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Determination of Risk Factors Predisposing Chicken to Influenza A Virus Infection at Uasin Gishu County, Kenya

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Abstract

Influenza A is a highly contagious virus of global health concern causing significant deaths in chicken. The virus is a threat to poultry production in many countries including Kenya, due to location along key wild bird's migratory routes. This study aimed at determination of risk factors predisposing chicken to Influenza A virus infection in Uasin, Gishu County, Kenya. To achieve this, age, sex, breed (hybrid and indigenous), vaccination status (avian flu vaccine), restocking source, management systems of chicken and seasonal weather variations were assessed to determine those that are associated with virus infection. The study was conducted at the Regional veterinary investigation laboratories (RVIL) in Eldoret. Oropharyngeal swab were collected from 305 sampled chicken brought in by farmers to the laboratory from all the sub-counties of Uasin, Gishu County, Kenya for screening of suspected zoonotic diseases. Real-time reverse transcriptase polymerase chain reaction (rtRT-PCR) was used to diagnose the virus. Face to face interviews with farmers who brought their chicken for screening in the laboratory were conducted to gather information on possible confounding factors such as the sex, age, breed, management system of chicken, seasonal weather variation, restocking source and vaccination status of chicken which were recorded using a structured questionnaire. Pearson chi square technique was employed to test for statistical significance on differences across data sets at a 95% confidence level. The result showed that there was significant difference in influenza A virus infection positivity between hybrid and indigenous breeds ($p = 0.000$), while age (p-0.6992), sex (p-0.879), management systems (p-0.5747), vaccination status (p-0.81), restocking source (p-0.549) and seasonal weather variation (p-0.42) were not significantly associated with Influenza A virus in chicken. In conclusion breed of chicken demonstrated a statistically significant effect as a predisposing factor on the infection by Influenza A virus in chicken in Uasin, Gishu County, Kenya. The study recommends that public health veterinary sectors within the ministry of agriculture from the County should create awareness to farmers on the transmission, symptoms, control and treatment for Influenza A virus among hybrid and indigenous breeds of chicken.

Keywords: Chicken, Diagnosis, Influenza A virus, Risk factors, rtRT-PCR

INTRODUCTION

Avian influenza A virus (AIV) is a highly contagious disease, characterized by an extensive circulation in several reservoir populations of wild aquatic birds (Paul et al., 2019). The virus is made up of eight gene segment, which is a single-stranded negative-sense ribonucleic acid (RNA) (Tan et al., 2015). Rearing methods in confined and free-range farming has been considered as a risk factor that causes influenza A virus in chicken where the highly pathogenic avian influenza virus found in free range chickens are usually associated with the transmission of the lowly pathogenic avian influenza virus from wild birds (Wang et al., 2017). Young and laying chicken got infected with highly pathogenic avian influenza in Pakistan which caused death of 3.2 million birds (Sarwar *et al.,* 2013).

A recent case of highly pathogenic H_5N_2 variant of avian influenza was observed in a layer breed in Iowa region of United States, it has been observed that broiler and layer breeders are more affected by the avian influenza virus (Zhao et al., 2019). Furthermore birds of older age have been observed to be more susceptible to AIVs than those of a younger age, however recovered birds have poor growth in the future (Cheema et al., 2011). The avian influenza virus predicted differential infections probabilities, with female chicken being less likely to test positive than males which may be due to increased testosterone levels during the breeding season, which has been shown to impair males' immune systems, making them more susceptible to the virus (Farnsworth et al., 2012). Additionally, for restocking, farmers purchase their bird for restocking from other counties in Kenya, and birds purchased from market places are frequently ones that have AIVs (Kariithi et al., 2020). Inactivated vaccines are widely used in China to protect poultry. Since 2012, the clade of AIVs has been mostly controlled in China by the inactivated H_5N_1 vaccination developed from the vaccine strain Re-6, which was created using reverse genetics and contains the HA and NA genes of the virus (Zeng et al., 2020).

