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Surveillance on avian influenza and new castle disease in rift valley water body areas of Ethiopia

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Abstract

HPAI (High Pathogenic Avian Influenza) is one of the most serious public-health threats to arise from the animal reservoir. The diseases can kill up to 90% of a flock and generate epidemics that can spread quickly, wreaking havoc on the poultry business and putting severe trade restrictions in place. New Castle disease (NCD) is also a persistent threat to the poultry business and is a limiting illness for poultry farmers around the world. It is one of the most serious diseases in Ethiopia, with high mortality and huge economic losses. For this and the like purposes risk-based surveillance was conducted in selected sites in Oromia, Amhara, and Southern Nation Nationality Peoples Region (SNNPR) rift valley water body areas and 5-10 km radius districts from January to April 2019. The goal was to assess the presence or absence of Highly Pathogenic Avian Influenza (HPAI) and New Castle Disease (NCD) in migratory birds and domestic chicken in water body areas, as well as to assess the level of community awareness in the study areas.

Total 6866 samples were collected, including 819 sera samples and 1836 tracheal and cloacal swab samples from backyard domestic chickens within a 5-10 km radius of rift valley lakes, and 4211 fecal droplet samples from wild/migratory birds. Avian influenza serum samples tested by HA/HI and Fecal droplet samples tested by RT PCR came back negative. The results for NDV swab 1336 samples tested by RT PCR were 182 samples positive, and 819 samples tested by HI were 72 samples positive. In the public health aspect, a questionnaire survey was completed by 180 members of the respective community. As a result, the respondents have a solid understanding of the migratory birds that visit their area.

Keywords: HPAI; HI; NCD; Risk based surveillance; RT-PCR.

Introduction

HPAI (High Patogenic Avian Influenza) is one of the most serious public-health threats to arise from the animal reservoir. The diseases can kill up to 90% of a flock and generate epidemics that can spread quickly, wreaking havoc on the poultry business and putting severe trade restrictions in place [1]. Newcastle disease (ND) is also a persistent threat to the poultry business and is a limiting illness for poultry farmers around the world. It is one of the most serious diseases in Ethiopia, with high mortality and huge economic losses [2].

The worldwide expansion of Avian Influenza (AI) viruses, particularly certain highly virulent AI viruses of the H5 and H7 sub types, has put the livelihood of small rural chicken operations, which were previously primarily endangered by New Castle Dis-

ease (ND) viruses, at peril. On a global scale, the presence of these two illnesses poses a threat to industrial poultry-farming methods as well as free-range operations. Because these diseases have certain characteristics, such as high flock mortality and specific clinical and pathological findings, they can be readily misdiagnosed or confused with one other, as well as with other viral or bacterial diseases [3].

The incidence of Avian Influenza (AI), bird flu has been a worldwide cause for concern since 1960s. Poultry farmers of poor households in developing countries, especially in Sub-Saharan Africa has been hit the worst by the outbreak of this illness [4]. Between 2013 and 2017, 12 distinct influenza A subtypes were reported in various places throughout the world. Europe had the most viral diversity (7 subtypes), followed by Asia and the Americas (6 subtypes each), and Africa (4 subtypes) (3 subtypes). H5N1, H5N2, and H5N8 were the most widely distributed subtypes, with cases documented in four of the five regions [5,6].

In January 2018, eighteen new HPAI outbreaks in domestic birds were reported in Asia, encompassing four different subtypes. The Asian lineage HPAI/H5N1 virus was still being found in poultry and wild birds in numerous Asian and African nations. Bangladesh and Cambodia have lately suffered new H5N1 epidemics in poultry. In Asia and Africa, the virus has become enzootic, causing outbreaks in poultry and sporadic human infections [6].

Avian influenza infection in poultry has recently been reported in a few eastern African nations, and there is a substantial danger of transmission to Ethiopia via migrating birds, diseased poultry, and poultry products. In addition, various factors, such as insufficient biosecurity (primarily free-range chicken) and the relatively high number of migratory water birds wintering in Ethiopia's Rift Valley Lakes and wetland, may play a role in the spread of Avian Influenza (AI). This could raise the risk of HPAI spreading into the country's chicken population (if carried by migratory birds). The types of exposure that cause human illnesses are poorly understood. In Ethiopia, risk assessment studies were not conducted and the awareness of the community and the risk of exposure for potential avian influenza is undetermined [7].

