Sokoine University of Agriculture

MPhil Dissertation

Epidemiological Study of Newcastle Disease Virus in a Live Bird Market in Morogoro Municipality, Tanzania

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EPIDEMIOLOGICAL STUDY OF NEWCASTLE DISEASE VIRUS IN A LIVE BIRD MARKET IN MOROGORO MUNICIPALITY, TANZANIA

Dissertation Submitted to Sokoine University of Agriculture in Partial Fulfilment of the Requirements for the Degree of Master of Philosophy

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EXTENDED ABSTRACT

Village poultry plays a vital role in providing essential nutrition and income for rural communities in Africa. In this context, poultry are typically traded through live bird markets, which serve as central trading hubs where producers connect with traders and consumers, facilitating the flow of poultry products along the value chain. While they serve as important trading hubs, these markets create an environment where pathogens, like Newcastle disease virus (NDV) and avian influenza, can easily emerge and spread. To date, half of NDV class II genotypes have been reported in Africa (I, IV, V, VI, VII, XI, XIII, XIV, XVII, XVIII, and XXI). The information on the circulating NDV genotypes is still scarce despite the endemic nature of ND in most countries on the African continent. Improving our understanding of the epidemiology of NDV in live bird markets is important for assessing the circulating genotypes, disease risks and identifying factors that contribute to its persistence. A one-year repeated cross-sectional study was employed to survey local chickens at the Mawenzi live bird market in Morogoro municipality for NDV presence, its temporal and spatial distribution, and risk factors for NDV infection. The oro-cloacal and blood samples were collected from 659 local chickens between June 2020 and May 2021. The sampling was conducted once every week and questionnaires administered to the middlemen and traders at the same time. The questionnaire targeted the information on the source of the chickens, vaccination status, whether homes from where chickens were bought keep mixed poultry species, transit time from the villages to the market, time taken from collection of chickens from the villages to arrival to the market. Newcastle disease virus was detected by using reverse transcription real-time polymerase chain reaction (RT-qPCR) and conventional PCR followed by sequencing of PCR products. Twenty-three percent of 659 local chickens sampled were positive for NDV based on PCR. Increased odds of NDV infection were identified in chickens that had been in the market for two or more days prior to sampling. Four significant spatiotemporal clusters of NDV-positive chickens encompassing 13 villages were detected between August and October 2020, illustrating geographic hotspots of infection when NDV was most prevalent. Furthermore, Sequencing and phylogenetic analysis revealed the presence of sub-genotype VII.2. Similar to other live bird markets, this market had enclosures with high densities of birds of mixed species, limited biosecurity, and the presence of birds with observable illness. Bird traders who source the chickens from the villages, described long transit times in mixed enclosures with limited sanitation practices without consideration of sick birds or vaccination status prior to arriving at the live bird market. The detected sub-genotype VII.2 has phylogenetic links to Zambian NDV strains implying a Southeast dissemination of the virus, considering that it was first detected in Mozambique. This study highlights the need to invest in infrastructure and biosecurity for live bird markets as well as training opportunities for increasing traders' knowledge on hygiene, sanitation, animal welfare, and poultry biosecurity measures. Moreover, the study underscores the need of active NDV surveillance to determine the distribution of this NDV genotype in the country and monitor its spread and contribution to the emergence of new ND virus strains.

IKISIRI KUU

Kuku wa kienyeji wana jukumu muhimu katika kutoa lishe muhimu na mapato kwa jamii za vijijini barani Afrika. Katika muktadha huu, kuku kwa kawaida huuzwa kupitia masoko ya ndege hai, ambayo hutumika kama vitovu kuu vya biashara ambapo wazalishaji huungana na wafanyabiashara na watumiaji, kuwezesha mtiririko wa bidhaa za kuku kwenye mnyororo wa thamani. Ingawa zinatumika kama vitovu muhimu vya biashara, masoko haya yanaunda mazingira ambapo vimelea vya magonjwa, kama vile virusi vya ugonjwa wa **Mdondo** (NDV) na mafua ya ndege (AI), vinaweza kuibuka na kuenea kwa urahisi. Hadi sasa, nusu ya jamii za virusi katika kundi la pili la NDV zimeripotiwa katika Afrika (I, IV, V, VI, VII, XI, XIII, XIV, XVII, XVIII, na XXI).

Taarifa kuhusu aina za jeni za mdondo zinazozunguka bado ni chache licha ya hali ya kawaida ya Mdondo katika nchi nyingi za bara la Afrika. Kuboresha uelewa wetu wa epidemiolojia ya virusi vya **Mdondo** katika masoko ya ndege hai ni muhimu kwa kutathmini aina za jeni zinazozunguka, hatari ya magonjwa na kutambua sababu zinazochangia kuendelea kwake. Utafiti wa mwaka mmoja unaorudiwa wa sehemu mbalimbali ulifanyika kuchunguza kuku wa kienyeji katika soko la ndege la Mawenzi katika manispaa ya Morogoro kwa uwepo wa mdondo, namna ugonjwa ulivyotawanyika kulingana na wakati wa mwaka na eneo, na sababu za hatari kwa maambukizi ya mdondo. Sampuli za kutoka mdomoni (mate au makohozi) nan jia ya haja kubwa na damu zilikusanywa kutoka kwa kuku 659 wa kienyeji kati ya Juni 2020 na Mei 2021.

Sampuli ilifanyika mara moja kila wiki na dodoso zilijibiwa na wachuuzi na wauzaji wa kuku sokoni kwa wakati mmoja. Dodoso hilo lililenga taarifa za chanzo cha kuku hao, hali ya chanjo, iwapo nyumba walikonunuliwa kuku wanafuga kuku mchanganyiko, muda wa kusafiri kutoka vijijini kwenda sokoni, muda kutoka vijijini hadi kufika sokoni. Virusi vya ugonjwa wa Mdondo viligunduliwa kwa kutumia unukuzi wa reverse-time polymerase chain reaction (RT-qPCR) na PCR ya kawaida ikifuatiwa na mpangilio wa bidhaa za

PCR. Asilimia 23 ya kuku 659 wa kienyeji waliochukuliwa walikuwa na virusi kwa kuzingatia PCR. Ongezeko la uwezekano wa maambukizi ya **mdondo** ulitambuliwa kwa kuku ambao walikuwa sokoni kwa siku mbili au zaidi kabla ya kuchukua sampuli. Maeneo manne muhimu walikotoka kuku waliopatikana na **mdondo** yanavyojumuisha vijiji 13 viligunduliwa kati ya Agosti na Oktoba 2020, ikionyesha maeneo yenye maambukizi ya kijiografia wakati **mdondo** ulikuwa umeenea zaidi. Zaidi ya hayo, Mfuatano na uchanganuzi wa filojenetiki ulibaini kuwepo kwa aina ya jeni VII.2. Sawa na masoko mengine ya ndege hai, soko hili lilikuwa na vizimba vilivyo na msongamano mkubwa wa ndege wa aina mchanganyiko, usalama mdogo wa viumbe hai, na kuwepo kwa ndege walio na ugonjwa unaoonekana. Wafanyabiashara wa ndege wanaopata kuku hao kutoka vijijini, walielezea muda mrefu wa kuku kuishi katika vizimba vyenye mchanganyiko na taratibu ndogo za usafi wa mazingira bila kuzingatia hali ya ndege wagonjwa au chanjo kabla ya kufika kwenye soko la ndege hai. Aina ya jeni VII.2 iliyogunduliwa ina uhusiano wa kifilojenetiki na aina ya **mdondo** ya Zambia ikimaanisha kuenea kwa virusi kutoka kusini kuelekea mashariki, ikizingatiwa kwamba iligunduliwa kwa mara ya kwanza nchini Msumbiji. Utafiti huu unaonyesha haja ya kuwekeza katika miundombinu na usalama wa viumbe hai kwa masoko ya ndege hai pamoja na fursa za mafunzo kwa ajili ya kuongeza ufahamu kwa wafanyabiashara kuhusu usafi, usafi wa mazingira, ustawi wa wanyama na hatua za usalama wa kuku. Zaidi ya hayo, utafiti unasisitiza haja ya ufuatiliaji hai wa virusi vya **mdondo** ili kubaini usambazaji wa aina hii au nyingine ya virusi vya **mdondo** nchini na kufuatilia kuenea na mchango wake katika kuibuka kwa aina mpya za virusi vya **Mdondo**.

DECLARATION

I, **JOHN BENJAMIN TSAXRA,** do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work and it has neither been nor concurrently been submitted for a higher degree award in any other institution.

03/05/2024

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DEDICATION

This research work is dedicated to my late beloved wife and parents, and my lovely daughters.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

%	Percentage
<	Less than
>	Greater than
AIC	Akaike Information Criterion
AID-OAA-A	Cooperative agreement
AOaV	Avian Orthoavulavirus
APMV	Avian paramyxovirus
bp	Base pair
℃	Degree Celcius
CI	Confidence Interval
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DPRTC	Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy
DSI	Department of Science and Innovation
e.g	For example
ELISA	Enzyme-linked Immunosorbent Assay
et al	And others
F gene	Fusion gene
Fam	Fluorescein amidites
FAO	The Food and Agriculture Organization
Fig	Figure
G	Gauge
GTR	General Time-Reversible
HI	Haemagglutination inhibition
HPAI	Highly Pathogenic Avian Influenza
ID	Identification
Km	Kilometer
L gene	Large protein gene (RNA-dependent RNA polymerase)
LBM	Live bird market
LMIC	Low and middle-income countries
log	Logarithm
M gene	Matrix gene
MAFFT	Multiple Alignment using Fast Fourier Transform

MGB	Minor groove binder
mL	Milliliter
MLBM	Mawenzi Live Bird Market
NCBI	The National Center for Biotechnology Information
ND	Newcastle disease
NDV	Newcastle disease virus
NP	Nucleoprotein
NRF	National Research Foundation
OR	Odd ratio
P	Probability
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
qGIS	Quantum Geographic Information System
RBC	Red blood cell
RNA	Ribonucleic acid
rRT-PCR	Real-time reverse transcription Polymerase Chain Reaction
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Reverse transcription real-time polymerase chain reaction
ssRNA	Single-stranded negative-sense Ribonucleic acid
SUA	Sokoine University of Agriculture
USA	United States of America
USAID	United States Agency for International Development
v/v	Volume per volume
vNDV	Velogenic Newcastle disease virus
WOAH	World Organization for Animal Health
μL	Microliter

STRUCTURE OF THE DISSERTATION

This dissertation consists of FOUR chapters, chapter ONE describes background information on Newcastle disease, problem statement and research justification and objectives. Chapter TWO (Manuscript ONE) describes spatiotemporal patterns and risk factors for Newcastle Disease Virus (NDV) among chickens at Mawenzi Live Bird Market (MLBM) in Morogoro, Tanzania**.** Chapter THREE (Manuscript TWO) describes molecular characterization of Newcastle disease viruses obtained from MLBM in Morogoro, Tanzania in 2020-2021**.** Chapter FOUR consists of General discussion, conclusions and recommendations.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

1.1.1 Local chicken husbandry in Tanzania

Traditional poultry keeping is widely practiced in rural areas of low and middle-income countries throughout the world. Local chicken production is a low-input system requiring minimal investment (Branckaert, 2007) making it accessible to resource-poor communities (Wong *et al*., 2017). Even households without land can raise chickens because they can be sustained through scavenging for food on communal village land (Sonaiya, 1990; Barua and Yoshimura, 1997; Kitalyi, 1998; Branckaert *et al*., 2000; Permin *et al*., 2001). Village poultry can provide households with earnings and a source of relatively inexpensive animal protein a nutritionally-rich food, thereby contributing to food security and poverty alleviation (Gueye, 2000). Furthermore, chickens play an important role in sociocultural practices as they are offered as gifts for friends and family, as a welcome meal for visitors, and for many other rituals (FAO, 2006).

