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Determination of the Seroprevalence of Newcastle Disease Virus (Avian Paramyxovirus Type 1) in Zambian Backyard Chicken Flocks

By

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Submitted in fulfilment of the requirements for the degree of Master of Science in the Department of Veterinary Tropical Diseases in the Faculty of Veterinary Science, University of Pretoria

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LIST OF ABBREVIATIONS

AI	:	Avian Influenza
ANOVA	:	Analysis of Variance
APMV-1	:	Avian Paramyxovirus Type 1
CFSPH	:	Centre for Food Security and Public Health
CI	:	Confidence Interval
CSO	:	Central Statistics Office
CVRI	:	Central Veterinary Research Institute
DVO	:	District Veterinary Officer
ELISA	:	Enzyme linked immunosorbent assay
HA	:	Hemagglutination
HI	:	Hemagglutination inhibition
K	:	Zambian Kwacha
LBM	:	Live bird markets
N	:	Total number of birds sampled
ND	:	Newcastle disease
NDV	:	Newcastle disease virus
nm	:	nanometres
OIE	:	Office International des Epizootes (World Organization for Animal Health)
P	:	Seroprevalence
S/P	:	Sample/Positive
USA	:	United States of America
%	:	Percentage
^{0}C	:	Degrees Centigrade
$	:	United States Dollar

Thesis Summary

Determination of the Seroprevalence of Newcastle Disease Virus (Avian ParamyxovirusType 1) in Zambian Backyard Chicken Flocks

By

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Promoter Associate Professor C. Abolnik
Department Veterinary Tropical Diseases
Degree MSc (Veterinary Tropical Diseases)

The specific objectives of this study were to determine the Newcastle disease virus (NDV) antibody titres from the chicken sera collected from various districts and provinces of Zambia and to determine the seroprevalence of ND in Zambian backyard chickens. Results showed that 73.9 % of the birds sampled tested positive for Newcastle disease (ND) antibodies. The seroprevalence of Newcastle disease virus (NDV) in Zambian backyard chicken flocks varied among the five provinces sampled, ranging from 82.6 % in Eastern Province to 48.3 % in Luapula Province. The seroprevalence of the virus also varied among the 11 districts sampled, ranging from 91.3 % in Monze District of Southern Province to 22.8 % in Mufulira District of the Copperbelt Province. The results indicated that the seroprevalence of ND in Zambia has increased since the last survey conducted in 1994. The data generated is expected to contribute towards a more clear understanding of the epidemiology of NDV that would ultimately contribute towards an improved ND control programme to benefit all stakeholders in Zambia. An improved ND control programme is expected to enhance flock numbers and ultimately improve the dietary requirements and income needs of many poor households in the country.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Newcastle disease (ND), notifiable to the Office International des Epizooties (OIE), is caused by virulent avian paramyxovirus type 1 (APMV-1) strains. It is a contagious disease of birds that is widely distributed throughout the world, affecting many domestic and wild avian species and causes severe economic losses in the poultry sector (Cattoli, Fusaro, Monne, Molia, Le Menach, Maregeya, Nchare, Bangana, Maina, Koffi, Thiam, Bezeid, Salviato, Nisi, Terregino & Capua, 2009).

The causal agent, Newcastle disease virus (NDV), is a negative-sense single-stranded RNA virus. NDV strains can be categorised as velogenic (highly virulent), mesogenic (intermediate virulence) or lentogenic (non-virulent). Velogenic strains produce severe nervous and respiratory signs, spread rapidly and cause up to 90-100 % mortality in susceptible birds. Mesogenic strains may cause symptoms such as coughing, decreased egg quality and production and result in up to 10 % mortality. Lentogenic strains produce mild signs with negligible mortality. While the chicken is the primary source of the virus, other domestic birds and certain wild birds are susceptible and may be sources of infection (Alexander, 1998; Jackson, 2010). Apart from the known presence of the virus in cormorants in North America, it was unclear whether virulent viruses are perpetuated in wild waterfowl. Isolation of virulent viruses from wild aquatic birds has been reported. It was known that wild waterfowl were a natural reservoir of avirulent viruses, but all isolates from wild waterfowl sequenced in their study were found in lineage 1 and lineage 6; which contain mainly avirulent isolates. No virulent isolates from aquatic birds were included. However, whether these virulent viruses emerged from within the wild bird population or were transferred to wild birds from domestic poultry was uncertain (Aldous, Mynn, Banks & Alexander, 2003).

Transmission of NDV occurs by exposure to faecal and other excretions from infected birds, and through contact with contaminated feed, water, equipment and clothing (Alexander, 1998). Control of the disease in the commercial poultry sector is achieved through biosecurity measures, hygiene, disinfection and vaccination (Alexander, 1995). In rural areas, the options for village poultry are limited and there is generally limited or lack of veterinary and technical input. The practical realities of poor adherence to the cold chain; infrastructure; and technical support critically limit the use of conventional vaccines and implementation of vaccination techniques in tropical countries (Spradbrow, 1993).