The timing of seasonal influenza virus epidemics varies globally, with the majority of influenza A virus infections in temperate regions occurring during the winter and rainy season in tropical regions (Hirve et al., 2016). Vaccination is an important measure used for control and prevention of the H_5N_2 highly pathogenic avian influenza (HPAI) among poultry in endemic countries (Swayne et al., 2020).

Kenyans had adverse negative impact on chicken production and trade as a result of the Highly Pathogenic Avian Influenza (HPAI) brought by the H_5N_1 in 2005, losing an estimated Ksh 2.3 billion (OIE, 2015). However, Kenya has little data on potential risk factors associated with the virus, meaning that the actual risk factors associated with influenza A virus infection in the country including Uasin, Gishu County are not yet established. The objective of the current study assessed the intrinsic and extrinsic factors that could be the predisposing factor for influenza A virus infection in chicken such as age, sex, breed (hybrid and indigenous types) vaccination status (avian flu vaccine), restocking source, management system of chicken and seasonal weather variation in Uasin, Gishu County, Kenya.

MATERIALS AND METHODS

Sampling Techniques

This was a cross sectional study involving chicken farmers from whole of Uasin, Gishu County who brought their chicken to be screened at Regional Veterinary Investigation Laboratory (RVIL) for suspected zoonotic diseases. The consecutive sampling method was used to collect samples from the chicken of all farmers seeking chicken screening services at RVIL who consented to the study. The research was carried out throughout the wet and dry seasons until the required sample size was attained. A total of 305 chicken samples were collected in accordance with the sampling criteria guidelines provided by Naing et al. (2006).

Sample Size Determination

This study used the sampling formula described by Naing et al. (2006)

$$n = \frac{(Z_\alpha)^2 p(1-p)}{d^2}$$

Where;

N = required sample size
z = Critical value for 95% confidence level (1.96)
p = prevalence of influenza A virus in chicken (0.8% based on Munyua et al., 2013)
d = margin of error (1%), thus;

$$n = \frac{(1.96)^2 0.008(1-0.008)}{0.01^2}$$

n=304.87

This study collected samples from 305 chicken and satisfied the margin of error (d) (1%)

Specimen Collection and Processing

Chicken specimens were obtained by gently swabbing the oropharyngeal area near the opening of the trachea using plastic shafted polyester tipped swabs. The specimens were placed in 2 ml cryo-vials that were labelled with individual chicken identification numbers, Uasin Gishu/ Oropharyngeal/ number (UG/OP/01 up to UG/OP/305) and maintained frozen at -70°C in the refrigerator. Diagnostic test for RNA virus was done using real time PCR method. Face-to-face interview were conducted to farmers who brought chicken from their farms to the RVIL for zoonotic disease screening. The interviews aimed to gather information on possible risk factors associated with chicken infection with influenza A virus, such as the sex, age, breed (Hybrid and indigenous), management system of chicken, seasonal weather variation, restocking source and vaccination status (avian flu vaccine) of chicken which were recorded using a structured questionnaire.

Procedures for RNA Extraction and Detection of Influenza A Virus

A total of 305 specimens were tested for influenza A virus using real-time reverse transcription polymerase chain reaction (rt RT-PCR) technique on a Kingfisher Flex system following the manufacturer's instructions (CDC, 2011). RNA was extracted from the oropharyngeal specimens using a MagMax viral RNA isolation kit (Ambion Inc, Applied Biosystems, CA, USA). The prepared specimens containing unknown RNA materials were loaded in a 96 well plate and were run against known positive and negative control in 7500 rt-PCR machine. The machine was programmed to run for 10 minutes at 45°C for reverse transcription and 10 minutes at 95°C to activate the Taq polymerase. Denaturation at 94°C for 20 seconds, annealing/extension at 80°C for 15 seconds, and final extension at 70°C for 5 minutes to make up a 45-cycle PCR. Results from the whole rtPCR were analyzed, read at the annealing and extension stage, and recorded as cycle threshold (CT) values.