Local chicken production is common among households in Tanzania, especially in resource-limited communities. In villages of Low and Middle-Income Countries (LMIC), such as some rural areas in Tanzania, the chickens are primarily kept in extensive scavenging systems (FAO, 2014). Diseases, predation, feed insufficiency, and shelter are among many factors affecting local chicken production in Tanzania.

1.1.2 Newcastle disease

Newcastle disease (ND) serve as a major constraint to poultry production in these settings (Alexander, 2000; Mwalusanya *et al*., 2002; Sonaiya, 2007; Ananth *et al*., 2008; Alfred *et al*., 2012). In Tanzania, vaccination against ND is the main means of controlling the disease (Alders *et al*. 2010). In Tanzania, however, it is challenging to implement effective vaccination programs in remote village settings (Campbell *et al*., 2018) due to a lack of experts and a cold chain system. In the extensive scavenging systems, poultry from different households, ages, and species comingle with each other and sometimes encounter wild birds presenting opportunities for pathogen transmission. In these settings, some of the birds may be vaccinated against ND, whilst others are not (Msoffe *et al*., 2010; Wong *et al*., 2017), thereby, the inconsistency of ND vaccination can increase the risk of NDV outbreaks among local chicken flocks.

1.1.3 Aetiology of Newcastle disease

Newcastle disease virus (NDV), the virus that causes ND is highly contagious, infecting both domestic and wild bird species, making the disease one of the most important socioeconomical disease of domestic poultry in Africa (Mngumi *et al*., 2022). The virus is classified into pathotypes; lentogenic (low virulence), mesogenic (medium virulence), and velogenic (high virulence) depending on their virulence (Suarez *et al*., 2020). All pathotypes of NDV occur in rural poultry, but velogenic strains are most commonly reported in Africa (Awan *et al*., 1994; Yongolo *et al*., 2011 and Msoffe *et al*., 2019). In susceptible chicken populations, velogenic NDV (vNDV) causes mortality up to 100% in affected flocks (Alexander *et al*., 2012).

The etiology of ND Avian Orthoavulavirus type- 1 (AOaV-1) previously known as avian paramyxovirus type- 1 (APMV-1) (Kuhn *et al*., 2019). It is a single-stranded negative-sense RNA (-ssRNA) virus (Alexander and Senne, 2008) with a genome of 15.2 kb base pairs and six genes. The genome encodes six proteins namely; nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and the RNA-dependent RNA polymerase (L) (de Leeuw and Peters, 1999; Seal *et al*., 2000). All NDV strains belong to a single serotype, but there is substantial genetic and antigenic variation across strains (Diel *et al*., 2012; Snoeck *et al*., 2013). AOaV-1 is subdivided into two classes:

class I and class II, with class I NDVs primarily encompassing lentogenic viruses commonly found in wild birds and less frequently in poultry and class II NDVs consists of all pathotypes (Suarez *et al*., 2020), these strains are detected in multiple wild birds and domestic poultry species worldwide. Class I viruses have only one genotype, while Class II viruses have 20 distinct genotypes (Dimitrov *et al*., 2019). In addition to genotype, the nucleotide sequence of the cleavage site of the F gene determines the pathogenicity of NDV (Diel *et al*., 2012; Dimitrov *et al*., 2019). The clinical signs caused by NDV infection in chickens are variable based on the pathogenicity of the strain. The signs range from none (asymptomatic infection) to severe as decreased egg production, depression, diarrhea, respiratory distress, and neurological signs (Dimitrov *et al*., 2019).

1.1.4 Marketing of local chickens in Tanzania

In Tanzania, indigenous chickens are mainly sold through live bird markets (LBMs), because of the lack of a cold chain to distribute chilled meat (Msoffe *et al*., 2019). Live bird markets congregate live chickens of mixed species from smallholder farmers located throughout the country. They provide a platform for small-scale poultry producers to access wider markets beyond their immediate communities.

1.1.5 Marketing systems of poultry as potential source of ND transmission

Because live bird markets maintain a constant influx of new birds and have minimal biosecurity, they can act as a source of pathogens, such as NDV and influenza A virus, for chickens, other species in the market. In light of this, they also pose a risk to humans due to the zoonotic potential of some viruses affecting livestock (Munyua *et al*., 2013; Mulisa *et al*., 2014; Ogali *et al*., 2018; Ipara *et al*., 2019; Msoffe *et al*., 2019). In these settings, local chickens can become infected and serve as reservoirs for other susceptible poultry species in commercial farms and wild species (Olabode *et al*., 1992).

1.1.6 Characterization of the NDVs

The NDV fusion (F) gene is commonly targeted for the classification of NDV into genotypes (Acheson *et al*., 2011; Dortmans *et al*., 2011; da Silva *et al*., 2020) using both partial and complete sequences (Miller *et al*., 2015; Butt *et al*., 2018).

In Africa, a range of NDV genotypes have been reported, including genotypes I, II, IV, V, VI, VII, XI, XIII, XIV, XVII, XVIII and XXI (Dimitrov *et al*., 2019; Zanaty *et al*., 2019; Mngumi *et al*., 2022). Over the past few decades, investigators in Tanzania have isolated and characterized both lentogenic and velogenic strains of NDVs (Loretu and Mkaria, 1981; Yongolo *et al*., 2011; Msoffe *et al*., 2019; da Silva *et al*., 2020), some of which were detected in live bird markets. Previous research has revealed a seroprevalence of 26% for ND in major live bird markets in two regions of Tanzania, Morogoro and Dar es Salaam (Chiwanga, 2012). The first isolation and pathotyping of NDV was performed by Loretu and Mkaria in 1981. More recently, researchers in Tanzania have isolated and characterized both velogenic and lentogenic NDV strains of genotypes V and XX from backyard chickens (Yongolo *et al*., 2011), and genotypes V and XIII.1.1 from live bird markets (Msoffe *et al*., 2019). In addition, da Silva *et al*. (2020) reported genotypes V, VII.2, and XIII in chickens. While NDV is endemic and causes devastating economic losses in indigenous chickens in Tanzania, our understanding of the diversity of NDV genotypes circulating among village poultry and in live bird market settings is still limited. Tanzania's borders, like those of many other countries in the region, allow the relatively unrestricted trade in live chickens within the sub-region and have resulted in the spread of ND across East Africa. This study aimed to investigate the prevalence and spatiotemporal patterns and risk factors for NDV among chickens in a live bird market, identify and molecularly characterize NDV genotypes circulating among local chickens obtained from a live bird market serving as a central poultry trading hub in Tanzania in 2020 and 2021.

1.2 Problem Statement and Research Justification

Local chickens are the dominant species of poultry kept in the developing countries including Tanzania. Production of the local chickens face challenges which includes; diseases, predation, feeds insufficiency and shelter. Newcastle disease is still the most devastating disease to local chickens causing up to 100% mortality in affected flocks. The disease is more common in local chickens than in commercial flocks. Live bird markets could be a reservoir for ND for the chickens. Epidemiological studies for the disease in local chickens are scarce. The live bird market offers a unique opportunity to encounter NDV from various parts hence increases the chances of elucidating their diversity. This provides necessary information on the virus prevalence, circulating viruses, spatiotemporal patterns and risk factors for NDV transmission among chickens in the live bird markets.

1.3 Objectives

1.3.1 General objective

To determine the prevalence, spatiotemporal patterns and genotype diversity of Newcastle disease virus in local chickens in live bird market in Morogoro Municipality, Tanzania.

1.3.2 Research questions

1. What are the sources of the chickens sold at Mawenzi live bird market?
2. How do spatial and temporal patterns influence NDV transmission across chicken population?
3. What NDV genotypes are circulating among local chickens at the live bird market?

1.3.3 Specific objectives

1. To determine the sources (focal points) of the local chickens brought for sale in the live bird market across the months.
2. To assess spatial and temporal patterns and risk factors for NDV among ND positive chickens in a live bird market.

3. To identify and molecularly characterize NDV genotypes circulating among local chickens obtained from a live bird market.

CHAPTER TWO

Manuscript One

2.0 SPATIOTEMPORAL PATTERNS OF DISTRIBUTION AND RISK FACTORS FOR NEWCASTLE DISEASE VIRUS AMONG CHICKENS IN A TANZANIA LIVE BIRD MARKET

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Abstract

Village poultry plays a vital role in providing essential nutrition and income for rural communities in Africa. In this context, poultry are typically traded through live bird markets, which serve as central trading hubs where producers connect with traders and consumers, facilitating the flow of poultry products along the value chain. While they serve as important trading hubs, these markets create an environment where pathogens, like Newcastle disease virus (NDV) and avian influenza, can easily emerge and spread. Improving our understanding of the epidemiology of NDV in live bird markets is important for assessing disease risks and identifying factors that contribute to its persistence. Local chickens at the Mawenzi live bird market in Morogoro municipality were surveyed for NDV presence, its temporal and spatial distribution, and risk factors for NDV infection. Twenty-three percent of 659 local chickens sampled over a one-year period were positive for NDV based on PCR. Increased odds of NDV infection were identified in chickens that had been in the market for two or more days prior to sampling. Four significant spatiotemporal clusters of NDV-positive chickens encompassing 13 villages were detected between August and October 2020, illustrating geographic hotspots of infection when NDV was most prevalent. Similar to other live bird markets, this market had enclosures with high densities of birds of mixed species, limited biosecurity, and the presence of birds with observable illness. Bird traders who source the chickens from the villages, described long transit times in mixed enclosures with limited sanitation practices without consideration of sick birds or vaccination status prior to arriving at the live bird market. This study highlights the need to invest in infrastructure and biosecurity for live bird markets as well as training opportunities for increasing traders' knowledge on hygiene, sanitation, animal welfare, and poultry biosecurity measures.

Key words: Spatio-temporal patterns, Newcastle disease virus, Prevalence, Local chickens, Live bird market, Morogoro, Tanzania

2.1 Introduction

Traditional poultry keeping is widely practiced in rural areas of low and middle-income countries throughout the world. Local chicken production is a low-input system requiring minimal investment [1] making it accessible to resource-poor communities [2]. Even households without land can raise chickens because the birds can be sustained through scavenging for food on communal village land [3, 4, 5, 7, 8]. Village poultry can provide households with earnings and a source of relatively inexpensive protein and nutritionally-rich food, thereby contributing to nutritional security and poverty alleviation [6]. Furthermore, chickens play an important role in sociocultural practices as they are offered as gifts for friends and family, as a welcome meal for visitors, and for other rituals [9].