Newcastle Disease was first formally described in Newcastle-upon-Tyne, England in 1926. (Alexander, 1991; Jackson, 2010). Songolo & Katongo (2000) reported that in Zambia, Newcastle disease was first reported in 1952. The disease was concentrated along the line of rail where the largest numbers of birds occurred. However, it is possible that despite the low numbers of poultry in the rural areas, the disease also existed there at the time. Due to an inadequate or lack of a proper reporting system at the time, the disease was not reported. With regards to the welfare of the family, poultry contribute greatly to the protein requirements of the rural population and also to the income generating power of the family. Unfortunately, Newcastle disease (ND) is the main limiting factor in rural poultry production systems. Little attention has been given to the control of ND in Zambia. Sharma, Hussein, Pandey and Shandomo (1985) reported that the government ceased implementing free ND vaccinations in chickens despite village chickens being a major food source for many households in Zambia. Despite this, it was reported that there was consensus that vaccination campaigns do increase the total household chicken numbers and consequently have a vital role to play in the improvement of household food security and family income (Bagnol, 2001). Newcastle disease has been cited as the most significant factor limiting village poultry production in Zambia (Bray & Moffat, 1990). Based on the haemagglutination inhibition (HI) titres of 2000 blood samples, Alders, Inoue and Katongo (1994) previously reported very high prevalence of antibodies to ND virus (29.2 % to 51.3 %) in village chickens in Zambia.

The poultry industry in Zambia is based on two distinct systems. The first is the commercial system where broilers or layers are obtained from hatcheries and reared on commercial feed in properly designed chicken houses. Strict vaccination schedules for important avian diseases are followed. Most commercial systems are situated mainly along the railway line in close proximity to major towns. The second is the village production system where chickens scavenge for food and basically subsist with little input from their owners. Vaccination schedules are rarely followed, if at all (Songolo & Katongo, 2000). The country report accounted by Mavale (2001) showed some similarities in village chicken rearing between Mozambique and Zambia. The report stated that village chickens obtain food predominantly by scavenging in the surrounding environment. Sometimes they receive household scraps and crop remainders which farmers often preserve and prepare later to feed their livestock and poultry after harvesting the main crop. Housing is not always provided, but when it is, it is made up of local materials. Sometimes, the owner's house provides night shelter for the chickens. The main intention is to provide protection from predation or theft during the night. Because of poor diet, predation and presence of other poultry diseases, fertility is affected; and as a consequence although a hen may lay more than ten eggs at a time, only two to three chicks will be raised to adulthood. Overall, the standard of husbandry is usually poor. In addition to ND, constraints to village chicken production can be summed up as poor nutrition and housing, predation, theft, low market prices or remoteness to lucrative markets as well as other poultry diseases. Despite these constraints, village chickens constitute more than 62 % of the actual poultry population and 72 % of households keep village chickens in Zambia. In seven of the nine provinces of Zambia, village chickens constitute between 85 % and 92 % of the poultry population (Central Statistics Office 2003).

1.1.1 Objective

The general objective of this study was to determine the seroprevalence of Newcastle disease in Zambian back yard chickens located along water sources, border areas and busy market places where chickens are sold.

1.1.2 Specific objectives

The specific objectives of this study were:

- To determine the NDV antibody titres of chicken sera collected from various districts and provinces of Zambia.
- To determine the seroprevalence of ND in Zambian backyard chickens at district and provincial level.

1.2 Literature Review

Newcastle Disease (ND) was first recognized in 1926, in Java, Indonesia and in Newcastle-Upon-Tyne, England. It was, however, reported that there were known outbreaks of the disease in poultry before 1926 with similar clinical signs to what we now know as Newcastle Disease (Alexander, 1991; Spradbrow, 2001a). The disease has been described by different terminologies including Pseudo-fowl pest, Pseudovogel-pest, Avian distemper, Avian pest, Ranikhet disease, Tetelo disease, Pseudo- fowl plague, Korean fowl plague and Avian pneumoencephalitis.

Traditional chicken rearing and characteristics of chicken rearing households in Zambia

Chicken rearing provides a critical income stream for households in both rural and urban Zambia. The 2002/ 2003 Post Harvest Survey of the Central Statistical Office (CSO, 2003) recorded a total of 720,013 households nationwide rearing chickens compared to 586,621 in the previous season. On average, this represents about 38 % of the total number of households in the country.