Data Management and Analysis

Data was entered into spread sheets in Microsoft Excel (2019). The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. Chi-square test and odds ratio values were used to analyse data sets for inferential interpretation. The results data were presented using tables and graphs.

RESULTS

Detection of Influenza A Virus

Four specimen samples tested positive for the influenza A virus at Cycle Threshold (Ct) values of 21.146, 22.373, 36.998 and 32.546, respectively, whereas 301 samples tested negative at Cycle Threshold (Ct) values of >40.0, (Figure 1).

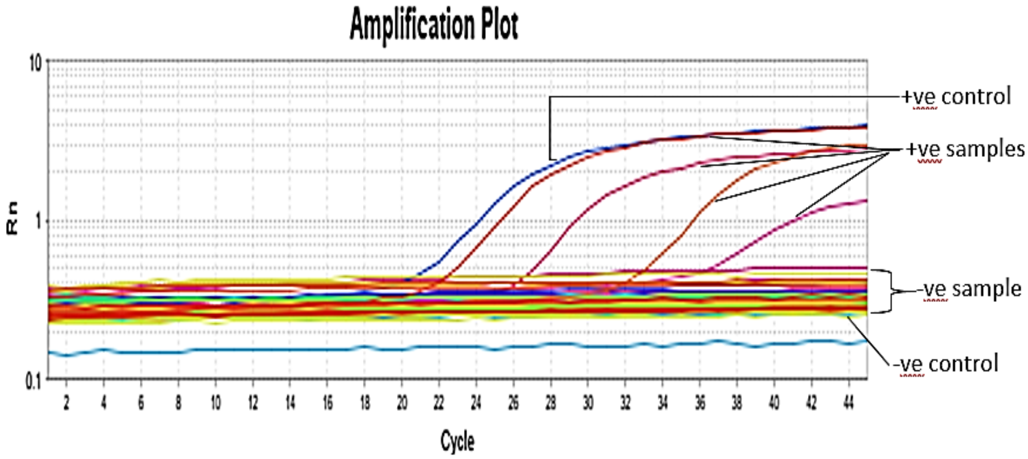

Figure 1: The Cycle Threshold (Ct) values of 21.146, 22.373, 36.998, and 32.546 indicate positive amplification for the influenza A virus, where values more than 40 indicate negative outcome for influenza A virus.

Association between Influenza A Virus Infection in Chicken and Assessed Intrinsic Risk Factors

Out of the 305 tested sampled chicken, specimens from three indigenous breeds and one hybrid breed tested positive for Influenza A virus. Chi-square analysis shows that breed type significantly influences the prevalence of the influenza A virus infecting chicken in Uasin Gishu County (p = 0.0000; OR =0.52) (Table1 and Table 2).

Three of the four confirmed cases of influenza A virus were in young chicks under the age of four months and only one of the confirmed case was an adult above the age of four months. The chi-square test suggested that there was no significant (p-value = 0.6992) association between age and the frequency of the influenza A virus infection, although the odds ratio shows that there was 1.55 more times for chicks (less than 4 months old) to test positive for influenza A virus compared to adult (more than 4 months old chickens (Table 1 and Table 2).

The influenza A virus infection was examined in 104 male and 201 female chicken. Each gender had two positive outcomes for influenza A virus. The Chi-square test yielded a p-value of 0.8790, and the odds ratio was 1.16 indicating that gender was not significantly associated with the viral infection (Table 1 and Table 2). This shows that the study found no evidence linking the sex of the chicken to the frequency of their infection with influenza A virus in Uasin Gishu County.