Local poultry production is common among households in Tanzania, especially in resource-limited communities. In Tanzania and elsewhere, smallholder poultry producers face a number of challenges when rearing village poultry flocks, including predation, poor husbandry, as well as disease [5, 10]. Newcastle disease (ND), in particular, is an important constraint to traditional poultry keeping in sub-Saharan Africa with high mortalities among local flocks every year [11-14]. In Tanzania, vaccination against ND is utilized as a means of controlling the disease [11]; however, it is challenging to implement effective vaccination programs in remote village settings.

Newcastle disease (ND) is caused by avian *orthoavulavirus* 1 (AOaV-1; formerly avian paramyxovirus- 1) which belongs to the genus Avulavirus, subfamily Paramyxovirinae, and family Paramyxoviridae [15]. ND virus is a negative sense single-stranded RNA-enveloped virus [16]. AOaV-1 viruses consist of virulent and avirulent strains which fall into a single serotype. The phylogenetic analysis of the fusion (F) gene shows that NDV can be grouped into two classes, Class I and Class II, with the former primarily including the avirulent viruses most commonly found in wild birds and the latter encompassing the virulent viruses found in both wild birds and poultry. Class II is further divided into 20 genotypes [17]. Furthermore, NDV can be divided into pathotypes; lentogenic (low virulence), mesogenic (medium virulence), and velogenic (high virulence) [18]. All pathotypes of NDV occur in rural poultry, but velogenic strains are most commonly reported in Africa [19-21]. Velogenic Newcastle disease virus (vNDV) is considered endemic among village chickens in most low-middle income countries [22, 23] and epidemics of vNDV result in devastating economic losses on poultry production [24].

Over the past few decades, investigators in Tanzania have isolated and characterized both lentogenic and velogenic strains of NDVs [20, 21, 25, 26], some of which were detected in live bird markets. Previous research has revealed a seroprevalence of 26% for ND in major live bird markets in two regions of Tanzania, Morogoro and Dar es Salaam [27]. Live bird markets congregate live chickens from smallholder farmers located throughout the country. They provide a platform for small-scale poultry producers to access wider markets beyond their immediate communities. Local chickens are sold largely through the live bird markets because of the lack of a cold chain for the distribution of chilled fresh meat directly to the markets in urban areas [21]. Because these markets maintain a constant influx of new birds and have minimal biosecurity, they can act as a source of pathogens, such as NDV and influenza A virus, for chickens, other species in the market and also, they pose a risk to humans due to the zoonotic potential of some viruses affecting livestock [21, 28-31]. In these settings, indigenous poultry can become infected and serve as reservoirs for more susceptible exotic breeds in commercial farms [32] as well as wild birds coming into contact with infectious birds and/or materials at the market. Epidemiological studies are therefore increasingly valuable to gain additional insights into the diversity of circulating NDV strains and the ecology of the virus in the market and village settings. This study aimed to investigate the prevalence and spatiotemporal patterns of and risk factors for NDV among chickens in a live bird market in Tanzania.

2.2 Materials and Methods

2.2.1 Study area and design

Samples were collected weekly over a one-year period (June 2020 to May 2021) from the Mawenzi live bird market located in Morogoro municipality in Tanzania, 196 kilometers west of Dar es Salaam, the country's largest city and commercial center, and 260 kilometers east of Dodoma, Tanzania’s capital.

The Mawenzi live bird market is located within the general food market. The live poultry are sheltered in wood and wire mesh cages stacked on top of each other. Birds are provided food (maize bran) and water in the cages. There is a small bird slaughtering and processing area within the market that is located next to the bird enclosures and is devoid of a water supply and sanitation facilities [21].

Prior to initiating this project, the objectives of the study were introduced to the local government authority, market management entities, as well as poultry traders and middlemen. With permission from the local government authorities, sampling was conducted once a week targeting days when chickens were offloaded into the market from the villages. Permission for conducting this research in chickens was obtained from the Sokoine University of Agriculture (SUA/DPRTC/R/186). Institutional review boards at the Sokoine University of Agriculture and the University of California, Davis (DPRTC/R/T/VOL XXII/13) approved the study procedures for human subjects.

2.2.2 Sample collection and processing

Over the period of one year, a total of 659 chickens were sampled at the Mawenzi live bird market. Oro-cloacal swabs (n=659) and sera samples (n=657) were collected from the chickens. The chickens were randomly selected (first and every sixth count afterward) for sampling each week. Most samples were collected immediately after chickens were offloaded at the market from the villages, but some chickens were sampled after the bird had been in the market for two or more days.

The oro-cloacal samples were collected using sterile polyester-tipped swabs (Puritan, USA). The swabs from each bird were placed in individual vials with phosphate buffered saline (PBS) and immediately transported to the laboratory in cooler to be stored at -80℃ until further analysis. Whole blood (approximately 1ml) was collected from the brachial vein into a 3ml syringe (23G needle), the blood was left to clot overnight. Sera were decanted and stored in 1.5 ml Eppendorf tubes at -20˚C until hemagglutination inhibition (HI) assays were performed.

During each weekly sampling event, the following data were recorded: date of sample collection, the sex, health status, source (geographic location), and type of chicken sampled. Data was also collected on the timing of sampling of the chicken, i.e., whether the bird was sampled immediately after it was offloaded at the market for sale or after it had been in the market for two or more days.

2.3 Questionnaire

Questionnaires were administered to 23 chicken traders (16 middlemen and 7 sellers) who gave their consent to participate in the study. A middleman was defined as a person who collects poultry from the source and sells them to the seller at the market, and a seller was defined as a person who sells live poultry from a stand at the market. The aims of the study were communicated in Swahili, and written informed consent was obtained from all study participants. Questionnaires were administered to collect demographic information on the trader as well as data on the source of the bird (village), whether the chickens were housed together with other bird species at the source and during transit, the traders' criteria for selecting birds to sell in the market, whether chickens were obtained from one source all year-round, length of time spent in transit to the market, length of time spent at the market prior to sale, temporal patterns of illness in the traded birds, and biosecurity practices used by the trader in transporting and housing the birds prior to sale (e.g., vaccination, cage disinfection).

2.4 Serological analysis

Sera collected from local chickens at the Mawenzi live bird market were analyzed for antibodies against NDV using the haemagglutination inhibition (HI) assay as previously described [33, 34]. The assay was performed according to the OIE procedure [35]. Briefly, 25 μL of PBS was dispensed into each well of a plastic V-bottomed microtiter plate. A total of 25 μL of serum was placed into the first well of the plate. Two-fold dilutions of the 25 μL serum were then made across the plate. Twenty-five (25 μL) of 4 haemagglutinating units of ND viral antigen (LaSota) was added to each well and the plate was left for 30 minutes at room temperature. Chicken red blood cells (RBCs) (25 μL of 0.5% (v/v)) were then added to each well and, after gentle mixing by tapping the sides of the plate, RBCs were allowed to settle at room temperature for 40 minutes. The HI titer of each serum sample was expressed as the reciprocal of the serum dilution and most conveniently expressed as the logarithm to base 2 ($\log_2$). The agglutination was assessed by tilting the plates with those wells in which the RBCs streaming was observed at the same rate as the control wells (positive serum, virus/antigen, and PBS controls) considered to show inhibition [35].

2.5 Real-time reverse transcription PCR (rRT-PCR)

Total nucleic acid was extracted from oro-cloacal swabbed sample using the IndiMag Pathogen Kit in an IndiMag™ 48 instrument, according to the manufacturer’s instructions. Real-time reverse transcription PCR (rRT-PCR) was performed with VetMAX Plus RT-PCR kits (Thermo Fisher Scientific, MA, USA) (half-reactions) according to the manufacturer's recommended protocol and a 53°C annealing temperature, with MGB/Fam-labeled NDV L-gene specific primers and probes described by Fuller et al. [36], in a StepOne Plus thermal cycler (Applied Biosystems). Cycle threshold (Ct) values < 40 were considered positive for NDV.

2.6 Statistical Analyses

The NDV prevalence and seroprevalence and respective 95% confidence intervals were estimated for the samples. Latitude and longitude coordinates were assigned to each chicken source based on the village centroid (the center point of the village) where the chicken was collected. Temporal and spatial clustering of NDV was evaluated among chickens using Bernoulli model elliptical scanning windows in SaTScan v.10.0 [37-39]. A maximum spatial and temporal cluster size of 25% of the population at risk was used for the spatiotemporal analysis, and overlapping clusters were not permitted. For the analysis, one month was set as the time aggregation unit. Clusters were mapped using qGIS [40].

Unadjusted bivariate associations between NDV infection, host factors, and spatiotemporal variables were evaluated using Chi-square tests of independence. These variables were further evaluated for multivariable associations with NDV infection using mixed effects logistic regression models to evaluate the influence of putative risk factors on the odds of infection in chickens at the live bird market. Factors evaluated for their association with NDV infection included sex of the chicken, month of sample collection, geographical (region) origin of the bird prior to being offloaded at the market, and status of chicken at the time of sample collection (new arrival to the market or chicken present for >2 days in the market prior to sample collection). For all multivariable models, putative risk factors with $P < 0.20$ in the univariable analyses were evaluated in the mixed effects logistic regression models using the lme4 package in R [41] and retained in the models if $P < 0.05$. The ID of the chicken trader was included in the model as the cluster variable to account for unmeasured correlation among multiple chickens from one source.

In all models, NDV infection by month was evaluated for a difference in magnitude across months, and significance of effect between months using the likelihood ratio statistic and similar months were collapsed into the following time periods: January-March, April-July, and August-October. Possible confounding variables and interaction effects were evaluated in the model building process. Confounding was evaluated in the model through assessing whether there was a greater than 10% change between unadjusted and adjusted odds ratio estimates for the other variables in the model. Final parsimonious models were selected using Akaike's Information Criterion (AIC) for comparisons between nested and non-nested models. Overall model fit was evaluated using Hosmer-Lemeshow goodness-of-fit test and measures of information criteria. Odds ratios were estimated with 95% confidence intervals. All analyses were performed using R statistical software version 4.2.2 (October 31, 2022) [42].

2.7 Results

A total of 659 chickens were sampled during a 1-year period (from June 2020 to May 2021) at the Mawenzi LBM in Morogoro municipality. On average, there were 316 birds housed at the market at a given sampling point. The birds were of mixed species, including guinea fowl, ducks, and indigenous chickens. The birds were sourced from villages within the Morogoro, Dodoma, Tabora, Shinyanga, and Manyara regions. On average, one chicken exhibited clinical signs of illness during each weekly sampling event, which ranged from drowsiness, mucoid, and watery discharge from the mouth and nostrils, and diarrhea to swollen eyes, cyanotic comb and wattles, gasping, rales, and torticollis.

