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A study of Newcastle disease virus in poultry from live bird markets and backyard flocks in Kenya

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A countrywide cross-sectional study was conducted to determine the presence of Newcastle disease virus (NDV) in poultry in live bird markets (LBMs) and backyard poultry farms in Kenya. A total of nine hundred and twenty two (922) poultry in backyard flocks and four hundred and fifty four (454) poultry in LBMs were examined. Overall, NDV was detected in 10.1% (46/454) of the poultry sampled in live bird markets. In backyard flocks, NDV was detected in 3.6% (33/922) of the poultry sampled. Regional variations in NDV occurrence was observed in both live bird markets and poultry flocks. Markets in major towns and cities had significantly ($p<0.05$) higher NDV detection rates. Higher NDV detection rates were observed in backyard farms in Lake Victoria Basin than other regions. Chicken had the highest NDV detection compared to other poultry species. The study detected NDV in apparently healthy chicken and brought forward the probable high importance of carrier birds in the circulation and transmission of NDV and in causing outbreaks. The study also points to the usefulness of reverse transcription polymerase chain reaction (RT-PCR) in screening for NDV to prevent the outbreaks and control of ND in Kenya.

Key words: Backyard poultry, chicken, Kenya, live bird markets, NDV, RT-PCR.

INTRODUCTION

Poultry farming is an important socio-economic activity in the developing countries. It contributes to food security and livelihoods in poor rural households. In Kenya, poultry is particularly important to women, the youth and other vulnerable groups such as human immunodeficiency virus (HIV) affected households.

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Economically, the poultry industry contributes 30% to the agricultural sector and 7.8% of the Gross Domestic Product of Kenya (MoALF, 2015).

The poultry population in Kenya is estimated at 30 million of which 80% are extensively managed backyard poultry (MoALF, 2015). Despite the role poultry plays in the livelihoods of the rural communities and the entire economy of Kenya, poultry production is hampered by a wide range of constraints. Infectious diseases are significant constraints to poultry production around the globe including Kenya (Assam et al., 2011; Chaka et al., 2012; Eze et al., 2015; Olwande et al., 2016; Sandhu et al., 2009; Singla and Gupta, 2012; Sultana et al., 2014). Among various infectious diseases, Newcastle Disease (ND) is the main challenge for the backyard poultry in most households across Kenya (Olwande et al., 2016).

Newcastle disease is a highly contagious and fatal disease of poultry which is notifiable to the World Organization for Animal Health (OIE, 2018). It is caused by Newcastle disease virus (NDV), classified under the genus *Avulavirus* within the family *Paramyxoviridae* (Amarasinghe et al., 2017). In developing countries, the disease is endemic and causes periodic outbreaks that wipe out entire flocks with severe impact on production (ACIAR, 2014) and heavy economic losses annually (Ashraf and Shah, 2014; Hugo et al., 2017). ND is highly prevalent in both commercial and backyard poultry in most countries in Africa (Chaka et al., 2013; Jibril et al., 2014) and Asia (Khan et al., 2011; Sultana et al., 2014). However, in backyard flocks, ND is particularly difficult to control because biosecurity measures are most often entirely lacking (Rimi et al., 2017).

In Kenya, Newcastle disease outbreaks are documented to occur throughout the year in both the cold and dry periods with peaks in April, June to July and September to November (Kemboi et al., 2013). Various management factors on backyard flocks such as keeping of mixed poultry species, mode of disposal of poultry waste and restocking with birds from markets are associated with the occurrence of ND outbreaks in Kenya (Njagi et al., 2010b).

Vaccination of poultry with ND vaccine boosts their immunity and reduces the occurrence of ND (ACIAR, 2014). However, vaccination of backyard poultry remains a challenge due to the complex production dynamics of these flocks (Alexander et al., 2013). Kenya is no exception; millions of backyard poultry remain unprotected and thus vulnerable to ND infection during outbreaks. Losses of up to 100% are reported in unvaccinated poultry population. ND control is therefore of importance in enhancing the productivity of backyard poultry.

A major step in the control of ND is understanding the dynamics of maintenance and spread of the viral agent in between major outbreaks. The virus is thought to be maintained in healthy birds which act as a source of infection to susceptible poultry in a flock. Human activity and increased turnover in live bird markets has been thought to aid in maintenance and transmission dynamics of NDV leading to outbreaks (Abdisa and Tagesu, 2017).

In order to enhance epidemiological knowledge on maintenance and transmission of ND, and develop sustainable and appropriate ND control strategies in Kenya, a study was conducted to examine poultry for the presence of NDV in apparently healthy backyard poultry flocks and live bird markets.