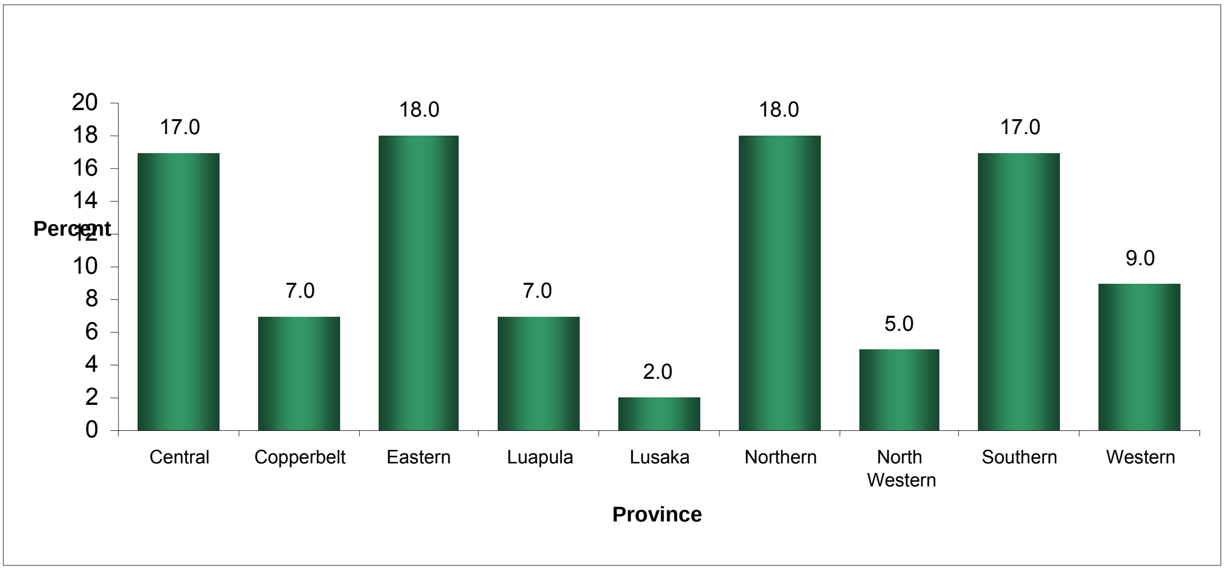

FIGURE 1.1 Chickens raised by Province, 2002/2003 (Source: CSO 2002/2003)

A total of 6,886,395 mainly traditional chickens were raised nationwide by the end of the season compared to 5,325,093 in the previous season. Eastern and Northern Provinces contributed the highest number of chickens with 18 % for either province. Southern Province contributed 17 % as did Central Province. Lusaka Province contributed the least number of chickens- only 2 %. On a 'per household' basis, Central Province was the largest chicken rearing province, recording an average of 17 chickens per chicken rearing household. This was followed closely by Southern Province, which recorded 15 chickens per rearing household (Central Statistics Office, 2003).

Ownership and economic value of traditional chicken rearing

Nationwide, an estimated 97.4 % of households (98.3 % and 89.3 % in rural and urban areas, respectively) were engaged in chicken rearing in the 2002-2003 agricultural season (Central Statistics Office, 2004). The ownership structure of chickens in Zambia was almost balanced between male members (51 %) and female members (49 %) of the households in that agricultural season. The turnover in all provinces totalled about K9 billion (or $2 million) from sales of 1,127,096 chickens. Southern Province reported the highest cash value (despite a relatively low average price of K7, 700 ($1.70) per chicken, followed by Central and Eastern Provinces. The highest average price per unit (per chicken), reported in Lusaka and Central Provinces was at about K11, 000 ($2.45). The lowest was reported in Eastern and Western Provinces at about K6, 000 ($1.56) and K7, 000 ($1.33) per chicken, respectively (CSO, 2003).

The evidence above indicates that there's high potential for village chicken production in the country. Chickens can be sold to make money easily and quickly. Village chicken buyers include both households and restaurant owners. Restaurant owners have discovered that when their clientele request a chicken meal, more often than not the village chicken is the preferred choice. It is not until the village chicken provisions have been exhausted that people reluctantly settle for the alternative; the commercial chicken. Thus, given a choice, most consumers wanting to buy a chicken would rather buy a village chicken than a commercial one. Due to this preference and demand, village chicken owners are increasingly realizing that they could earn even more income from selling their chickens not only to individual buyers, but to restaurant owners as well. The money obtained would be used for various activities in the home such as buying everyday items like food and paying school fees for their children. However, although the village chicken is the easiest source of protein and income to which the smallholder poultry farmers have access, the protein component of their (the farmers') diet is lacking. Their children provide the evidence, especially the under five year old age group, as it results in protein-energy malnutrition, which as a consequence inhibits their growth. Malnutrition increases the risk of illness, and because the children are constantly sick, their mental development is affected and this subsequently reduces their school performance and labour productivity (Alders, 2001). Despite the income generated from the sale of their chickens, smallholder farmers rarely sell their chickens in large numbers because they have very small flock sizes due to mortalities and other causes such as theft. If their flock sizes were bigger, the farmers would probably find it easier to sell more as well as to slaughter some of the chickens for consumption. Because ND exists in the country at present, some of the birds have antibodies against NDV. In a study conducted by Alders *et al.* (1994), 2000 blood samples were collected from all the nine provinces of Zambia. The results revealed very high prevalence of antibodies to ND virus (29 % to 51.3 %) in village chickens in Zambia. It was suggested that an endemic form of NDV infection occurs, with the virus being maintained in a partially immune population. The virus spreads slowly among the susceptible portion of the flock, and the occasional deaths are neither stressful to the owners nor are they sufficiently serious to attract official attention (Spradbrow, 2001b). However, any efforts to control the disease should not be disregarded as clinically healthy birds that are incubating the disease cannot be detected as potential transmitters of the disease to other flocks (Spradbrow, 2001b).