Table 1: Influenza A virus infection in relation to age, breed and sex of chicken

Factor	Type	No. Examined	Positive cases	P –values
Age	Adults	104	1	0.6992
	Chicks	201	3	
	Total	305	4	
Breed	Hybrid	45	1	0.0000
	Indigenous	260	3	
	Total	305	4	
Sex	Female	104	2	0.8790
	Male	201	2	
	Total	305	4	

Chi square test p – values at 95% confidence interval

Table 2: Odds ratio relationships between influenza A infection with the assessed intrinsic risk factors of chicken

Risk Factors	**Influenza A**			
Age (months)	**N**	+ve	-ve	**OR**
Chicks (< 4 months)	201	3	198	**1.55**
Adult (> 4 months)	104	1	104	
TOTAL	305	4	301	
Gender				
Female	164	2	162	**1.16**
Male	141	2	139	
TOTAL	305	4	301	
Breed				
Hybrid	45	1	044	**0.52**
Indigenous	260	3	257	
TOTAL	305	4	301	

Effect of Extrinsic Factors on Infection of Influenza A Virus in Chicken

The influence of seasonal weather variation (wet and rainy season) revealed three positive cases out of 168 sampled chickens during the rainy season (1.79%) compared to only one positive case out of 137 sampled chickens during the dry season (0.73%). Despite the rainy season having a higher number of positive cases, the viral infection did not change significantly between the dry and wet seasons (Table 3; p = 0.42)

Out of the 305 chicken, 93 (30.5%) were vaccinated with avian flu vaccine and 212 (69.5%) were not vaccinated. Three (1.4%) of the unvaccinated chicken tested positive for influenza A virus while only one (1,1%) chicken of the population that had received vaccination tested positive for influenza A virus (Table 3). Chi-square test result revealed that there was no significant difference for influenza A virus infection rate in chicken that had received vaccinations compared to chicken that had not been vaccinated. Despite the observation that more positive cases of Influenza A virus occurred in the unvaccinated chickens compared to the vaccinated chickens in Uasin Gishu County, the Chi-square test result (p = 0.81) indicates that both vaccinated and unvaccinated chickens still had an equal likelihood of getting the virus infection and thus vaccination did not significantly protect the chicken from the infection (Table 3).

Three (1.8%) of the positive cases of chicken with the influenza A virus were from free range management system while only one (0.7%) case was from confined management system. Chi-square test result (p = 0.5747) showed that despite free range management system having more positive cases compared to confined management system, management systems did not significantly influence the frequency of Influenza A virus amongst the chicken in Uasin Gishu County.

There was no significant difference between the various sources from where the farmers obtained supply to restock their chicken as evidenced by the Chi-square test result (p = 0.549) indicating that chicken may be infected with influenza A viruses, regardless of where the new supply came from (Table 3).

Table 3: Association between Influenza A Virus infection in Chicken and Extrinsic Environmental Risk Factors

Factor	Type	No. Examined	Positive cases	p-value
Season	Dry	137	1	0.4200
	Wet	168	3	
	Total	305	4	
Vaccination (avian flu vaccine)	Vaccinated	93	1	0.8100
	Unvaccinated	212	3	
	Total	305	4	
Management Systems	Confined	138	1	0.5747
	Free range	167	3	
	Total	305	4	
Restocking	Market	28	0	0.5490
	Home village	171	2	
	Own chicken	106	2	
	Total	305	4	

Chi square test at 95% confidence interval

DISCUSSION

Influence of Intrinsic and Extrinsic Risk Factors on the Infection of Chicken with Influenza A Virus

According to the current study, Statistical analysis supported the finding that breed type (hybrid and indigenous) had a statistically significant impact on the influenza A virus infection in chicken, various breeds of chicken have varying levels of resistance to influenza A virus infection as previously been documented (Sarwar et al., 2013). This observation can also be explained by the fact that the different breeds of chickens receive distinct management strategies, with hybrids being housed with more concern compared to indigenous breed types by Akanbi & Taiwo (2014). Study finding conducted in Nigeria, between the years of 2006 and 2008, hybrid chicken was more infected with the highly pathogenic avian influenza (HPAI) H_5N_1, with mortality rates of 11.11 percent, however, the findings of the current study differ from the findings reported by Metras, et al. (2013).