MATERIALS AND METHODS

Study area and design

A cross-sectional study was conducted between November 2015 and March 2016 in backyard flocks and live bird markets in Kenya. Semi-structured questionnaire interviews with poultry keepers and live bird market sellers were conducted to establish the management and trade practices and dynamics as well as health and disease status of birds. At the same time, selected poultry were sampled to establish their NDV status.

Backyard poultry flocks were sampled in three agro-ecological zones; Western Highlands, Lake Victoria basin and Coastal zones were purposively selected based on high population density of backyard poultry in these Zones (KNBS, 2016). Western Highlands is an agricultural hub of Kenya. It is a vast zone and stretches from Kisii and Bomet in the South to Tranzoia, Uasin Gishu, and Mt Elgon area of Bungoma County through to Nandi, Kakamega and Vihiga. This zone lies above 1500 m above level and experiences heavy rains averaging 1500 mm most part of the year and temperatures of between 11 to 20°C. The zone holds half of the country's backyard poultry population (KNBS, 2016).

The Lake Victoria basin is a zone located in the Southwest part of Kenya around Lake Victoria which includes Busia, Siaya, Kisumu, Migori and Homabay counties. This zone is hot and humid and experiences average rainfall of 500 to 1000 mm that occurs twice per year. Population of backyard poultry in the area is estimated at 6 million (KNBS, 2016). On the other hand, the Coast region lies within the Coastal lowlands, which may extend from sub-humid to arid zones.

The population is approximately 3.3 million within an area of 79,686 km^2. Average annual rainfall is poorly distributed and unreliable and ranges from 500 to 750 mm. The mean annual temperature is higher than 24°C, and mean maximum temperature is lower than 33°C.

To sample live bird markets; major markets were selected from five zones namely; Western Highlands, Lake Victoria Basin, Coastal strip, Eastern and Nairobi metropolitan. The sampled markets included; Western Highlands (Chwele, Bungoma, Kitale, Kericho, Kakamega and Bomet); Lake Victoria Basin (Bumala, Kisumu, Homabay, Migori); Coast (Majengo, Marikiti, Kilifi); Eastern (Meru, Makueni) and Nairobi metropolitan (Burma, Kibera, Kawangware, Machakos and Kitengela). The LBMs were categorized into three market types; City, major town and trading centre markets. LBMs selected from Nairobi, Kisumu and Mombasa accounted for the city markets. Figure 1 shows the location of sampled villages and live bird markets.