1.2.1 Species affected by Newcastle Disease

Many avian species are affected by Newcastle disease viruses. Of poultry, chickens are the most susceptible and ducks and geese are the least. Inapparent infections and carrier states can occur in psittacine and some wild bird populations. Man, environmental reservoirs and the characteristics of NDV play a considerable role in the persistence of the disease in village chickens. When mesogenic or avirulent strains of NDV are involved in the infections or when the chickens are only partially immune, recovered birds may shed the virus for a period after recovery, as would latently affected chickens. Vaccinated birds may also be a source of the virus to susceptible birds. However, clinically diseased birds may constitute one of the most important sources of infection. Spradbrow (2001b) reported that some strains of ND can produce conjunctivitis in humans- especially for those that regularly work in close contact with chickens such as laboratory workers and abattoir staff. However, serious human symptoms are rarely encountered.

1.2.2 Transmission of Newcastle Disease

Newcastle disease can be spread by mechanical means or by other species of poultry, pets and wild birds (Hafez, 2005). Direct contact with sick, susceptible birds, faeces and respiratory discharges or contamination of the environment including food, water, equipment, and human clothing spreads the virus. Newcastle disease viruses can survive for long periods in the environment, especially in faeces. Generally, the virus is shed during incubation, during the clinical stage and for a short time during recovery. Some psittacine species can shed the virus intermittently for a year or more. Virus is present in all parts of the carcass during acute infection and at death (Alexander, 1998). In the village chicken production system, Mavale (2001) stated that the most common route of transmission in village chickens is probably the fecal-oral route. Transmission by the respiratory route is important mainly in intensively managed systems. Circulating strains can cause up to 100 % mortalities (Alders & Spradbrow, 2001). Mavale (2001) also reported that the factors that influence outbreaks of ND in village chickens include the immune status of the flock, the nutritional status of the flock and the seasonality of the disease. Some factors that influence the seasonal occurrence of ND in village chickens may be scarcity of feed at certain times of the year, different age ranges of village chicken flocks, climate stresses, incidence of other infections and seasonal peaks in market activity.

1.2.3 The Aetiological agent of Newcastle Disease

Newcastle disease (ND) is caused by viruses of the Avian Paramyxovirus type 1 (APMV-1) serotype of the genus *Avulavirus,* family *Paramyxoviridae* (OIE, 2010). There are ten avian paramyxovirus serotypes designated APMV–1 to APMV–10 (CFSPH 2005; Miller, Afonso, Spackman, Scott, Pedersen, Senne, Brown, Fuller, Uhart, Karesh, Brown, Alexander & Swayne, 2010; OIE, 2010). NDV (APMV-1) may show some antigenic cross-relationships with some of the other avian paramyxovirus serotypes, particularly APMV-3 and APMV-7. However, these problems can be resolved by the use of suitable antigen and antiserum controls (OIE, 2010).

APMV-10 was recently isolated from rockhopper penguins (*Eudyptes chrysocome*) in a study conducted in the Falkland Islands by Miller *et al.* (2010). The study showed biological, serological, and genomic characterization of the paramyxovirus. This penguin virus resembled other APMVs by electron microscopy; however, its viral hemagglutination (HA) activity was not inhibited by antisera against any of the nine defined APMV serotypes. In addition, antiserum generated against this penguin virus did not inhibit the HA of representative viruses of the other APMV serotypes (Miller *et al.* 2010).

1.2.4 *Classification of Newcastle Disease Virus*

Multiple systems of classifying NDV are currently utilized worldwide with no consensus as to which is more appropriate. In the preliminary characterization studies of NDV, six lineages (1 to 6) were determined using restriction enzyme analysis. These groupings have subsequently been confirmed, and two further lineages (7 and 8) and several sublineages within these have been identified through nucleotide sequencing studies (Aldous *et al.* 2003). Only a single serotype of APMV-1 exists (Czegledi, Ujvari, Somogyi, Wehmann, Werner & Lomniczi, 2006).