Chicken of both sexes showed similar infection rates in the current study, with each sex having only two positive infection outcomes. The Chi-square test indicates that there is no significant relationship in influenza A virus infection between the male and female sex of the chicken. The finding of this study contradict those of Morgan & Klein's (2019), who suggested that biological and socio-cultural differences between male and female influence how influenza infections and treatments differ between the sexes, with females developing stronger immune responses and thus having better resistance to the virus.

The current study found a higher rate of positivity in chicks below 4 months of age compared to older chicken although the difference was not statistically significant. However, findings of Cheema et al. (2011), suggested that older chicken become more vulnerable to Avian Influenza A virus (AIV) compared to younger chicken, contradicting the current findings. This may be due to the general lack of sanitation in chicken houses (increased crowded exposure with increasing age) and the fragility of young chicks whose immune has not fully matured to respond against infections including influenza A virus infection. The outcome also differs with that of Nooruddin et al. (2006), who found that the frequency of infection was higher in birds 34 weeks of age and lower in birds 8 to 12 weeks of age. This discrepancy may also be the result of inadequate sanitation in the majority of chicken homes and the susceptibility of chicks to the influenza A virus as immunity was not completely established as previously noted

(Brown et al., 2013). Furthermore, due to lack of stock records kept by the majority of farmers, it is challenging to get the correct age group of chicks or grown adult chickens. Therefore, a mistake in the classification of age might be the cause of the differences in these findings.

Despite higher number of cases observed during the wet season, statistical analysis found no significant difference between the seasons. This means that seasonal weather variation had no effect on the incidence of influenza during the wet and the dry seasons which correspond to the cold and hot seasons. These findings agree with those of Liang et al. (2020) who found little evidence on impact of temperature on HPAI H_5N_1 outbreaks. However, it disagrees with Zhang et al. (2014) who documented that viruses that caused the influenza A pandemic in Hong Kong were first isolated during the cold-weather season (Si et al., 2010).

The current study found that vaccinated and unvaccinated chicken had equal chances of contracting influenza A virus. This suggests that the available vaccine may not be effective in protecting the chicken against the virus probably due to development of variants that are resistant to the elicited immune response to the antigens in the vaccine. Vaccine failure may also be the cause of non-protection. This may be due to improper handling and administration of the vaccine which is more probable when farmers don't utilize professional services offered by veterinary personnel due to the costs involved. This result also shows that the vaccine used or the method of administration may not be effective in protecting the chicken.

Out of the four positive cases of influenza A infections, three were detected in young chicks while only one was in an adult chicken. The lower number of adults testing positive for Influenza A virus may result from the fact that adults may have already been naturally exposed to influenza viruses early in life and therefore may have developed some immunity (Munyua et al., 2013).

The findings of this study in chicken re-stocking sources indicate higher positive cases from own chicken and home village sources for chicken supply. However, re-stocking sources of chicken by farmers had statistically insignificant differences between all sources of supply meaning that the chicken can contract Influenza A virus irrespective of the source of the new stock. This study contradicts that of Hasan et al. (2019), whose finding indicated that most of the infected cases of chicken stock in Pakistan were purchased in markets.

CONCLUSION AND RECOMMENDATION

During this study, Infection of influenza A virus in chicken brought to RVIL in Uasin Gishu County was assessed in relation to intrinsic and Extrinsic risk factors. Of all the assessed possible risk factors, only the breed (hybrid and indigenous types) of chicken demonstrated a statistically significant effect on the occurrence of Influenza A virus in Uasin Gishu County Kenya. This study has put to light the risk factor of chicken breed types on the infection of AIVs in Uasin Gishu County Kenya probably due to relaxed management system practice. Thus, this study recommends that proper management and control measures should be put in place by ministry of agriculture to reduce the economic loss to farmers.

This study also recommends that future research should involve field survey observations in poultry farms by researchers to clearly capture the possible risk factors on infection of influenza A virus in chicken. This will ensure proper programme sensitization among poultry farmers on symptoms, transmission, control and prevention of the virus.

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