Sample size and selection of study birds

A total of 922 poultry were sampled from 225 backyard flocks. The

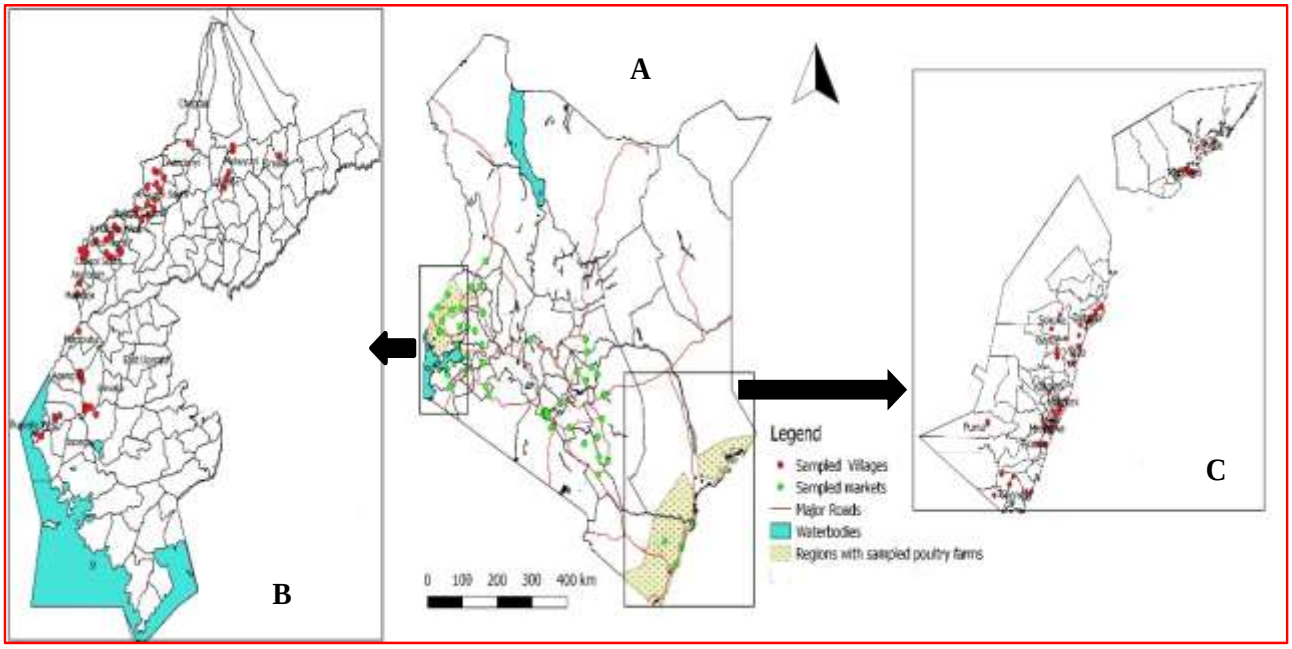

Figure 1. Geographical location of sampled backyard poultry flocks and live bird markets in Kenya, November 2014 to March 2016; (A) A map of Kenya showing the location of sampled live bird markets and poultry flocks (B) Geographical representation of sampled backyard poultry flocks in Western Kenya (C) Geographical representation of sampled backyard poultry flocks in Coastal region of Kenya.

sample size for each of the three zones was calculated based on the formula (Charan and Kantharia, 2013).

$$n=1.962\ p\ (1\text{-}86\ p)/L2$$

Where:

n= required sample size, p= prevalence of ND and L= precision.

Prevalence of ND was estimated to be 30% from a previous study (Njagi et al., 2010a; Olwande et al., 2016), a confidence level of 95% and precision of 5% was used. A multistage sampling criterion was used to select poultry to be sampled. Sub-locations were listed from the three selected zones (Western Highlands, Lake Victoria Basin and Coastal strip) and 5 were randomly selected from each zone. From each sub-location backyard poultry keepers were listed with the help of the field extension staff. Fifteen (15) poultry keepers were selected randomly from each sub location. From each farm, 4 adult birds were sampled. For farms with mixed species of birds; 2 birds of each of the other species were also sampled.

In live bird markets, a total of 454 birds were sampled from 124 traders. The number of birds sampled in each market was 20, assuming a market size of 50 to 100 birds, a minimum expected prevalence of 10% and confidence interval of 95%. Twenty (20) live bird markets (LBM) were selected from the five zones. In the LBMs, five sellers were selected randomly and four birds of were randomly selected per seller for sampling. However, in 7 live bird markets that had high bird turnover, we sampled 7 traders each. This included Kisumu, Kericho, Majengo, Burma, Meru and Chwele markets. Table 1 shows the number of birds sampled in poultry farms and LBMs. To sample birds, we collected tracheal and cloacal swabs in 1000µl of RNAlater®. We transported samples in a cool box and stored at −80°C until processing.

Newcastle disease virus screening

Screening for the presence of NDV in swab samples collected from birds was done using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) (Ganar et al., 2014). This involved extraction of Ribonucleic acid (RNA) from samples using Trizol LS Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Complementary DNA was synthesized from RNA using Superscript® III (M-MLV) reverse transcriptase of the First-strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). Briefly, for each reaction; 5 µl of sample total RNA was combined with 3 µl of 50 ng/µl of random hexamer, 1 µl of 10 mM dNTP mix, 2 µl of DEPC treated water and heated at 65°C for 5 min. We placed the mixture on ice immediately for 1 min to anneal the primers to the 3' terminal sequences of the RNA. The SuperScript™ III reverse transcription mix was then prepared according to the manufacturer's instructions. Each reaction containing; 1µl of 50 U of Superscript™ III Reverse Transcriptase, 1µl of 40 U of RNaseOUT™ Recombinant Ribonuclease Inhibitor, 2µl of 0.1 M DTT, 2µl of 10X First Strand Buffer and 4µl of 25mM $MgCl_2$ were annealed at 25°C for 10 min, extended at 42°C for 50 min. Thereafter, we incubated at 70°C for 15 min to inactivate the reverse transcription enzyme, and later chilled at 4°C. Amplification of NDV was done by conventional PCR using primers previously published by Liu et al. (2008) which target a 535bp region of the virus. The PCR was performed using Taq DNA polymerase and 5 µl of cDNA with the cycling parameters starting with a denaturation step of 95°C for 3 min; followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 3 min, followed by 72°C for 10 min. PCR products were visualized by agarose gel (1.5% w/v)

Table 1. Number of birds sampled in markets and poultry farms in different regions of Kenya between 2014 and 2016.

Regions	Areas with sampled households	Sampled markets	Number of birds sampled	
			Households	Markets
Lake Victoria basin	Aktes, Aturet, Aterait, Budalang'i; Amukura	Bumala, Kisumu, Homabay, Migori	288	58
Western Highlands	Kimilili, Cheptais, Kaptama,Kabuchai, Chwele	Chwele, Bungoma, Kitale, Kericho, Kakamega and Bomet	324	135
Coastal	Dabaso, Gede,Matsangoni, Mkomani, Shella	Majengo, Marikiti, Kilifi	310	71
Eastern	-	Meru, Makueni	-	64
Nairobi metropolitan	-	Burma, Kibera, Kawangware, Machakos and Kitengela	-	126
Total	-	-	922	454

electrophoresis and UV illumination after staining with GelRed™ (Biotium).