Lineage 1 probably represents the primordial form of NDV and contains avirulent viruses primarily from waterfowl, but also from chickens. Strains have been used as live vaccines. Lineage 2 and sub-lineages 3a and 4b were involved in the first pandemic of ND which started in mid-1920s and subsided in the late 1950s. Strains from lineage 2 have been used as live vaccines (e. g LaSota/46 and Hitchner/B1). Strains from sub-lineage 3c are considered to be composed of isolates from or considered to be from the second NDV pandemic in the 1970's which was influenced by trade in exotic birds. Birds of the families Psittacidae (Parrots), Sturnidae or starlings (mynahs), and other caged birds such as Pittidae (pitas) that moved in commercial channels were the principle source of infection during the 1970-72 pandemic (USA) of the velogenic viscerotropic form of the Newcastle disease (Alexander, 1998). Sub- lineage 4b is composed solely of viruses associated with the ongoing panzootic in pigeons. Lineages 3 and 4 emerged during the second pandemic of the 1960s and 1970s. In a study conducted by Lomniczi, Wehmann, Herczeg, Ballagi-pordany, Kaleta, Werner, Meulemans, Jorgensen, Mante, Gielkens, Capua and Damoser in 1998, strains derived from sporadic cases in Denmark, Sweden, Switzerland and Austria were classified into lineage 4, the same group which caused outbreaks in the Middle East and Greece in the late 1960's and in Hungary in the early 1980's. Sub-lineage 4b of pigeon origin was responsible for the third pandemic during the 1980s. Two more types; lineage 5 and sub-lineage 3d, appeared in late 1980s. An outbreak of APMV-1 (lineage 4) occurred in pigeons in the USA and the UK during 1984 (Alexander, 1998) and 1990s in East Asia, Europe and South Africa (Aldous *et al.* 2003; Xiufan & Yongzhong, 2005). In contrast, viruses that caused epizootics in Germany, Belgium, The Netherlands, Spain and Italy could be classified into lineage 5, hitherto undetected in Europe. It is possible that lineage 5 viruses originated in the Far East because they showed a high genetic similarity (97 %) to NDV strains isolated from Indonesia in the late 1980’s. It has been suggested that since 1927 there have been four or more panzootics of Newcastle disease (Alexander, 1991). Several genetically distinguishable viruses can be co-circulating at any one time. However, two panzootics, the one occurring in poultry and pet birds in the early 1970s and the panzootic in pigeons, were clearly distinct entities. Isolates considered from or derived from the panzootic that occurred in the early 1970s were all placed in sub-lineage 3c. The outbreaks of virulent NDV that affected cormorants in the US and Canada in the 1990s are also represented in sub-lineage 3c, but form a discrete branch. This cormorant epizootic led to infections in domestic poultry (Aldous *et al.* 2003).

Waterfowl are usually considered to be resistant even to the most virulent strains for chickens, however clinical diseases with high mortality caused by certain lineages of NDVs in domestic geese and in wild double-crested cormorants have emerged since 1990s in China and North America respectively. The novel ND entity in the goose was caused by viruses of lineage 5, whereas the epizootics in comorants were linked with viruses representing an unclassified novel genotype closely related to sub-lineage 3c. It seems that the increased virulence of ND viruses to some species of waterfowl happened concurrently with the emergence of new lineages which have evolved in particular ecological and geographical areas. The emergence of sub-lineage 5d viruses in China in the late 1990s was concomitant with the sudden appearance of clinical ND outbreaks in goose flocks. Most of the NDVs isolated from outbreaks in goose flocks since the late 1990s, together with most of the chicken isolates during the same period, have been classified as sub-lineage 5d. The viruses showing high virulence for geese fall into lineage 4, lineage 5, and 3d respectively, while the far less pathogenic (to geese) strain of Herts/33 is a member of sub-lineage 3b and very virulent for chickens (Aldous *et al.* 2003; Xiufan & Yongzhong, 2005).

In many African countries, ND has been officially and unofficially reported and remains the main poultry disease in commercial and rural chickens in Africa. Virological and epidemiological information concerning NDV strains circulating in the western and central regions of Africa has, in the past been scarce. However, in recent years, molecular studies have been conducted in West Africa and in countries such as Sudan, Ethiopia and Madagascar that have produced more information regarding the disease in Africa. Sequence analysis, pathotyping and detailed genetic characterization of virulent ND strains detected in rural poultry in West and Central Africa revealed the circulation of new genetic lineages, distinguishable from the lineages described in the eastern and southern parts of the continent (Snoeck, Ducatez, Owoade, Faleke, Alkali, Tahita, Tarnagda, Ouedraogo, Maikano, Mbah, Kremer & Muller, 2009). Partial sequence analysis was conducted on 44 Newcastle disease virus (NDV) strains obtained between 2002 and 2007 from different poultry species in Nigeria, Niger, Burkina Faso and Cameroon. Lineage 2 viruses identified were genetically identical or similar to the locally used LaSota vaccine strain and were mostly detected in commercial farms. Lineage 1, 3 and 4 strains were only sporadically found, and their origin was less clear. Twenty-one strains from backyard farms and live bird markets (LBMs) formed three new clusters within lineage 5, tentatively named 5f, 5g and 5h. A more recent study also reported the identification of these three unique sub-lineages in West Africa, and sub-lineages 5f and 5g in particular in non-commercial farms in Nigeria. In this study, 33 NDV isolates, which included NDVs recovered from LBMs in Nigeria, during active surveillance from 2007 to 2008 and viruses recovered from outbreaks in backyard and commercial chicken farms within the same period were analysed. The close genetic similarities identified, provided evidence for the first time of the epidemiological link between the viruses circulating in the LBMs and those recovered from outbreaks in backyard and commercial chicken farms in Nigeria between 2007 and 2008 (Solomon, Abolnik, Joannis & Bisschop, 2011).