NCD is caused by virulent strains of Newcastle Diseases Virus (NDV) or Avian Paramyxovirus type 1 (APMV-1) that belongs to the genus Avula and family Paramyxoviridae [8]. NCD is one of the most infectious diseases of poultry and induces up to 100% mortality as well as a decrease in egg production and poor egg quality. it is also highly contagious, septicemic, fatal and destructive disease which attack chiefly chickens and turkeys usually in an acute, sometimes in sub-acute or even chronic form. Occasionally human being and even wild birds may be also infected with the virus [9]. Isolation and characterization of suspected pathogenic strains of the virus should be conducted in a virus-secure laboratory. Vaccination is the most important method of disease control which results in a quite significant increase in chick survival [10]. The epidemiology of NCD in village birds in Ethiopia is not clearly understood. Nonetheless it appears that NCD is the most important reoccurring epidemic every year [11].

Poultry play an important economic, nutritional and socio-cultural role in the livelihoods of poor rural households in developing countries, including Ethiopia. The total poultry population in Ethiopia is estimated at 48.9 million, 81.7% of which are village chickens. Poultry rearing is particularly important to women, who often own and manage chickens and the resulting income is often used to support education of children [12].

Ethiopia has a diversified ecology, wet-land and lakes which located in rift valley basin which host several migratory birds in different seasons. So that pathogenic strain of avian influenza virus is expected to threaten for the emerging through migratory birds from other suspected continents and countries. The domestic chicken in village 10 km radius around the lakes are risk for contamination of the virus. The living style in Ethiopia is also expected to increase the risk to the pandemic. - consists of traditional low input systems which play an important part in the poverty reduction and food safety of rural families - People are living with their poultry most of the time in the same house or in an attachment where there is no strong barrier. - Poultry also mixes regularly with the people their droppings in every quarter of the compound often mixing with food items. This will make Ethiopia at the fore front to face the problem of this possible pandemic (Non published Ethiopian fact status).

Avian influenza outbreaks show a seasonal occurrence in high-risk areas, which coincided with migratory activity. It is; therefore, this risk-based assessment is designed with the aim of assessing avian influenza and NCD in wild birds and domestic chicken in the rift valley lakes Ethiopia. And to assess the level of the community awareness and the risk for human exposure to avian influenza viruses in the wet land areas of Ethiopia.

Objectives

→ To assess risk based active surveillance along migratory birds and domestic chicken for early detection of highly pathogenic avian influenza (HPAI) and Newcastle disease in the selected regions of Ethiopia.

→ To assess the level of the community awareness and the risk for human exposure to avian influenza and Newcastle viruses in the selected regions of Ethiopia.

→ To increase the development of regional and national government preparedness plan for an early detection highly pathogenic avian influenza/HPAI/ in the migratory season of wild birds

Material and methods

Study area

The study was conducted from January 2019 to April 2019 in 3 regions namely Amhara, Oromia and SNNPR in the rift valley lakes and selected districts which are located 5-10 km radius to the water bodies (Figure 1). From Amhara regions selected eight woredas namely Bahir dar liyu zone, Bahir dar zuria, Mecha & North Achefer (West Gojjam Zone), Libokemekem, Fogera, Dera (South Gondar Zone), and Dembya (North Gondar Zone) in Northwestern Ethiopia. Those woredas were selected based on their close proximity to Lake Tana and surrounding wetlands which are often used as resting sites for migratory birds. From SNNPR regions selected 7 woredas and their districts Arbaminch Zuria wereda shele and mela. Birbir