Data analysis

Data on management and trade dynamics captured through the questionnaires were entered in a spreadsheet (Microsoft Excel) and linked with the results from NDV screening. Descriptive analysis on management and market practices and NDV positivity was carried out using R version 3.2.3 (R CRAN). NDV detection with 95% confidence intervals (CI) was calculated as the proportion of the number of NDV positive birds on RT-PCR to the total number of samples tested. Frequencies (percentages) for categorical variables were calculated and Chi-square test was used for comparison. Statistically significant difference was accepted at a probability (p) of $p<0.05$.

RESULTS

General Information of sampled backyard poultry farms and live bird markets

Two market types were encountered; open air (64.7%) and enclosed (35.3%). In the open-air markets, birds were placed outdoors either in cages, traditional baskets or tethered to a pole. Enclosures were mainly permanent or temporary structures and birds were kept in stalls or cages. Majority (64.7%) of the sampled markets had between 100 and 200 birds. LBMs were categorized as city, major town or trading centre markets. Two-thirds of the sampled live bird markets routinely offered slaughter services, that is, traded both, live and slaughtered birds. However, only 17.7% of these had designated slaughter facilities. Chicken was the predominant poultry species kept by 98.2% (221/225) of sampled backyard farms, two thirds (65%) of the households also kept other poultry species including ducks, guinea fowls, turkeys, pigeons and geese. Similarly, sampled live bird sellers predominantly sold chicken (98.4%:122/124). While all sampled backyard farms kept only local chicken ecotypes, almost half of market sellers (46%) sold both local chicken ecotypes and exotic chicken (Table 2).

Occurrence of NDV in sampled birds

Figure 2 is a gel image showing the NDV negative and positive samples. Overall, NDV was detected in 3.6% (33/922; range 2.0 to 7.4) of the sampled backyard poultry in farms. Of the positive birds, 32 were chicken and 1 duck. There were regional variations in household NDV detection; Lake Victoria basin had significantly higher NDV detection ($p<0.05$) compared to the other zones (Coast and Western Highlands) (Table 3). Aterait, Aturet and Aktes had significantly higher proportion of NDV positive birds. NDV was not detected in any of the birds sampled in Gede, Amukura and Shella sub-locations. Overall, NDV was detected in 10.1% (46/454) of the sampled birds. NDV detection was significantly higher ($p<0.05$) in open air markets (21.2%) compared with enclosed markets (8.1%). Similarly, there were significant ($p<0.05$) differences in NDV detection across the five sub-regions with Nairobi having the highest detection rate and Coast region having the lowest detection rate (Table 4). In addition, LBMs in the cities and major towns presented higher detection rates than those from trading centres ($p<0.05$). Overall, 19 out of 22 LBM (86.3%) had at least one bird that tested positive for NDV. Amongst the LBMs, Kibera (58.3%, OR = 7.7, $p<0.04$), Homabay (45.5%, OR = 4.6, $p = 0.12$), Kitengela (38.5%, OR = 3.4, $p = 0.197$), Bungoma (40.0%, OR = 3.7, $p = 0.16$) and Burma (33.3%, OR =2.8, $p = 0.303$) had the highest NDV detection rates in that order. NDV was not detected in birds sampled from LBMs of Chwele, Kisumu and Marikiti (Table 4).

DISCUSSION

This study utilized the RT-PCR to detect the presence of

Table 2. Characteristics of sampled live bird markets and poultry farms.

Characteristics	No. households (N=225)	Proportion sampled	SE
Farm type			
Chicken only	113	50.2 (37.3-61.8)	0.04
[1]Mixed species	112	49.8 (38.2-62.7)	0.04
Chicken flock size			
Less than 10	136	60.4 (45.9-72.3)	0.03
11-30	62	27.6 (18.8-34.1)	0.04
>30	23	10.2 (7.1-24.8)	0.04
Type of treatment			
Conventional	72	32.0 (19.7-41.2)	0.04
Herbal	58	25.8 (13.4-32.0)	0.04
None	95	42.2 (23.1-55.5)	0.04
Vaccination			
Yes	53	23.6 (16.6-31.8)	0.04
No	172	76.4 (68.2-83.5)	0.04
Market characteristics	**No of markets (N=20)**	**-**	-
Market enclosure			
Open air	12	60.0 (46.6-71.8)	0.11
Enclosed	8	40.0 (38.2-53.5)	0.11
Bird population			
<100	5	25.0 (16.8-32.7)	0.12
100-200	7	35.0 (22.6-41.9)	0.11
>200	8	40 (28-53.1)	0.11
Slaughter services			
No	8	40.0 (35.2-52.5)	0.11
Yes	12	60.0 (48.2-69.7)	0.11
Category of market			
Trading centre	4	20 (15.5-27.4)	0.13
Major town	9	45.0 (37.3-57.9)	0.11
City market	7	35.0 (29.0-41.8)	0.11

