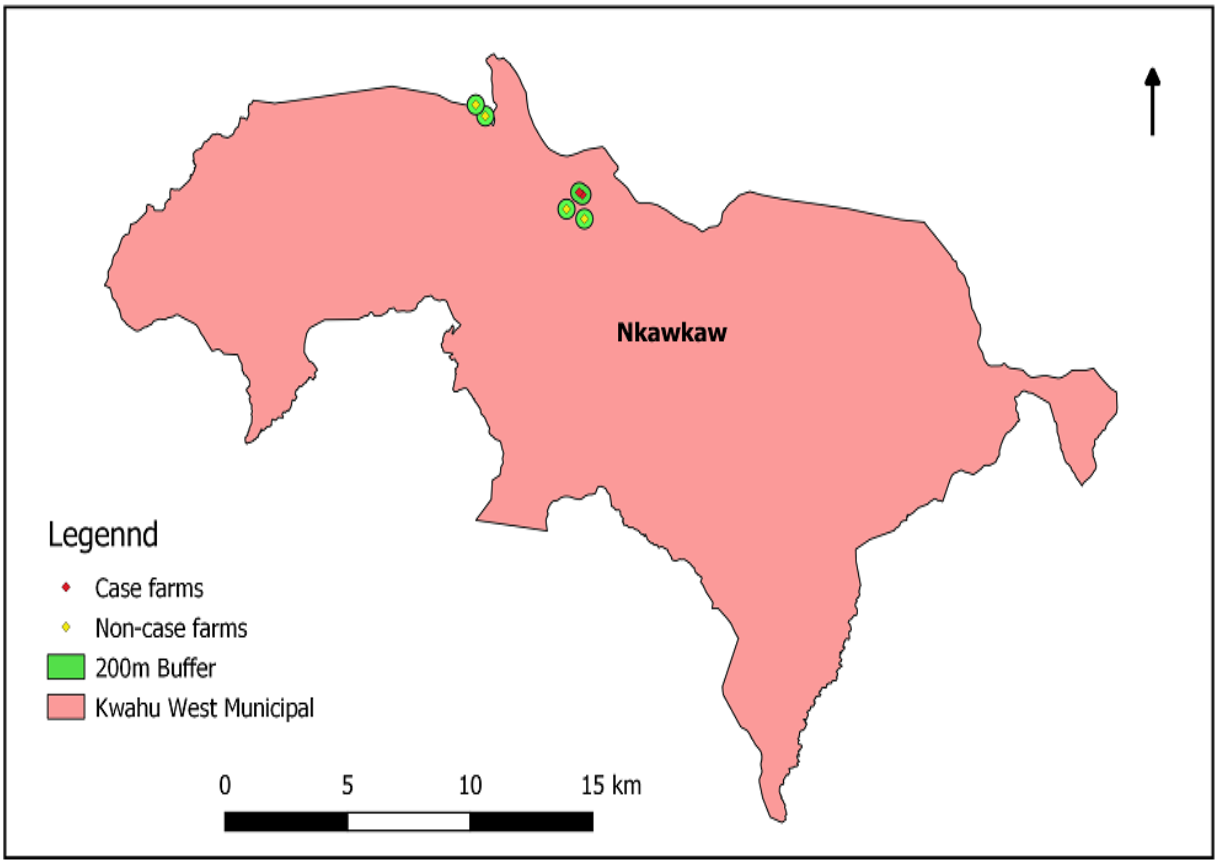

Figure 3: Distribution of Highly Pathogenic Avian Influenza infected and non-infected farms about the index case farm