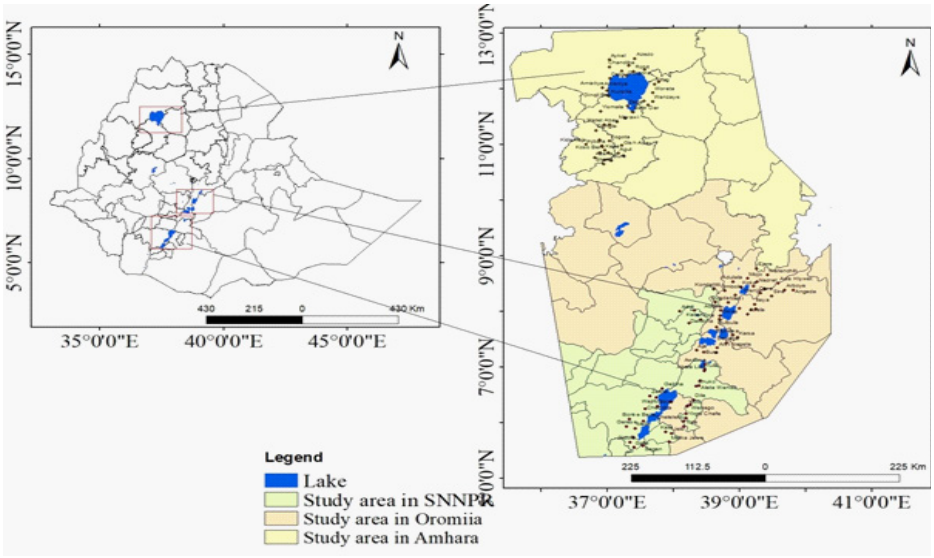

Figure 1: Map showing study area (Amhara, SNNPR and Oromia region) (by Arc GIS 10.4.1).

Town - Genta kanchama and Lante, Mirab. Abaya wereda, Fura, Feregosa and Mole kebeles. Humbo wereda Guricho and Abaya Mareka kebeles of Tullo kebele of Hawassa town, Jara damuua, Galelcha, Galo Argessa, Jara Hinessa and sama ejersa of Hawassa zuria wereda, watara kechema and woshana soyama Wondo genet wereda, Alfecho kebele of Amaro weredas. From Oromia region selected the rift valley lakes areas; Zway, Langano, Abiyata, Shala and the surrounding wet lands, which were found in 150kms radius; Lume, Adama, Dhera, Z/Dugda, Zway, Jido kombolcha, Arsi Negele. The study area includes 18 Kebeles from 10 districts. Generally, most part of the study areas are midland and are favorable for poultry raising.

Study population and sample size

Migratory/wild birds and domestic chickens were the study's subjects. All of the chickens in the study village were frequently kept outside to scavenge all day near the owners' house. Domestic poultry had not been immunized against any diseases. People who own poultry and those who live near the rift valley lakes make up the community.

Study design

In and around the rift valley lakes, risk-based surveillance was conducted to determine the presence or absence of avian influenza and Newcastle disease.

Questionnaire survey in animal health

Bahir Dar Regional Veterinary Laboratory developed a detailed structured questionnaire format to collect data on seasonal flyways, resting and sheltering sites of wild/migratory birds, common interface links with backyard poultry, occurrence of mass death among birds and backyard chickens, major poultry diseases, and bird flu knowledge. Five farmers from each peasant organization completed the questionnaires. A standard questionnaire was utilized to examine the community's awareness level of potential dangers, routes of disease transmission, sanitary conditions practiced, and feasible infection prevention and control methods from the household where samples from animals were taken and health care providers.

Sample collection

Blood samples for serum: The sum of the three regions 819 Blood samples (for serum) were taken from unvaccinated, seemingly healthy free range domestic chickens of various ages (pullets, cockerles and layers). Using sterile 5 ml disposable syringes and needles, approximately 3-5 ml of blood was drawn from each bird's wing vein (Figure 2). To allow clotting, the disposable syringes were labeled and set tilted on a table overnight at room temperature. After that, serum was extracted by decanting. The serum was placed in a cryogenic vial with proper labeling and stored at -20°C until the HA/HI assays were completed.