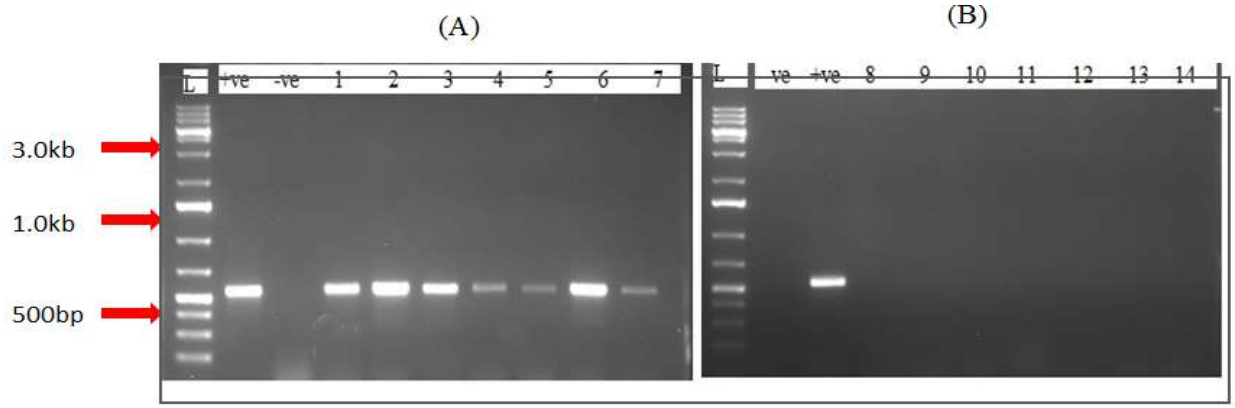

Figure 2. Gel images of amplified products showing (A) Positive samples (1-7) with band size of 535bp (B) Negative samples (8-14); L-represents the marker/ladder used (1KB plus) to estimate the product sizes as indicated by the red arrows; +ve - Positive control; -ve - Negative control.

Table 3. Number and proportion of birds (n=922) that tested NDV positive by geographical location.

Factor/variable	No of sampled birds(N)	No. NDV positive birds (n)	Proportion positive birds (%)	SE	p-value
Region					
Coastal strip	310	4	1.3 (0.5-3.5)	-	0.01**
Western Highlands	324	8	2.5 (1.5-4.9)	-	0.03**
Lake Victoria basin	288	21	7.3 (5.3-9.1)	-	Reference
Sub-location					
Aktes	53	4	7.6 (4.9-10.3)	-	0.01**
Ateriat	57	10	17.5 (12.7-22.9)	-	0.001**
Amukura	58	0	0	-	
Aturet	59	6	10.2 (4.5-14.8)	-	0.001**
Budalangi	60	1	1.7 (0.06-2.9)	-	
Kimilili township	60	3	5.0 (3.5-8.7)	-	0.02**
Cheptais	69	2	3.3 (2.9-7.1)	-	0.03**
Kaptama	62	1	1.6 (0.4-2.2)	-	0.06
Kabuchai	62	1	1.6 (0.4-2.2)	-	0.06
Chwele	75	1	1.3 (0.2-2.6)	-	0.1
Gede	60	0	0	-	
Dabaso	60	1	1.7 (0.06-2.9)	-	0.062
Matsangoni	65	2	3.1 (1.9-4.7)	-	0.03**
Mkomani	67	1	1.5 (0.06-3.6)	-	0.07
Shella	58	0	0	-	Reference

**Proportion with asterisks are different compared to others ($p<0.05$); Parentheses represent 95% confidence intervals.

NDV in poultry in live bird markets and poultry farms in various regions in Kenya in order to establish the NDV status of backyard poultry in between outbreaks. We detected NDV in approximately 3.6% of the sampled poultry in backyard flocks.

Olwande et al. (2016) and Njagi et al. (2010a) reported higher prevalence of ND in backyard poultry flocks in Western and Eastern Kenya. The difference could be due to the difference in the assays used. While Njagi et al. (2010a) and Olwande et al. (2016) used an antibody assay to estimate prevalence, our study utilized a PCR assay. Antibody assays test for long term immunoglobulins and are therefore bound to detect more positives than antigen based tests like PCR (Chaka et al., 2013). However, the antibody tests detect exposure to infection rather than presence of the viral agent as detected by PCR (OIE 2015). This study detected NDV in 10% of sampled poultry in live bird markets. Similar NDV prevalence has been reported in LBMs in other countries in the Eastern Africa region (Byarugaba et al., 2014; Chaka et al., 2013; Mulisa et al., 2014).