The overall NDV seroprevalence and prevalence, based on HI and PCR, among the chickens at the market over the study period were

22.1% (95% CI: 19.2%–25.5%) and 23.8% (95% CI: 21.1%–27.2%), respectively (Table 1). The Ct values for PCR positive samples ranged from 17.6 to 39.3 with a median of 31.1. NDV strains identified through genotyping are reported in a follow-up study.

Table 2.1: Summary of seropositive (by HI) and PCR positive samples for NDV broken down by time at which bird was sampled.

Sample collection time	Total sampled (659)	Seropositive samples (146)	PCR positive samples (157)
Immediately after offload	515	118(80.8%)	131(83.4%)
At 2-5 days after offload	144	28(19.2%)	26(16.6%)

In the multivariable analysis, chickens that had been held at the market for two or more days prior to sampling were three times more likely to test positive for NDV as compared to chickens that had just been offloaded for sale at the Mawenzi market (Table 2.2, Odds Ratio (OR) = 3.0 (95% CI: 1.4-6.8), P = 0.007). In addition, the odds of NDV infection were highest during the period extending from August to October followed by the period of April to July relative to the months of November through March (Table 2.2). Sex of the chicken was not significantly associated with NDV infection among birds in this study.

Table 2.2: Host and seasonal factors associated with NDV infection in the chickens sampled at the Mawenzi live bird market from June 2020 to May 2021.

Factor	OR	(95% CI)	P value
Status of chicken (reference group = Chicken as new arrival at market)			
Chicken in market $\geq$2 days	3	(1.4-6.8)	0.007
Time period (reference group = November-March)			
April-July	3.8	(1.3-6.7)	0.014
August-October	25.5	(9.7-66.7)	<0.001

In evaluating the temporal distribution of NDV infection in the live bird market chickens, results revealed a peak in the prevalence of the virus among chickens between August and October (Figure 2.1).

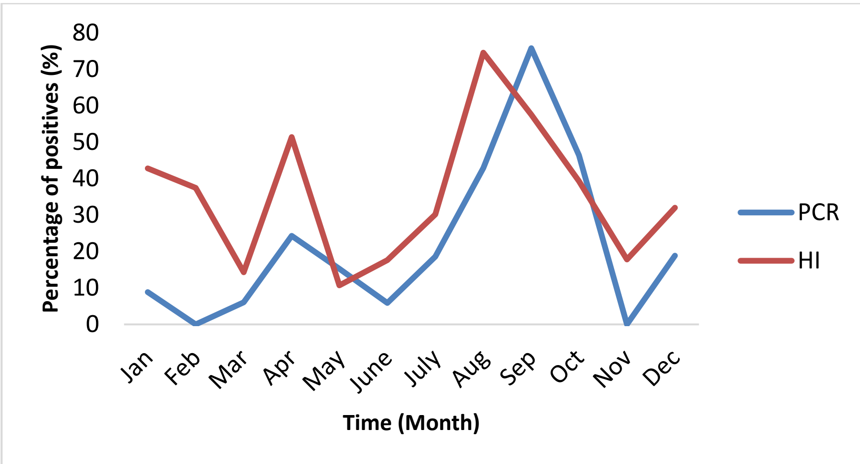

Figure 2.1: Monthly prevalence (PCR and HI based) of Newcastle disease virus and antibodies detected in chickens at the Mawenzi live bird market in Morogoro, Tanzania from June 2020 to May 2021.

Four significant spatiotemporal clusters of NDV-positive chickens (Figure 2.2) were detected with radii of 0 km, 83.7 km, 58.42 km, and 30.47 km with $P < 0.0001$, $P < 0.0001$, $P = 0.002$, and $P = 0.01$, respectively. The clusters, encompassing 13 villages in Morogoro (6), Manyara (1), Dodoma (2), Tabora (3), and Shinyanga (1) region were detected between August and October 2020.

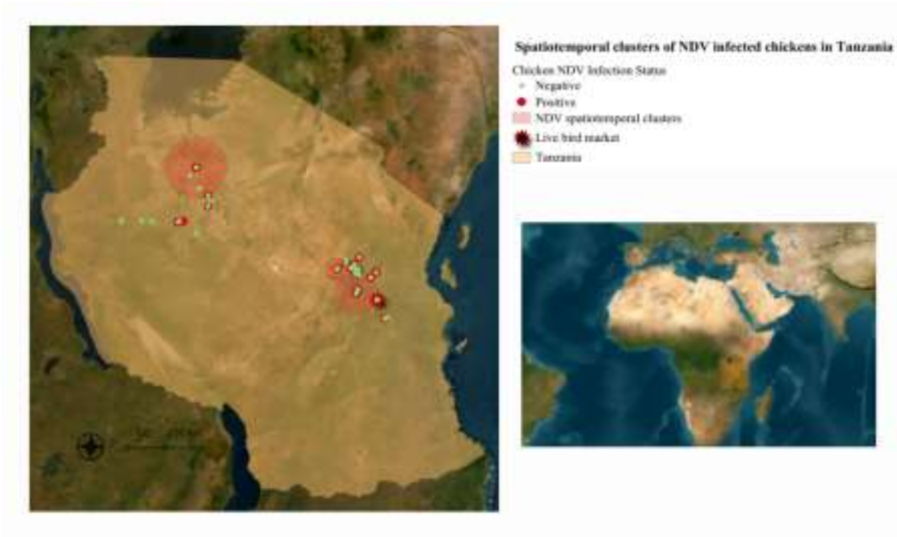

Figure 2.2: Map showing significant spatiotemporal clusters of Newcastle disease virus infected chickens transported and offloaded at the Mawenzi live bird market in Morogoro, Tanzania from June 2020 to May 2021

A total of 23 chicken traders (16 middlemen and seven sellers, all male) in the Mawenzi LBM provided responses to the questionnaire. The data stemming from questionnaires administered to these individuals revealed that chickens from different sources were mixed (pooled) in enclosures before and during transportation to the market. The average transit time of birds from the source to the market was 1–5 days. Middlemen collected and transported birds by public buses and/or trucks within cages with some birds tethered to the outside of the cages when space was limited. Chickens from different sources were mixed during transportation and at the point of sale in the market, including mixing of newly acquired birds with birds that were already present in the market. The questionnaire also revealed that chicken cages (collection and/or transport cages) were not disinfected between bird collections. Chickens were also pooled together at the market regardless of their health status. All of the respondents indicated that they were able to distinguish between ill and healthy birds. The respondents reported that they often encounter ill birds during the collection of chickens. When asked how sick birds are managed, 6% of traders indicated that sick birds were provided herbal remedies at the households. In addition, 18% of traders indicated that ill birds were slaughtered for home use and 76% reported that sick chickens were transported to the market for sale. Regarding the criteria of selection of chickens by the live bird traders, only 12% of the traders considered an ill bird to be unfit for transport and sale at the market. In addition, the traders indicated that vaccination history for the birds was unknown. When asked about seasonality of illness among collected birds, live bird traders reported that illness was most prevalent from June to October, compared to the other periods of the year.

2.8 Discussion

This study revealed that the local chicken trade associated with the Mawenzi live bird market in Morogoro Municipality serves as a source of circulating NDV and provides an ideal environment for virus transmission and regional spread among chickens and possibly other avian species in Tanzania. The NDV prevalence and seroprevalence among chickens sampled at the Mawenzi live bird market for this study were in the range of previously reported estimates in Tanzania (Table 2.1). Other ND studies which were conducted in the villages in Tanzania reported seroprevalences of 46.8% [43] and 13.3% [44]. The study conducted by Msoffe et al. [21] in live bird markets found a 32% prevalence of NDV. Mngumi and Bunuma reported a prevalence of 26% in the villages in Njombe and Bahi districts in Tanzania [45]. At Mawenzi live bird market chickens are brought from different sources within and outside of Morogoro region. In this study, chickens were sourced from parts of Morogoro, Shinyanga, Tabora, Dodoma, and Manyara regions. In Tanzania, the local poultry trade is unregulated with limited to no biosecurity along the market chain [46]. The absence of active NDV surveillance and biosecurity practices along the trading chain of local chickens poses a significant challenge for disease prevention and control strategies and may contribute to regular outbreaks of ND in the country [21]. Many studies have illustrated the potential role of unregulated poultry trade through live bird markets on ND activity. For example, congregating poultry originating from different areas along with transportation and poor biosecurity measures have been associated with NDV outbreaks in Ethiopia and Kenya [28, 29].

This spatiotemporal study has revealed that, with the exception of one month (February) (Fig 2.1), NDV is circulating year-round among the local chickens coming into the live bird market providing further evidence of the endemicity of this virus and the live bird market as a continued source of infection among birds originating from different parts of the country. The temporal and spatiotemporal patterns indicated seasonality of NDV activity with the highest prevalence of NDV infected birds during the period from August to October (Fig 2.1). Live bird traders indicated that incidences of illness in chickens were relatively significant during the period from August to October. Unfortunately, we were not able to differentiate whether antibodies in the birds were due to previous exposure or ND vaccination. During this period, there were multiple geographic hotspots of NDV infection implying that there were multiple sources of virus in the Mawenzi live bird market (Fig 2.2). Unfortunately, the exact focal point of infection could not be established in this study because chickens were not sampled at homes or farms of origin but rather at the destination market [47]. Seasonality of NDV activity, as corroborated by other studies [48-53], might be due to the dry weather during the period of August to October in most parts of the country during which poultry feeds are scarce, a stress that cause immune suppression to the chickens. Other studies conducted in East Africa and in Asia have reported similar patterns with ND peaks during and at the end of the dry seasons [54-58]. In this study, the range of time spent during transport from the geographic origins of the chickens to Morogoro live bird market was between one to five days. This transit time aligns with the length of the incubation period for ND. As a result, susceptible chickens could become infected while in mixed cages during transportation and present to the market with subclinical and/ or clinical disease depending on the individual immunity and time of arrival at the market. Mixing of birds during transportation and upon arrival at the live bird market has been previously associated with disease spread and outbreaks [28, 31]. The traders' practices of mixing birds from different places and housing them in mixed species cages at the market regardless of their health status may result in contact between infected and susceptible birds, increasing the risk of transmission. In addition, results from this study indicate a higher odd of infection in birds that have been present at the market for two or more days (Table 2.2) highlighting that co-housing of new arrivals with resident market birds is a significant risk factor for NDV spread. The vaccination history of the chickens arriving at the market was unknown and further increasing the risk of NDV emergence and spread. Furthermore, the cages used to bring chickens to the live bird market were taken back to the villages without disinfection in between bird collections. This heightens the risk of virus carriage back to the villages or from one village to the other via the chicken collection cages. Lastly, traders reported that sick chickens are often included in those birds selected to be sold in the market, further escalating the risk for disease spread among chickens congregating in live bird markets.

The Mawenzi live bird market encompasses a chicken slaughtering service whereby the visceral contents and feathers are improperly disposed of which may perpetuate the dissemination of NDVs and other pathogens [59] in the market and to the surrounding areas. Not unexpectedly, many studies have found that improper disposal of waste (e.g. carcasses, fecal matter) in market settings is associated with greater incidence of disease among flocks [28, 29, 50]. Many live bird markets are air open, do not have the appropriate infrastructure and equipment for sanitary slaughter of birds and therefore pose a risk for spillover of NDVs between market birds and wild birds and contamination of the premises [21, 60].