All of these strains were predicted to be virulent based on their F protein cleavage site sequence. Minimal genetic distances between new and previously established sub-lineages ranged from 9.4 to 15.9 %, and minimal distances between the new sub-lineages were 11.5 to 17.3 %. Their high genetic diversity and their presence in three different sub-Saharan countries suggest that these new sub-lineages represent the NDV variants indigenous to West Africa. In Central Africa, a molecular epidemiological study of avian paramyxovirus type 1 isolates carried out by Aldous *et al.* (2003) indicated the presence of sub-lineage 3c (largely composed of isolates derived from the 2nd pandemic of the 1970s) viruses that were obtained from a Tanzanian duck and chicken and sub-lineage 3d, composed of velogenic viruses derived from 1965 to 1994 in South Africa, Asia and Europe was isolated from chickens and turkeys (Aldous *et al.* 2003; Abolnik, 2007). Another virus isolated from a duck in Tanzania in 1995 was grouped under lineage 4a. Isolates from South Africa and Mozambique were grouped under sub-lineage 5b. The same study included two Zambian strains isolated from domestic fowl in 1995. Both were found to belong to lineage 2 (Cattoli *et al.* 2009).

There was an overall tendency towards chronological and geographical links between isolates placed in the same lineages and sub-lineages. However, the distribution of NDV is greatly influenced by international trade in poultry, poultry products and other birds such as captive caged birds and pigeons. It is likely that many of the natural factors that affect the epidemiology and evolution of NDV will be masked or influenced by these man-made factors. Precise factors affecting the epidemiology and evolution of NDV are unclear (Aldous *et al.* 2003). Investigations reported by Cattoli *et al.* (2009) and Snoeck *et al.* (2009) provide important information on the epidemiology, diagnosis and control of NDV in Africa and highlight the importance of supporting surveillance in developing countries for trans-boundary animal diseases. The rapid detection and assessment of the virulence of NDV is essential to ensure that outbreaks are contained and their impact minimized.

1.2.5 *Seroprevalence of Newcastle Disease*

Various studies have been conducted to determine the seroprevalance of ND in various countries in Africa. In a study conducted in Zambia by Alders *et al.* in 1994, the seroprevalence of NDV infection in chickens was 36.9% (based on the haemagglutination inhibition (HI) titres of 2000 blood samples). Seroprevalence varied between provinces, ranging from 29.2% in Northern Province to 51.3% in Copperbelt Province.

In Ethiopia, Tadesse, Ashenafi and Aschalew (2005) reported that the seroprevalence rates of 28.57 %, 29.69 %, and 38.33 % were found in Debre Berhan, Sebeta, and Nazareth areas respectively. A Newcastle disease (ND) seroprevalence rate of 43.68 % in central Ethiopia was reported among local scavenging chickens kept under a traditional management system (Ashenafi, 2000). Another study was conducted in two districts of eastern Shewa zone, Ethiopia by Chaka, Goutard, Bisschop and Thompson (2012) in 2010 to estimate the seroprevalence of ND (and other poultry diseases not being considererd in this study) in the wet and dry seasons. The overall seroprevalence of ND was 5.9 % during the dry season and 6.0 % during the wet season.

In Nigeria, a study carried out in rural chickens by Ezeokoli, Umoh, Adesiyun and Abdu (1984), showed a 73 % prevalence of antibodies against NDV in traditionally managed backyard flocks in Zaria. A similar study conducted by Eskoli (1984) reported a 72 % seroprevalence of NDV in traditionally managed, non-vaccinated village chickens. Sixty-three percent seroprevalence was reported by Orajaka, Adene, Anene and Onuoha (1999) in south eastern Nigeria. In south western Nigeria around Ibadan, 38% seroprevalence was reported by Oyewola, Ogundipe and Durojaiye (1996). In their study, Musa, Abdu, Dafwang, Umoh, Sa'idu, Mera, & Edache (2009) reported that 51.9 % of chickens had detectable antibodies to NDV among the 1, 208 chickens reared under extensive management systems in the four Local Government Areas (LGAs) of Plateau State that were used for the study.