The presence of NDV in LBMs has also been reported in other regions of Africa (Jibril et al., 2014; Omony et al., 2016; Solomon et al., 2012) and Asia (Barman et al., 2016). LBMs are reported to contribute to the persistence and spread of NDV and serves as a source of infection to backyard poultry flocks (Jibril et al., 2014). This finding therefore indicates the important role played by LBMs in the epidemiology of NDV in Kenya.

The study detected NDV in apparently healthy poultry in both LBMs and backyard flocks. This suggests the possibility of presence of poultry that are carriers of NDV (Munir et al., 2012). This has grave epidemiological implications because apparently healthy poultry mix with other birds. These NDV positive but apparently healthy birds may be incubating the virus with no obvious clinical signs and may transmit the virus to other birds (Ashraf, and Shah, 2014).

Variation in NDV detection in both LBMs and poultry flocks were observed on the basis of the geographical location. For instance, LBMs in major towns and city markets tended to have a higher frequency of poultry testing positive to NDV than those from trading centre. It is highly likely that this is associated with the volume and diversities of poultry trading and patterns that favour the maintenance and circulation of NDV. For instance, Nairobi metropolitan is a large urban area that attracts poultry centripetally from most parts of the country and as far as from Uganda. Poultry with unknown disease status are mixed, and transported over long distances from various sub-regions and arrive at Nairobi city stressed and immunocompromised with increase susceptibility to

Table 4. Number and proportion of birds (n=454) that tested NDV positive in different zones and markets.

Variable	No. of birds sampled	No. of NDV positive birds	Proportion (%) positive birds	p-value
Sampled regions				
Nairobi	104	24	23.1 (16.8-34.1)	Reference
Western Highlands	153	16	10.5 (7.3-15.6)	0.023**
Lake Victoria Basin	82	6	7.3 (3.6-9.1)	0.021**
Eastern	41	4	9.8 (4.5-11.7	0.032**
Coast	74	4	5.4 (3.6-8.3)	0.013**
Sampled markets				
Kitengela	26	5	19.2 (11.7-22.8)	Reference
Bungoma	17	6	35.3 (19.7-57.6)	0.163
Chwele	28	0	-	-
Kitale	27	0	-	-
Kericho	41	7	17.1 (10.9-29.3)	0.947
Kakamega	22	1	4.6 (3.1-9.6)	0.003**
Bomet	18	2	11.1 (8.1-14.4)	0.146
Bumala	20	0	-	-
Kisumu	28	2	7.1 (5.9-15.6)	0.01**
Migori	16	1	6.3 3.8-13.5)	0.005**
Homabay	18	3	16.7 (10.1-23.7)	0.406
Majengo	31	1	3.2 (0.8-4.8)	0.004**
Marikiti	26	0	-	-
Kilifi	17	3	17.7 (11.3-27.3)	0.746
Meru	17	0	-	-
Makueni	24	4	16.7 (9.9-28.6)	0.654
Burma	25	3	12 (6.8-17.4)	0.303
Kibera	25	8	32.0 (15.5-40.3)	0.035**
Kawangware	28	2	7.1 (5.5-12.2)	0.02**
Machakos	23	6	26.1 (13.0-37.9)	0.045**
Market type				
Open air	237	29	12.2 (6.2-17.9)	Reference
Enclosed	240	25	10.4 (5.2-14.9)	0.976
Bird population				
<100	112	17	15.2 (8.9-18.2)	Reference
100-200	177	14	7.9 (5.2-12.9)	0.079
>200	212	27	12.7 (8.4-17.9)	0.139
Slaughter services				
No	133	16	12.0 (7.3-19.8)	Reference
Yes	321	38	11.8 (9.7-13.1)	0.967
Category of market				
Trading centre	82	4	4.9 (2.0-13.5)	Reference
Major town	206	31	15.5 (12.3-19.4)	0.003**
City market	189	19	10.1 (6.6-17.2)	0.032**

**Proportion with asterisks are different compared to others ($p<0.05$); Parentheses represent 95% confidence intervals.

infectious pathogens (McCarron et al., 2015). Geographical variation as seen in this study could also indicate the locality variation in epidemiology and ecology of NDV, which results in some areas experiencing the inter-epidemic period with low virus activity while others experience high viral activity in early infection (Roy, 2012).