The findings from this study can be applied to other pathogens of importance in poultry in live bird market settings. For example, influenza A virus has been found to persist in live bird markets as a result of poor or inadequate sanitation, the constant presence of chickens in the market, and the practice of mixing birds in enclosures [21, 61-63]. An increased focus on disease prevention and control measures is needed to mitigate these risks, especially given the impacts of the current unprecedented global HPAI outbreak among poultry and wild birds.

2.9 Conclusion

This study provides further evidence that ND is endemic in Tanzania and circulates among village chickens year-round with peaks of NDV activity during the dry season. Determining the actual burden of the disease based on the source (focal point) might have been overestimated as chickens from different sources were mixed prior to reaching the market. In this case, point-source sampling is suggested in order to more accurately pinpoint spatial patterns. This study identified a number of risk factors for NDV among birds entering the live bird market that highlight potential points of intervention to mitigate risk. NDV vaccination is recommended periodically to protect chickens from ND by reducing infection and shedding of the virus. There is a need for greater awareness among the stakeholders (live bird sellers and middlemen) in the poultry value chain regarding best practices for biosecurity and sanitation during transportation and at the point of sale in the markets to reduce risk of NDV and other high consequence poultry diseases. Traders should also receive training on how to recognize clinical signs of illness in birds and the proper steps to take when a sick bird is identified. There is also a need for greater investment in market facilities and equipment (e.g., proper enclosures and slaughter and sanitation facilities) to improve hygiene and biosecurity and animal welfare, failure to implement proper biosecurity practices in live market in Tanzania poses a serious risk to spread the NDV to small-scale farm chickens in rural areas. There is a need in developing strategies to improve the biosecurity and to know more about the virus circulation and epidemiology. Lastly, policies to ensure that markets are regularly inspected by authorities and meet basic sanitation and safety standards are sorely needed to mitigate risk. As a limitation of the study, due to budget constraint it was not possible to use the Enzyme-linked Immunosorbent Assay (ELISA) for serologic testing, which has higher sensitivity for detection of antibodies. Therefore, the seropositivity reported here may be an underestimation of seroprevalence among this population. In addition, NDV strains identified through genotyping are reported in a follow-up study.

Data availability All data generated in this study are available upon request and uploaded in the USAID Development Data Library.

Conflict of interest Authors declare no conflict of interest. The submitting and corresponding author declares no conflict of interest with co-authors.

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CHAPTER THREE

Manuscript Two

3.0 MOLECULAR CHARACTERIZATION OF NEWCASTLE DISEASE VIRUS OBTAINED FROM MAWENZI LIVE BIRD MARKET IN MOROGORO, TANZANIA IN 2020-2021

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Abstract

Newcastle disease (ND) is among the most important poultry diseases worldwide. It is the major threat to poultry production in Africa and causes major economic losses for both local and commercial chickens. To date, half of ND class II genotypes have been reported in Africa (I, IV, V, VI, VII, XI, XIII, XIV, XVII, XVIII, and XXI). The information on the circulating NDV genotypes is still scarce despite the endemic nature of ND in most countries on the African continent. A total of 659 oro-cloacal swabs were collected from local chickens in Mawenzi live bird market located in Morogoro, Tanzania between June 2020 and May 2021. Newcastle disease virus was detected by using reverse transcription real-time polymerase chain reaction (RT-qPCR) and conventional PCR followed by sequencing of PCR products. Sequencing and phylogenetic analysis revealed the presence of sub-genotype VII.2. The detected sub-genotype VII.2 has phylogenetic links to Zambian NDV strains implying a Southeast dissemination of the virus, considering that it was first detected in Mozambique. This study underscores the need of active NDV surveillance to determine the distribution of this NDV genotype in the country and monitor its spread and contribution to the emergence of new ND viruses.

Key words: Newcastle disease, Genotypes, Phylogeny, Local chickens, Live bird market, Tanzania

3.1 Introduction

Newcastle disease virus (NDV) is highly contagious, infecting both domestic and wild bird species, and causes the most economically and socially important disease of domestic poultry in Africa (Mngumi et al. 2022). In susceptible chicken populations, velogenic NDV causes mortality up to 100% in affected flocks (Alexander et al. 2012). In villages of low and middle-income countries (LMIC), such as in some rural areas in Tanzania, chickens are primarily kept in extensive scavenging systems (FAO, 2014), where diseases like ND serve as major constraints to poultry production (Alexander, 2000; Mwalusanya et al. 2002; Sonaiya, 2007; Ananth et al. 2008; Alfred et al. 2012). In extensive scavenging systems, poultry from different households, ages, and species comingle with each other and sometimes encounter wild birds presenting opportunities for pathogen transmission. In these settings, some of the birds may be vaccinated against ND, whilst others are not (Msoffe et al. 2010; Wong et al. 2017), thereby, the inconsistency of ND vaccination can increase the risk of NDV outbreaks among village flocks. In Tanzania, indigenous chickens are mainly sold through live bird markets (LBMs), because of the lack of a cold chain to distribute chilled meat (Msoffe et al. 2019). Most LBMs receive chickens, guinea fowl, and ducks from different regions of the country, making this environment conducive for the emergence and spread of viruses, such as influenza A viruses and NDVs (Msoffe et al. 2019).

Newcastle disease virus, an Avian Orthoavulavirus type- 1 (AOaV-1) and previously known as avian paramyxovirus type- 1 (APMV-1) (Kuhn et al. 2019), is a single-stranded negative-sense RNA (-ssRNA) virus (Alexander and Senne, 2008). The genome encodes six proteins namely; nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and the RNA-dependent RNA polymerase (L) (de Leeuw and Peters, 1999; Seal et al. 2000). All NDV strains belong to a single serotype, but there is substantial genetic and antigenic variation across strains (Diel et al. 2012; Snoeck et al. 2013).

The NDV fusion (F) gene is commonly targeted for the classification of NDV into genotypes (Acheson et al. 2011; Dortmans et al. 2011; da Silva et al. 2020) using both partial and complete sequences (Miller et al. 2015; Butt et al. 2018). AOaV-1 is divided into two classes: class I and class II, with class I NDVs primarily encompassing lentogenic viruses commonly found in wild birds and less frequently in poultry and class II NDVs consisting of lentogenic (low virulence), mesogenic (medium virulence), and velogenic (highly virulent) pathotypes (Suarez, 2020), these strains are detected in multiple wild birds and domestic poultry species worldwide. Class I viruses have only one genotype, while Class II viruses have 20 distinct genotypes (Dimitrov et al. 2019). In addition to genotype, the nucleotide sequence of the cleavage site of the F gene determines the pathogenicity of NDV (Diel et al. 2012; Dimitrov et al. 2019). The clinical signs caused by NDV infection in chickens are variable based on the pathogenicity of the strain, and range from none (asymptomatic infection) to severe as decreased egg production, depression, diarrhea, respiratory distress, and neurological signs (Dimitrov et al. 2019).

In Africa, a range of NDV genotypes have been reported, including genotypes I, II, IV, V, VI, VII, XI, XIII, XIV, XVII, XVIII and XXI (Dimitrov et al. 2019; Zanaty et al. 2019; Mngumi et al. 2022). In Tanzania, the first isolation and pathotyping of NDV was performed by Loretu and Mkaria in 1981. More recently, researchers in Tanzania have isolated and characterized both velogenic and lentogenic NDV strains of genotypes V and XX from backyard chickens (Yongolo et al. 2011), and genotypes V and XIII.1.1 from live bird markets (Msoffe et al. 2019). In addition, da Silva et al. (2020) reported genotypes V, VII.2, and XIII in chickens. While NDV is endemic and causes devastating economic losses in indigenous chickens in Tanzania, our understanding of the diversity of NDV genotypes circulating among village poultry and in live bird market settings is still limited. Tanzania's borders, like those of many other countries in the region, allow the relatively unrestricted trade in live chickens within the sub-region and have resulted in the spread of ND across East Africa. This study aimed to identify and molecularly characterize NDV genotypes circulating among local chickens obtained from a live bird market serving as a central poultry trading hub in Tanzania in 2021 and 2022.

3.2 Materials and Methods

3.2.1 Study site

The Mawenzi live bird market is in Morogoro municipality in the eastern part of Tanzania (Figure 3.1). Morogoro is located 196 kilometers west of Dar es Salaam which is the country's largest city and commercial center, and 260 kilometers east of Dodoma, the country's capital city.

Figure 3.1: The map of Tanzania showing the location of Morogoro (blue dot)

The Mawenzi live bird market is located within the general food market. It is an open-air market where various species of live poultry (indigenous chickens, ducks, guinea fowl) are kept in mixed-species enclosures made of wood and wire mesh and stacked on top of each other. Birds are provided with maize bran mixed with food leftovers and water. The market sells more than 300 birds (mixed species) per week. These birds originate from multiple districts within Morogoro region and other regions of the country and are transported in cages using trucks to the market via middlemen to be sold to consumers by live bird vendors who are based at the market. The birds are collected and offloaded for sale in the market regardless of their vaccination and health status presenting challenges for NDV prevention and control. A mini slaughtering and processing area is located next to the cages, which does not have a water supply or sanitary facilities. In general, biosecurity measures are severely lacking further illustrating the potential for disease emergence and spread among birds housed in the market.

3.2.2 Collection of Oro-cloacal swabs

Oro-cloacal swabs were collected from chickens at the live bird market during the period from June 2020 to May 2021. The samples were collected on a weekly basis from the first and sixth chicken from each cage. Samples were collected using sterile polyester-tipped plastic swabs (Puritan, USA). A swab was inserted in the oral cavity including the choanal cleft and back of the throat in circular motions. The same swab was then used in a circular motion against the mucosa of the cloaca. The swabs were immediately placed into a cryovial containing 0.5mL sterile phosphate buffered saline (PBS) and stored in a cool box before transport to at Sokoine University of Agriculture laboratory to be saved at -80 C°.

3.2.3 RNA Extraction and Real-time Reverse Transcription Polymerase Chain Reaction (RT-qPCR).

Viral RNA was extracted from the swabs using the IndiMag Pathogen Kit in an IndiMag automated extraction instrument (Indical Bioscience, USA), following manufacturer's instructions. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed to detect the presence of the NDV, using the primers and probes described by Fuller et al. (2010) that detects both class I and II AOaV-1 viruses, and VetMAX Plus RT-PCR kits (Thermo Fisher Scientific). The RT-qPCR cycling conditions consisted of a 53°C annealing temperature for 45 seconds followed by one cycle at 95°C for 15 minutes, and 40 cycles at 95°C for 10 seconds, 50°C for 30 seconds and an extension temperature of 72°C for 30 seconds. RT-qPCR was performed in a StepOne Plus thermal cycler (Applied Biosystems). Cycle threshold (Ct) values < 40 were considered positive.