In Tanzania, 13.3 % of the chickens were seropositive when HI test was performed prior to vaccination against ND from 120 chickens > 4 months of age from five villages (Minga, Katule, Maeda & Musasa, 1989). In another study conducted by Yongolo (1996) in Tanzania, seroprevalence varying from 25 % to 81.5 % was reported. In Mauritania, serum samples were obtained from 80 chickens in rural poultry flocks in each of three different regions. Antibodies against NDV were detected in 4.6 % of chickens. A serological study was also conducted in Benin in three ecologically different regions in the south, centre and north. Seropositivity of chickens for NDV was 56, 75 and 69 %, respectively (Bell, 1992). In a study to determine the epidemiology and characterization of ND in smallholder poultry conducted in Mozambique by Perttula (2009), 41 % of the unvaccinated chickens were positive for antibodies against NDV. Studies have shown that there is a variation in seroprevalence in the various regions of Africa. Various factors may account for this.

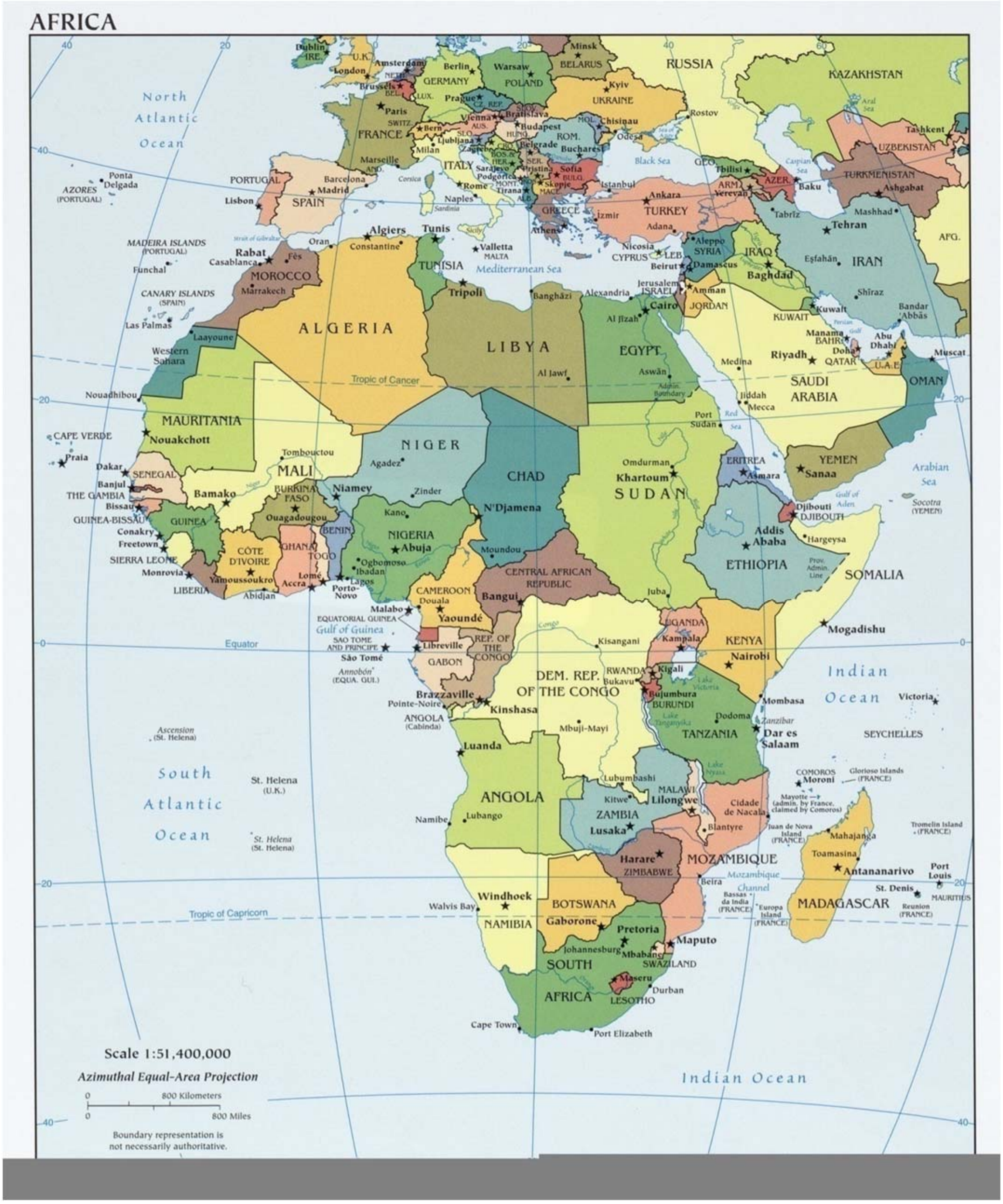

FIGURE 1.2 Map of Africa (Source: Worldatlas)