The study revealed low awareness and use of ND vaccination among poultry keeping households in the study area. This is in agreement with Ndegwa et al. (2015) who reported low adoption of ND vaccination in village poultry flocks in Kenya. Vaccination is the most effective control method for ND (ACIAR, 2014), however various socioeconomic factors are thought to limit its uptake in village poultry flocks (Copland and Alders, 2013). This highlights the need for intervention to improve uptake and sustainability of ND vaccination in village poultry.

In this study, reverse transcription-polymerase chain reaction (RT-PCR) allowed rapid detection of NDV directly from diagnostic tracheal and cloacal swabs without the need to first isolate the virus in embryonated eggs. Although virus isolation in embryonated chicken eggs remains the “gold standard” method of NDV identification, it is time-consuming (5 to 10 days) and requires additional period to determine the isolate’s pathogenicity (OIE, 2015). Using RT-PCR, we detected NDV within 24 h. This suggests that the approach could significantly reduce the time required to respond to the introduction of Newcastle disease outbreaks (ND) and impact on the spread of the disease from farms or across regions (Abdisa and Tagesu, 2017). It can also become a powerful tool where targeted control of ND is needed.

Ethical considerations

All interviewed persons gave their informed consent prior to their inclusion in the study. All procedures performed in studies involving handling and slaughter of birds was in accordance with the ethical standards of the animal welfare committee of the Veterinary Research Institute-Kenya Agricultural and Livestock Research Organization.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abdisa T, Tagesu T (2017). Review on Newcastle Disease of Poultry and its Public Health Importance. Journal of Veterinary Science and Technology 8:1-7.

Australian Centre for International Agricultural Research (ACIAR) (2014). Newcastle disease control in Africa, ACIAR impact assessment series. http:// aciar.gov.au/files/ias_87-web.pdf

Alexander DJ, Bell JG, Alders RG (2013). A Technology Review: Newcastle Disease. Journal of Chemical Information and Modeling 53:1689-1699.

Amarasinghe GK, Bejerman N, Kim-Blasdell BR, Alisa Bochnowski B, Thomas BB, Alexander BB, Maisner A, Payne SL, Wahl-Jensen V, Peter W BJ, Werren JH, Anna WBE, Kuhn JH (2017). Taxonomy of the order Mononegavirales: update 2017. Archives of Virology 162:2493-2504.

Ashraf A, Shah MS (2014). Newcastle Disease: Present status and future challenges for developing countries. African Journal of Microbiology Research 8:411-416.

Assam A, Abdu PA, Joannis TM, Nok AJ (2011). Influenza A antigen, Newcastle and Gumboro diseases antibodies in apparently healthy local poultry. Bulletin of Animal Health and Production in Africa 59:25-35.

Barman LR, Sarker RD, Das BC, Chowdhury EH, Islam PMD (2016). Avian influenza and Newcastle disease virus in dead chickens in markets in Dhaka , Bangladesh in 2011-2012. 33:8-15.

Byarugaba DK, Mugimba KK, Omony JB, Okitwi M, Wanyana A, Otim MO, Kirunda H, Nakavuma JL, Teillaud A, Paul MC, Ducatez MF (2014). High pathogenicity and low genetic evolution of avian paramyxovirus type I (Newcastle disease virus) isolated from live bird markets in Uganda. Virology Journal 11:173.

Chaka H, Goutard F, Bisschop SPR, Thompson PN (2012). Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia. Poultry Science 91:862-869.

Chaka H, Goutard F, Gil P, Abolnik C, de Almeida RS, Bisschop S, Thompson PN (2013). Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. Tropical Animal Health and Production 45:705-714.

Charan J, Kantharia N (2013). How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics 4:303.

Copland JW, Alders RG (2013). The Australian village poultry development programme in Asia and Africa. World's Poultry Science Journal 61:31-38.

Eze I, Amos I, Chibuogwu A (2015). The Serological status for Newcastle Disease in Local Chickens of Live bird Markets and Households in Nsukka, Enugu State, Nigeria. Nigerian Journal of Microbiology 29:3096-3104.

Ganar K, Das M, Sinha S, Kumar S (2014). Newcastle disease virus: Current status and our understanding. Virus Research 184:71-81.

Hugo A, Makinde OD, Kumar S, Chibwana FF (2017). Optimal control and cost effectiveness analysis for Newcastle disease eco-epidemiological model in Tanzania. Journal of Biological Dynamics 11:190-209.

Jibril AH, Umoh JU, Kabir J, Saidu L, Magaji AA, Bello MB, Raji AA (2014). Newcastle Disease in Local Chickens of Live Bird Markets and Households in Zamfara State, Nigeria. Hindawi Publishing Corporation. http://dx.doi.org/10.1155/2014/513961

Kemboi DC, Chegeh HW, Bebora LC, Maingi N, Nyaga PN, Mbuthia PG, Njagi LW, Githinji JM (2013). Seasonal Newcastle disease antibody titer dynamics in village chickens of Mbeere District, Eastern Province, Kenya. Livestock Research for Rural Development 25(10). Article #181 http://www.lrrd.org/lrrd25/10/kemb25181.htm

Kenya National Bureau of Statistics (KNBS) (2016). Economic Survey 2016. http://www.knbs.or.ke

Khan MY, Arshad M, Mahmood MS, Hussain I (2011). Epidemiology of Newcastle disease in rural poultry in Faisalabad, Pakistan. International Journal of Agriculture and Biology 13:491-497.