3.2.4 Conventional RT-PCR and Sanger DNA sequencing

For samples where the Ct value after RT-qPCR was ≤ 30, a 1,100 bp portion of the NDV genome that spans the 3’ end of the M gene and 5’ end of the F gene (including the F_0 cleavage site) was amplified using primers NDV M610 (forward) and F581 (reverse)

(Abolnik et al. 2004). RT-PCR was also performed with a second set of oligonucleotides, AIIs (forward) and AIIs (reverse) that amplifies a 362 bp region of the F gene spanning the F_0 cleavage site (Wang et al. 2001). RT-PCR products were separated in 1% agarose gel, purified (QIAquick PCR Purification Kit, Qiagen), quantified with a Nanodrop spectrophotometer, and submitted to Inqaba Biotech (Pretoria, South Africa) for Sanger DNA sequencing.

3.2.5 Ion Torrent Sequencing and Analysis

Transcriptomic libraries were prepared using the Sigma Whole Transcriptome Amplification Kit (Sigma, Germany), according to the manufacturer's recommendation. DNA libraries were shipped on ice packs to the Stellenbosch University Central Analytical Facility (Stellenbosch, South Africa) for Ion Torrent sequencing. Ion Torrent reads were assembled in the CLC genomics workbench software v.22. Multiple sequence alignments of complete or partial consensus genomes were performed in MAFFT v.7. Reference partial and full NDV F gene sequences were used for classification (Dimitrov et al. 2019) and phylogeny, including relevant sequences on the analysis. RAxML phylogenetic trees were constructed in Geneious Prime 2023.1.2 (Biomatters Ltd) using the GTR GAMMA I nucleotide model with the rapid bootstrapping and search for best scoring maximum likely hood tree algorithm using 1000 bootstrap replicates, a parsimony random seed of 456, and starting with a complete random tree (Stamatakis, 2014). The obtained sequences were uploaded to NCBI sequence read archive and can be accessed at the BioProject accession number PRJNA987660.

3.3 Results

Six hundred and fifty-nine (659) chicken samples were collected from the live bird market. A total of 155 (23.5%) chickens tested positive for the presence of NDV-specific RNA by RT-qPCR. Of the positive samples, 77 had cycle threshold (Ct) values less than 30 and were suitable for further genetic characterization. Using the AIIs/AIIe primers (Wang et al. 2001), we obtained 42 amplicons that matched the 362 bp expected fragment size. DNA was extracted from the gel and purified with the QiaQuick Gel Extraction kit (Qiagen, USA) and 37 PCR amplicons were of sufficient DNA concentration for Sanger sequencing. Sequences were obtained from 27 samples; however, 18 were non-specific bacterial DNA. The nine remaining sequences were all characterized as NDV genotype VII.2 with a velogenic F_0 cleavage site sequence of RRRKRF. Whole transcriptome libraries were prepared from these nine samples and submitted for Ion Torrent sequencing. Sequencing results are summarized in Table 3.1.

Table 3.1: NDV direct deep sequencing on swab samples focusing on the full NDV genome and the F gene.

Sample	Complete genome[1] (no. reads mapped) [% coverage]	F gene[2] (no. reads mapped) [% coverage]	BLAST result
J-122 (August, 2020)	**9,806 (141) [64.5%]**	**563 (4) [33.9%]**	**Genotype VII.2**
J-145 (September, 2020)	**11,299 (189) [74.4%]**	**868 (7) [52.2%]**	**Genotype VII.2**
J-156 (September, 2020)	6,101 (64) [40.2%]	153 (3) [9.2%]	Genotype VII.2
J-168 (September, 2020)	**13,220 (302) [87%]**	**1,449 (22) [87.2%]**	**Genotype VII.2**
J-196 (September, 2020)	0	0	
J-219 (October, 2020)	8,166 (85) [53.8%]	334 (3) [17.5%]	Genotype VII.2
J-403 (January, 2021)	1,116 (12) [7.3%]	0	Genotype VII.2
J-489 (March, 2021)	**14,349 (1,270) [94.5%]**	**1,619 (71) [97.4%]**	**Genotype VII.2**
J-578 (April, 2021)	6,884 (64) [45.3%]	116 (1)	Genotype VII.2

[1]15192 bp reference sequence; [2]1662 bp reference sequence; bolded samples were included in the phylogenetic analysis.

Partial NDV genomes recovered from the swab samples varied up to 94.5% coverage. One of the issues encountered was the fragmentation of the recovered reads affecting sequencing depth and coverage. Recovery of over 30% of the F-gene sequence was accomplished in four out of the nine samples. These sequences were included in the phylogeny for comparison with previous NDVs detected in Tanzania, new relevant NDV sequences, and other genotype VII.2 references (Supplementary Figure 1). Figure 3.2 depicts the phylogenetic tree prepared with a 598bp segment of the

F gene. The viruses from 2020-2021 cluster together, sharing a recent common ancestor with previously reported Tanzanian and Mozambique strains, as well as strains from Zambia and Zimbabwe from 2015 and 2013, respectively, although the bootstrap values are low.

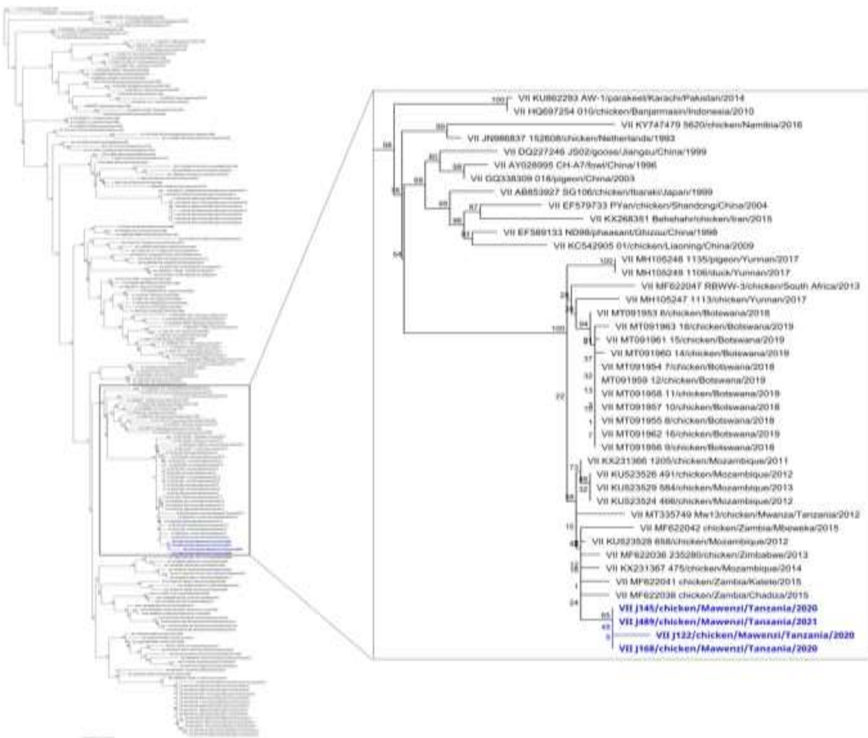

Figure 3.2: Maximum likelihood phylogenetic tree of the partial F gene sequences (598 bp). The tree is focusing on genotype VII strains. Tanzanian strains are highlighted in blue.

To confirm the results of the phylogenetic tree reconstructed with a partial F gene sequence, we prepared a second tree using a 1687bp segment of the F gene (Supplementary Figure 2). Figure 3.3 confirms the phylogenetic grouping with strains from Tanzania, Zambia, Zimbabwe, and Mozambique. The genotype VII strain recovered from Mwanza maintains its relationship with our strains considering a larger gene sequence.

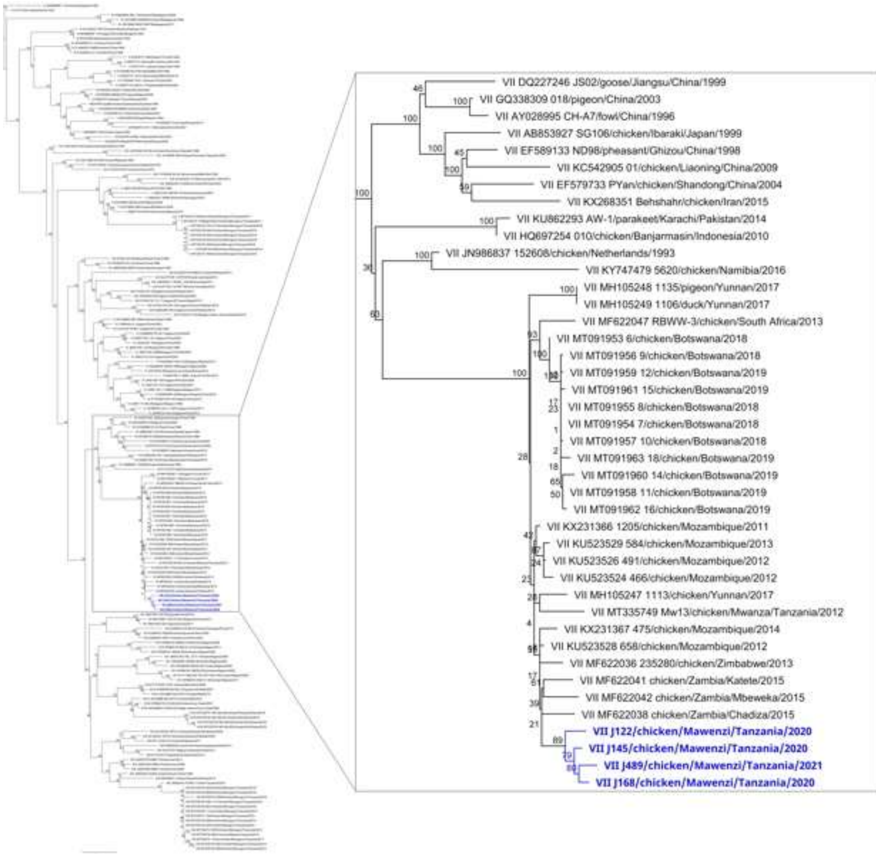

Figure 3.3: Maximum likelihood phylogenetic tree of the fusion (F) gene sequences (1 687 bp). The tree is focusing on genotype VII strains. Tanzanian strains detected in this study are highlighted in blue.

3.4 Discussion

This study detected NDV sub-genotype VII.2 (Table 3.1) and confirmed the continued circulation of the NDV sub-genotype VII.2 in Tanzanian chickens in 2020-2021. While our sequences showed fragmentation and poor depth due to the direct sequences obtained from swabs rather than isolates, they allowed us to clearly genotype the NDV infecting chickens in the Mawenzi market in Morogoro.