Swabs and Fecal dropping samples: In all, 1836 swab samples were obtained from three regions. Aseptically collected tracheal and cloacal swab samples from 5-6 birds, and 4211 fresh wild bird fecal dropping samples were taken early in the morning from the resting areas of wild/migratory birds. The swab samples and feces were then carefully placed in a labeled sterile cryogenic vial containing viral transport media and maintained at -20°C until the molecular RT-PCR assay at Animal Health Institute. Number of samples collected in each of the three regional lab was as follows: regional from Amhara (Bahirdar) Serum 306, Swabs (Tracheal and cloacal) 540, Fecal droplet 2715. From Oromia (Asela) Serum 200, Swabs (Tracheal and cloacal) 670, Fecal droplet 765. From SNNPR (Sodo), Serum 313, (Tracheal and cloacal) 626 and Fecal droplet 731. Hence the total Serum, Swabs (Tracheal and cloacal) and Fecal droplet in the three-study area was 819, 1836 and 4211 respectively. As a total sample of 6866 was collected.

Laboratory analysis

Haemagglutination-inhibition test: The Haemagglutination-Inhibition (HAI) test was carried out in the regional veterinary laboratories of Bahir Dar, Sodo, and Asala. The HA/HI tests for serum samples were performed using the NAHDIC HA/HI test, which was developed by Beard (1985) and the OIE (2000). The test was carried out on a V bottomed micro titer plate with two-fold dilutions of equal amounts (0.025 ml) of Phosphate Buffered Saline (PBS) and test serum (0.025 ml).

Each well received 4 haemagglutinating units (4 HAU) of virus/antigen, and the plate was kept at room temperature for at least 30 minutes. Finally, 0.025 ml of 1 percent (v/v) chicken Red Blood Cells (RBC) were added to each well, and the RBCs were allowed to settle for 30-40 minutes at room temperature following moderate mixing. The maximum dilution of serum was

used to determine the HI titer, which resulted in full suppression of 4 HAU of antigen. By tilting the plates, the agglutination was determined. Only those wells in which RBCs flow at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) were regarded to exhibit inhibition after a value of more than or equal to 4 (log to base 2) was considered positive. Antigens for H5N8, H7N1, and H9N2 were employed in HA/HI serological testing.

Molecular diagnostic test (RT-PCR): At NAHDIC, avian influenza and NDV viral nucleic acid were detected using Real-time Polymerase Chain Reaction (RT-PCR) on fecal droplets and Swab samples. The RNA was extracted using the Qiagen viral RNA micro kit according to the manufacturer's instructions. The Applied Bio System rapid 7500 PCR machine was used to amplify DNA.

Results

Questionnaire survey result in Animal health

Ninety respondents from the individual localities or PAs provided basic information on the overall situation of migrating birds and domestic poultry (in this case five from each). There were 21 females (23 percent) and 69 males (77 percent) with various educational backgrounds. 28(31.11%) illiterate, 21(23.33%) "Primary education" church educated 6(6.66%), primary school 21(21.33%), and high school and above 14 (14.55%). Almost 80% of the people polled had no idea what avian flu was.

According to the data gathered from the research regions, all of the respondents have a thorough understanding of the migrating birds that visit their area. Many of the local names provided by the responders are the same, such as "Shimela." Many of these birds spend the entire year in the area. Only slight modifications in their dynamics occur from time to time. Migratory birds rest and seek shelter on the shores of rift valley lakes and on rocky islands located a significant distance from the shore (with the exception of Mecha, which was taken into account as a wetland site of the "Koga" irrigation scheme along the river "wotet Abay" and its tributaries), and they prefer areas with little or no human and animal presence. The smaller wild birds prefer to rest and take refuge in larger trees near homesteads, according to the responses. The members of the investigation team were also present when the farmers reacted.

During the sampling period, there were no mass deaths among the birds. In the visited peasant communities, periodic devastation of backyard poultry was a typical occurrence from March to May.

Result RT PCR and heamaglutination test for avian influenza and NCD

All 819 serum samples examined by heamaglutination inhibition method for avian influenza antigens were determined to be negative against specific antibodies of H5,H7 and H9. The HI test method was used to test 819 samples for NDV, and 72 samples were determined to be positive. RT-PCR was used to examine 1836 swab samples (from the same chicken that tasted for HI Serum) and 4211 fecal droplets from wild birds. All facial droplet and swab samples tested negative for avian influenza virus nucleic acid (subtypes H5 and H7), according to the results. NDV swab 1336 samples analyzed by RT PCR 182 samples yielded positive results (Table 1).