Liu H, Wang Z, Wu Y, Wu Y, Sun C, Zheng D, Xu T, Li J (2008). Molecular characterization and phylogenetic analysis of new Newcastle disease virus isolates from the mainland of China. Research in Veterinary Science 85:612-616.

McCarron M, Munyua P, Cheng PY, Manga T, Wanjohi C, Moen A, Mounts A, Katz MA (2015). Understanding the poultry trade network in Kenya: Implications for regional disease prevention and control. Preventive Veterinary Medicine 120:321-327.

Ministry of Agriculture Livestock and Fisheries (MoALF) (2015). MoALF-Strategic Plan 2013-2015. http://dx.doi.org/10.4172/2157-7579.1000441

Mulisa DD, Kiros MKW, Alemu RB, Keno MS, Furaso A, Heidari A, Chibsa TR, Chunde HC (2014). Characterization of Newcastle Disease Virus and poultry-handling practices in live poultry markets, Ethiopia. Springerplus 3:459.

Munir M, Abbas M, Khan MT, Zohari S, Berg M (2012). Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. Virology Journal 9:46.

Ndegwa JM, Mead R, Norrish P, Shepherd D, Kimani C, Wachira A, Siamba D (2015). Evaluating Interventions Uptake in Indigenous Chicken Production in a Participatory Research with Smallholder Farmers in Kenya. Journal of Agricultural Studies 3:145.

Njagi LW, Nyaga PN, Mbuthia PG, Bebora LC, Michieka JN, Kibe JK, Minga UM (2010a). Prevalence of Newcastle disease virus in village indigenous chickens in varied agro-ecological zones in Kenya. Livestock Research for Rural Development P 95.

Njagi LW, Nyaga PN, Mbuthia PG, Bebora LC, Michieka JN, Minga UM (2010b). A retrospective study of factors associated with Newcastle disease outbreaks in village indigenous chickens in Africa. Bulletin of Animal Health and Production in Africa 58:22-33.

OIE (2018).OIE-listed diseases, infections and infestations in force in 2018. http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2018

OIE (2015). Newcastle Disease: General Inf. disease sheets. http://www.oie.int/fileadmin/Home/eng/Media_Centre/docs/pdf/Disease_cards/NEWCAS-EN.pdf

Olwande PO, Okuthe SO, Ogara WO, Bebora LC (2016). Participatory epidemiological assessment of factors that limit indigenous chicken productivity under free-range system in south western Kenya. Livestock Research for Rural Development P 183.

Omony JB, Wanyana A, Mugimba KK, Kirunda H, Nakavuma JL, Otim-Onapa M, Byarugaba DK (2016). Disparate thermostability profiles and HN gene domains of field isolates of Newcastle disease virus from live bird markets and waterfowl in Uganda. Virology Journal 13:103.

Rimi NA, Sultana R, Muhsina M, Uddin B, Haider N, Nahar N, Zeidner N, Sturm-Ramirez K, Luby SP (2017). Biosecurity Conditions in Small Commercial Chicken Farms, Bangladesh 2011–2012. Ecohealth 14:244-258.

Roy P (2012). Diagnosis and control of Newcastle disease in developing countries. World's Poultry Science Journal 68(4):693-706.

Sandhu BS, Brar RS, Brar APS, Sood NK, Singla LD (2009). Prevalence and pathology of parasitic gastrointestinal infections of poultry in Punjab. Indian Veterinary Journal 86:1276-1277.

Singla LD, Gupta SK (2012). Advances in diagnosis of coccidiosis in poultry. In: *Veterinary Diagnostics: Current Trends,* Gupta RP, Garg SR, Nehra V and Lather D (Eds), Satish Serial Publishing House, Delhi pp. 615-628.

Solomon P, Abolnik C, Joannis TM, Bisschop S (2012). Virulent Newcastle disease virus in Nigeria: Identification of a new clade of sub-lineage 5f from livebird markets. Virus Genes 44:98-103.

Sultana R, Hussain SA, Ali R, Zaidi FH, Anjum R (2014). A study on prevalence of economically important viral diseases in poultry flock in district Lahore. Science International 26:333–335. http://www.sci-int.com/pdf/156239210060--Razia_VET[1]-POULTARY---LAHORE--19-oct.pdf.