The molecular epidemiology of NDV genotype VII.2 in Africa was first investigated in 2017 (Abolnik et al. 2017). At that time, the phylogenetic link of these strains with isolates obtained from Zambia was detected. The source of sub-genotype VII.2 to the African continent is thought to be the movement of infected poultry, poultry products, or fomites from South-East Asia through Mozambique (Mapaco et al. 2016; Abolnik et al. 2017; Abolnik et al. 2018). The other possible ways in which sub-genotype VII.2 has spread from its source in Indonesia and Malaysia to other Asian countries and to the African continent could be through the movement of wild birds (Cappelle et al. 2015). Since its detection in Africa, sub-genotype VII.2 has been reported in Namibia (Molini et al. 2017), South Africa, Zimbabwe, Mozambique, Malawi, Zambia, Botswana (Abolnik et al. 2018), Tanzania (da Silva et al. 2020), and Democratic Republic of Congo (Twabela et al. 2021). Our previous report of a VII.2 strain (da Silva et al. 2020) is phylogenetically linked to strains from Mozambique, Zambia, Zimbabwe and groups in the same genotype as our current detections (Fig 3.2 and 3.3). Moreover, Kibasa (2020) reported the isolation of NDV genotype VII from chickens in Iringa Tanzania which is located at the Southern highland. The NDV obtained was 98% homologous to the virus obtained in Mozambique further confirming the porosity of the borders. These countries are near Tanzania suggesting the virus spread due to proximity. Sequences from Botswana and Yunnan (China) are recent additions to GenBank and help explain the potential distribution of these viruses not only in Africa but also in Asia. A meta-analysis reported by Mngumi (2022) categorizes sub-genotype VII.2 as a widespread NDV genotype in Africa with reports in East, West, South, and Central African countries. In Tanzania, vaccination of chickens against ND is the key to fighting the Newcastle disease as in many other countries worldwide. The vaccination practices in indigenous chickens are low and irregular as compared to the commercial chickens which partly results from limited access to veterinary services contributing to the emergence and maintenance of viruses in the poultry populations (Campbell et al. 2018). In addition, the mix of poultry species and population and movement through the live bird markets perpetuate and maintain the virus (Cappelle et al. 2011). Although chickens are vaccinated, sub-genotype VII.2 has been implicated to have the ability to cause outbreaks (Nooruzzaman et al. 2022). This may happen due to inadequate vaccinations which results in inadequate immune responses, or concurrent infection with immunosuppressive agents which compromise the mounting of adequate immune response (Alexander et al. 2012)

NDV genotype VII has been of global economic importance due to its diverse nature and recurrent outbreaks in Eastern Europe, the Middle East, and Asia and sporadic outbreaks in Africa and South America (Miller et al. 2015). This genotype is the virus responsible for the fifth NDV panzootic (Gaurav et al. 2021; Steensels et al. 2021; Twabela et al. 2021; Nasir et al. 2022). The panzootic nature of sub-genotype VII.2 was predicted (Miller et al. 2015; Rehmani et al. 2015) due to its nature and rapid spread from its source in Indonesia, to Pakistan, Israel, and Eastern Europe (Fuller et al. 2015). The isolation of sub-genotype VII.2 in Tanzania suggests the spread of this virus from neighboring countries and is evidence of the porosity of the country's borders. Biosecurity measures at this level including but not limited to poultry and poultry product import regulations, might help reduce the permeability of the borders and protect the country's poultry health status. In addition, the live bird market dynamics including the long distances traveled by birds to be sold at live bird markets and the lack of biosecurity along this commute contributes to virus dissemination. Biosecurity improvements and continued surveillance would help limit dissemination and improve our understanding of the geographical distribution of this genotype and others. In addition, it will inform the establishment of control measures to limit spread and subsequently reduce losses caused by NDV. This study contributes to the understanding of the circulating NDV strains in Tanzania.

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Author contribution Peter Msoffe, Terra Kelly, Rodrigo Gallardo, Amandus Muhairwa and Huaijun Zhou conceptualized and designed the study; John Tsaxra collected data; John Tsaxra, Celia Abolnik and Thandeka Phiri conducted lab assays; John Tsaxra, Augustino Chengula, James Mushi, Terra Kelly, Rodrigo Gallardo, Rachel Jude, and Celia Abolnik analysed the results; John Tsaxra wrote the first draft of the manuscript; Terra Kelly and Rodrigo Gallardo revised the contents; All authors revised and commented on the subsequent versions of the manuscript; All authors read and approved the manuscript.

Data availability All the data generated during this study are included in this manuscript.

Ethical approval This approval for sampling chickens for this research was obtained from the Sokoine University of Agriculture (SUA/DPRTC/R/186). Institutional review boards at the Sokoine University of Agriculture and the University of California, Davis (DPRTC/R/T/VOL XXII/13) approved the study procedures for human subjects.

Conflict of interest Authors declare no conflict of interest.

Consent to participate All participants gave their informed consent to participate in the study.

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CHAPTER FOUR

4.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 General Discussion

Generally, NDV prevalence and seroprevalence among chickens sampled at the Mawenzi live bird market for this study were in the range of previously reported estimates in Tanzania (Table 2.1). Previous ND studies conducted in Tanzania reported the seroprevalences and prevalences of 46.8% (Yongolo *et al*., 1996) and 13.3% (Minga *et al*., 1989) 32% (Msoffe *et al*., 2019) and 26% (Mngumi and Bunuma, 2022). Studies conducted elsewhere have illustrated the potential role of unregulated poultry trade through live bird markets on ND activity (Mulisa *et al*., 2014 and Ipara *et al*., 2019).

The spatiotemporal study has revealed that, with the exception of one month (February), NDV is circulating year-round among the local chickens coming into the live bird market providing further evidence of the endemicity of this virus (Fig 2.2) and the live bird market as a continued source of infection among birds originating from different parts of the country. The temporal and spatiotemporal patterns indicated seasonality of NDV activity with the highest prevalence of NDV infected birds during the dry period from August to October (Fig 2.1) as reported in other studies (Spradbrow, 2000; Yongolo *et al*., 2002; Otim *et al*., 2004; Zeleke *et al*., 2005; Njagi *et al*., 2010; Kemboi *et al*., 2013).

Traders' practice of mixing chickens from different sources and housing them in mixed species cages regardless of their health status which increases the risk of disease transmission has also been reported elsewhere (Ipara *et al*., 2019 and Ogali *et al*., 2018). In addition, results from this study indicate a higher odd of infection (Table 2.2) in birds that have been present at the market for two or more days highlighting that co-housing of new arrivals with resident market birds is a significant risk factor for NDV spread.

On the other hand, molecular characterization revealed the presence of NDV sub-genotype VII.2 (Table 3.1) and confirmed the continued circulation of the NDV sub-genotype VII.2 in Tanzanian chickens. The molecular epidemiology of NDV genotype VII.2 in Africa was first investigated in 2017 (Abolnik *et al*., 2017). Since its detection in Africa, sub-genotype VII.2 has been reported in Namibia (Molini *et al*., 2017), South Africa, Zimbabwe, Mozambique, Malawi, Zambia, Botswana (Abolnik *et al*., 2018), Tanzania (da Silva *et al*., 2020), and Democratic Republic of Congo (Twabela *et al*., 2021). The source of sub-genotype VII.2 to the African continent is thought to be the movement of infected poultry, poultry products, or fomites from South-East Asia through Mozambique (Mapaco *et al*., 2016; Abolnik *et al*., 2017; Abolnik *et al*., 2018).

Our previous report of a VII.2 strain (da Silva *et al*., 2020) is phylogenetically linked to strains from Mozambique, Zambia, Zimbabwe and groups in the same genotype as our current detections (Fig 3.2 and 3.3). Kibasa (2020) reported genotype VII with 98% homology to the virus obtained in Mozambique further confirming the porosity of the borders. Sequences from Botswana and Yunnan (China) are recent additions to GenBank and help explain the potential distribution of these viruses not only in Africa but also in Asia.

A meta-analysis reported by Mngumi (2022) categorizes sub-genotype VII.2 as a widespread NDV genotype in Africa with reports in East, West, South, and Central African countries (Fig 3.2 and 3.3). Although chickens are vaccinated, sub-genotype VII.2 has been implicated to have the ability to cause outbreaks (Nooruzzaman *et al*., 2022). This may happen due to inadequate vaccinations which results in inadequate immune responses, or concurrent infection with immunosuppressive agents which compromise the mounting of adequate immune response (Alexander *et al*., 2012)

This genotype is the virus responsible for the fifth NDV panzootic (Gaurav *et al*., 2021; Steensels *et al*., 2021; Twabela *et al*., 2021; Nasir *et al*., 2022). The panzootic nature of sub-genotype VII.2 was predicted (Miller *et al*., 2015; Rehmani *et al*., 2015) due to its nature and rapid spread from its source in Indonesia, to Pakistan, Israel, and Eastern Europe (Fuller *et al*., 2015).

4.2 Conclusion and Recommendations

4.2.1 Conclusion

This study provides further evidence that ND is endemic in Tanzania and circulates among village chickens year-round with peaks of NDV activity during the dry season. This study identified a number of risk factors for NDV among birds entering the live bird market that highlight potential points of intervention to mitigate risk. However, determining the actual burden of the disease based on the source (focal point) might have been overestimated as chickens from different sources were mixed prior to reaching the market.

4.2.2 Recommendations

In this case, point-source sampling is suggested in order to more accurately pinpoint spatial patterns. Regular NDV vaccination to protect chickens from ND by reducing infection and shedding of the virus. There is a need for greater awareness among the stakeholders (live bird sellers and middlemen) in the poultry value chain regarding best practices for biosecurity and sanitation during transportation and at the point of sale in the markets to reduce risk of NDV and other high consequence poultry diseases. There is also a need for greater investment in market facilities and equipment (e.g., proper enclosures and slaughter and sanitation facilities) to improve hygiene and biosecurity and animal welfare, failure to implement proper biosecurity practices in live bird market in Tanzania poses a serious risk to spread the NDV to commercial small-scale farms and chickens in rural areas. Tightening the biosecurity measures including the importation of poultry and poultry products regulations. There is a need to conducting regular and continued surveillance to know more about the virus circulation and geographic distribution of the viruses. Lastly, policies to ensure that markets are regularly inspected by authorities and meet basic sanitation and safety standards are sorely needed to mitigate risk.

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APPENDICES

Questionnaire

Appendix 1: Epidemiological study of Newcastle Disease Virus in a live bird Market in Morogoro Municipality, Tanzania.

Part I: Questions to live birds' sellers/traders.

***Gender a) Male b) Female**

1. What regions, villages or towns, do you obtain chickens from?
2. What are the criteria or specific factors you rely on when selecting chickens for sale at bird markets?
   a) Body weight
   b) Body conformation
   c) Price
   d) Demeanor
   e) Illness
   f) Vaccination status
   g) Any other?
3. a) In which places do most of the chickens generally meet your criteria of interest? And why?
   b) Are there any places where you have completely stopped collecting/ buying chickens from?
   c) If yes, could you please mention the places and the reasons?
4. Do you source chickens from the same places all year-round?
5. If you change the source of the chickens, what prompts you to change?
6. a) How long does it take from buying chickens from the villages to when they reach the market of destination?
   b) On average, how long do chickens stay at the market from offload to when they are all gone/bought?
7. Do you have any experience with diseases affecting chickens you sell at this market? (Yes or No).
8. Do you have experience with deaths of chickens that you sell?