Table 1: The test result Real time PCR and eamaglutination inhibition test.

The test result Real time PCR					
Tested disease	**Sample type**	**No sample tested**	**No of Positive**	**No of Negative**	**Prevalence %**
Newcastle disease	Swab samples	1336	182	1154	13.6%
Avian Influenza	Swab samples	1836	0	1836	0
	Fecal droplet	4211	0	1836	0
The result Heamaglutination Inhibition test					
Newcastle disease	Serum Samples	819	72	747	8.79%
Avian Influenza	Serum Samples	819	0	819	0

Discussion

NCD can strike at any time of year in Ethiopia, and velogenic forms of the virus are widely spread across the country. It is therefore critical that further extensive investigations focus on identifying NCD virus strains so that preventive and control methods can be developed [13].

In this investigation, we discovered that Newcastle disease is endemic in the study area, with a prevalence of 13.6 percent in the current study utilising virus isolation and RT-PCR. The Adama woredas (15%) had the highest prevalence of this finding by the RT-PCR assay, followed by Bishofitu (13.9 percent). This finding is consistent with Chaka et al. (2012)'s [14] findings in Eastern Shewa, where the prevalence was 24.2 percent in the dry season and 14.2 percent in the wet season, respectively.

The overall prevalence of new castle disease was 8.79 percent in the current study utilising the HI test. This is lower than the prevalence reported by [15,16], who found 38.33 percent in Centeral Ethiopia and 23.4 percent in Bishofitu Town, respectively.

This disparity in Newcastle disease prevalence could be attributable to differences in the research areas' agroecologi al zones, stress factors, nutritional deficiency, lack of vaccination, and poor sanitary conditions. Furthermore, the researchers' differing sample sizes and analyses may have influenced the prevalence of Newcastle disease.

Furthermore, the free-range poultry management approach used in the study locations may allow for the continuous spread of infection among the flocks. According to [15] the chicken was also susceptible to infection via wild birds, and local outdoor marketplaces where large numbers of chicken are congregated could act as continual foci of infection.

Conclusion

The absence of AIV antibodies among domestic poultry in the study area was revealed by serum samples for a Hemaglutination Inhibition test conducted on the prevalence of avian influenza. A negative result was discovered. Avian influenza nucleic acid was found to be absent in samples swabs and fecal droplets in a molecular assay. Out of 819 serum samples tested for NDV by hemaglutination inhibition test, 72 were positive with an 8.7% frequency, and out of 1336 swab samples tested by RT PCR, 182 were positive with a 13.6 percent prevalence.

This study found that farmers and other locals in the investigated areas are extremely aware of the seasonal flyway, as well as the migratory birds' resting and sheltering spots. Nonetheless, many of the res ponders either haven't heard of bird flu or haven't heard enough about it to know anything about the disease's infection and spread. Participants have a low level of knowledge of the risks and exposure that AI poses. Female participants, those aged 19-40, and those who were illiterate had lower levels of awareness. The majority of the subjects had close interaction with domestic animals (exposure). Female housewives in the 19-40 age group were found to have the most exposure (contact).

Based on results obtained the following recommendations are forwarded

√ Seasonal observation in the rift valley lakes and surrounding districts is recommended.

√ The spread of the disease from Asian, European, and African countries could result in the virus being introduced into Ethiopia via migrating birds. As a result, further AVI surveillance techniques should be implemented in order to track the incidence of AI viruses in wild birds.

√ As a type of early warning system, this could be useful.

√ People living in high-risk locations should be made aware of their surroundings.

√ Surveillance and information exchange systems for one health (human, animal, and environment) must be strengthened.

√ Veterinary diagnostic capacity in national and regional laboratories should be improved.

√ More research should be done to identify important poultry diseases and their risk factors.

Conflict of interests: The authors have not declared any conflict of interest.

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