9. Have any of your chickens at this market shown clinical signs of disease or illness?
10. a) If yes, what clinical signs do you often see?
    b) Among the clinical signs you mentioned, which one(s) are most common?
    c) Do you know what disease those clinical signs represent?
    d) Among the diseases you mentioned, which three are the most common?
    e) Among the diseases you mentioned, which one do you think is/are most dangerous and why?
11. What do you do with chickens that appear ill after buying from your source?
12. What season/time of the year do birds fall sick most often?
13. What poultry are you selling today?
    a. Chickens
    b. Doves
    c. Ducks
    d. Turkey
    e. Guinea fowl
14. Do you house them together? Yes/No
15. Who buys the live birds you sell?
    a. Customer for home use
    b. Restaurant/hotel
    c. Customer who resells at another market
    d. Other (please specify)
16. What do you do with dead chickens?
17. Do you do any disinfection of the bird enclosures?
18. How often do you clean/ disinfect the poultry shelter/cage?
19. Do you have any information of whether these birds were vaccinated or not vaccinated?
20. Do you vaccinate your chicken upon arrival? If yes against what disease?
21. If you obtain chickens from middlemen, from which places did they buy chickens?
22. Do you have names and contacts of the middlemen?

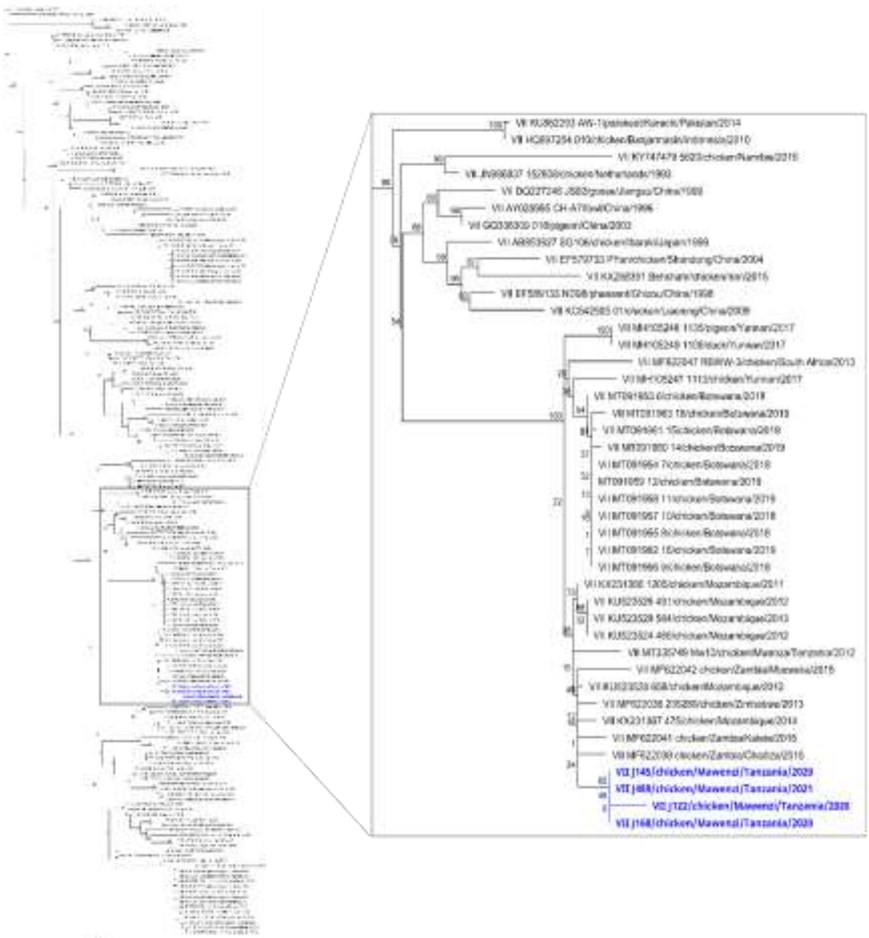
PART II: QUESTIONS FOR MIDDLEMEN

***Gender a) Male b) Female**

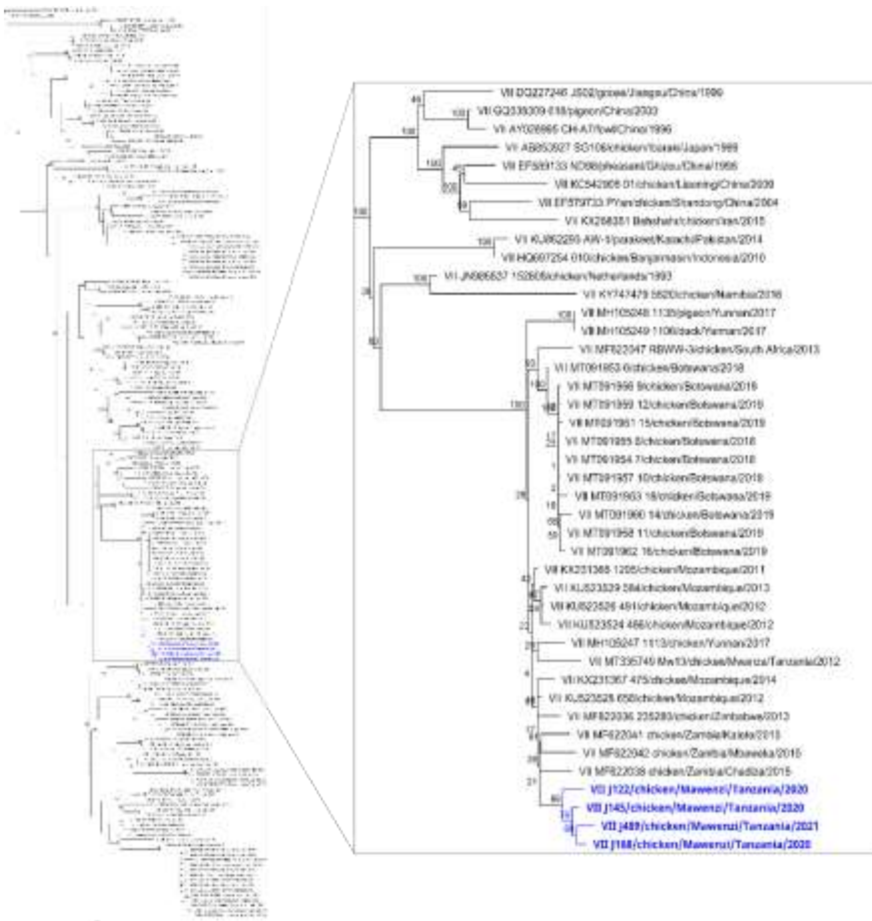
1. Where do you exactly obtain (village) chickens you sell?
2. Do the houses where you obtain chickens keep birds other than chickens?(Yes/No)
3. If yes, which species/types?
   a. Doves
   b. Ducks
   c. Turkey
   d. Guinea fowl
4. If yes, do they house them together?
5. When selecting chickens from different sources, what are the criteria or specific factors you rely on?
   a) Body weight
   b) Body conformation
   c) Price
   d) Demeanor
   e) Disease or illness
   f) Vaccination status
   g) Any other
   6. Among the places where you usually obtain chickens, which areas do the chickens generally meet your criteria? And why/how?
7. Where do you keep the chickens from the first day of collection to the day they are submitted to the market?
8. a) What means of transport do you usually use to transport chickens from the house where you obtain them to the primary collection points before transporting them to the market?
   b) What means of transport do you use to transport chickens from the primary collection points at the villages to the market?
9. How long does it take from the first day of collection to the day they reach the destination market?
10. Do you have any information of whether these birds are vaccinated or not vaccinated? If yes, how do you get this information?

11. Do you provide any care/management to the collected chickens before transporting to market places?
12. Do you vaccinate chicken just after purchase/ buying from household? If yes, against what disease?
13. What are the challenges during purchase, transportation and keeping chickens before reaching the market?
14. Are you able to distinguish between a healthy and a sick bird? If yes how?
15. Have you ever come across diseases when collecting chickens from the sources? (Yes or No).
16. If yes, in your knowledge what were the diseases and their clinical signs?
17. What is the most important disease in your opinion and why?
18. What season/time of the year birds fall sick more often?
19. What do you do with sick birds?
20. Do you purchase/sell different types of birds other than chickens?
21. If yes, which species/types?
    a. Doves
    b. Ducks
    c. Turkey
    d. Guinea fowl
22. If yes, do you house the different species together?
23. Do you know what other middlemen do with sick or dead chickens?

Appendix 2: Supplementary fig S1 a phylogeny tree of virus strains obtained in this study for comparison with previous NDVs detected in Tanzania, new relevant NDV sequences, and other genotype VII.2 references.



Appendix 3: Supplementary fig S2 to confirm the results of the phylogenetic tree reconstructed with a partial F gene sequence, we prepared this tree using a 1687bp segment of the F gene



Appendix 4: Plagiarism report

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Kuhusu Tasnifu Hii

Utafiti huu umelenga kutathmini epidemiolojia ya mlipuko wa ugonjwa wa Mdondo katika soko la uuzwaji wa kuku katika manisapaa ya Morogoro. Ugonjwa wa Mdondo (ND) husababishwa na virusi aina ya orthoavulavirus 1 (AOaV-1; zamani paramyxovirus-1) ambayo ni ya jenasi Avulavirus, jamii ndogo ya Paramyxovirinae, na familia Paramyxoviridae. Virusi vya ND ni hisia hasi ya virusi iliyofunikwa na RNA yenye nyuzi moja. Utafiti huu unatoa ushahidi zaidi kwamba Mdondo ni ugonjwa ambao upo nchini Tanzania na huzunguka kati ya kuku wa kijijini mwaka mzima na umekuwa na athari kubwa wakati wa kiangazi. Utafiti huu ulibaini sababu kadhaa za hatari kwa Mdondo kati ya ndege wanaoingia kwenye soko la ndege hai ambazo huangazia uwezekano wa kuingilia kati ili kupunguza hatari. Hata hivyo, kubainisha eneo halisi ambapo ugonjwa upo kulingana na chanzo (kiini) kunaweza kukadiriwa kupita kiasi kwani kuku kutoka vyanzo mbalimbali walichanganywa kabla ya kufikishwa sokoni. Hii inaruhusu watafiti na watunga sera kujua njia bora ya kukabiliana na magonjwa na uboreshaji wa ubora wa soko letu hai na bidhaa za kuku. Tanzania ina hatari kubwa ya kuenea kwa NDV kwa mashamba madogo ya kibiashara na kuku katika maeneo ya vijijini. Kwahivyo, kuna uhitaji wa kuimarisha hatua za usalama wa viumbe hai ikiwa ni pamoja na kanuni za uingizaji wa kuku na bidhaa za kuku. Kuna haja ya kufanya ufuatiliaji wa mara kwa mara na unaoendelea ili kujua zaidi kuhusu mzunguko wa virusi na usambazaji wa kijiografia wa virusi. Hatimaye, sera za kuhakikisha kuwa masoko yanakaguliwa mara kwa mara na mamlaka na kufikia viwango vya msingi vya usafi wa mazingira na usalama zinahitajika sana ili kupunguza hatari.