1.2.6 *Control of Newcastle Disease*

The control of ND in village chickens involving strict quarantine or strict biosecurity cannot achieve good results because of the difficulty in controlling the movement of people and chickens, as well as the control of the seasonal peaks in the marketing of the birds. The use of vaccines could be the one method that would be feasible in the control of the disease in village chickens. This method can only be helpful if the most challenging problem of thermostability is dealt with, as refrigeration is a huge setback in rural areas (Spradbrow, 2001). Currently in Zambia, the thermolabile, live lentogenic LaSota/46 vaccine strain is widely used by poultry producers in the commercial sector. The other control method would be to provide poultry farmers with written livestock messages that are comprehensive, but concise. These extension messages should include information such as the importance of segregation of unhealthy birds, the proper disposal of dead birds as well as viscera and feathers that remain if the birds are eaten and ensuring that the meat is properly cooked before consumption. Thus, for the successful control of ND, new vaccination technologies (such as the thermostable vaccines that have been tried and tested and have scored successes in countries such as Mozambique) and appropriate extension methods will have to be adopted.

Bell (2001) outlined the relative advantages and limitations of the different types of vaccines that could be employed to protect village chickens against ND. Conventional vaccines (for example Hitchner B1 [HB1], La Sota/46 and Komarov strains) have the disadvantage of requiring a cold chain to maintain their efficacy. Maintenance of the cold chain during distribution can be very difficult in village settings, particularly in locations with high ambient temperatures. Heat-resistant vaccines, also termed thermo-tolerant vaccines of which the NDV4-HR and the I-2 strains are examples, have the advantage in the village situation in that it is not critical to refrigerate the vaccine during transit.

The use of thermo-tolerant vaccines in village chickens has proved to be effective in rural areas of Asia and Africa (Alders and Spradbrow 2000; Bell, 2001; Wambura, Kapaga & Hyera 2000, Dieleman, 2001). Following successful trials, the I-2 vaccine is now produced in Mozambique, Tanzania and Malawi, where it is currently being employed to vaccinate village chickens against ND. Local production of the vaccine adds to the significant advantage of low cost (Bell, 2001), but the use of extension methods and vaccination as control measures for ND would have to be tailored to each country (Spradbrow, 2001). At present, the I-2 vaccine is not being utilized to vaccinate chickens in Zambia. It is anticipated that a combination of the aforementioned methods in the control of ND in village chickens in Zambia would be more effective limiting mortalities, improving flock numbers and ultimately improving food security and generating much-needed income for many poor households in the country.

1.2.7 The Problem/ Hypothesis

Newcastle disease is considered the major cause of mortalities of chickens in Zambia (Alders, Mudenda, Katongo and Inoue, 1994). The disease hinders production in village chickens with implications for food security and income generation. The majority (about 72 %) of employed persons in Zambia are engaged in the agricultural sector, mainly as small-scale farmers residing largely in rural areas. Unfortunately, despite being a major part of the Zambian population, small scale farmers are currently among the poorest in society (Central Statistics Office, 2004).

1.2.7.1 Benefits arising from the project

The benefit arising from this project is the determination of the seroprevalence of NDV antibodies in backyard village chickens. Recognizing that small-scale farming has the potential to make an important developmental contribution to the national economy, the government is now considering improving support to the agricultural sector thus spurring sectoral growth and productivity. It is anticipated that the right agricultural policy environment will improve sectoral incomes and savings, and contribute to poverty reduction.

Thus, the data generated is expected to contribute towards a more clear understanding of the epidemiology of NDV in Zambia. Providing updated information on the seroprevalence of NDV in Zambian village chickens would be a starting point to justify an official policy for a ND vaccination programme. Vaccination of village chickens against ND would limit mortalities, improve flock numbers and ultimately improve the diet requirements, provide food security and generate much-needed income for many poor households in the country.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Experimental Design

2.1.1 Sample collection

In a cross-sectional survey, serum samples used in this study were collected from various randomly selected, apparently healthy, unvaccinated, local chicken breeds in various districts of Zambia between the months of June and December, 2009 and 2010. Only chickens that had not been vaccinated against ND and were apparently healthy at the time of sample collection were included in the survey.

2.1.2 Sample size determination

The formula of Cannon & Roe (1982) was used in determining the sample size. This was done with an assumption that the seroprevalence of ND in the country was 25 % with 95 % probability of detecting at least one infected chicken. Eleven districts from five provinces were sampled. Twenty-nine households from each district were included in the study. This resulted in a sample size of about 1,595 samples. However, due to logistical problems, only 1,012 samples were obtained. Two millilitres of blood samples were collected from the wing vein of each bird. The blood was allowed to clot at room temperature after which sera was separated and stored at -20 ^{o}C until needed for laboratory analysis.

FIGURE 2.1 Zambia's Nine Provinces (Bized, 2001)

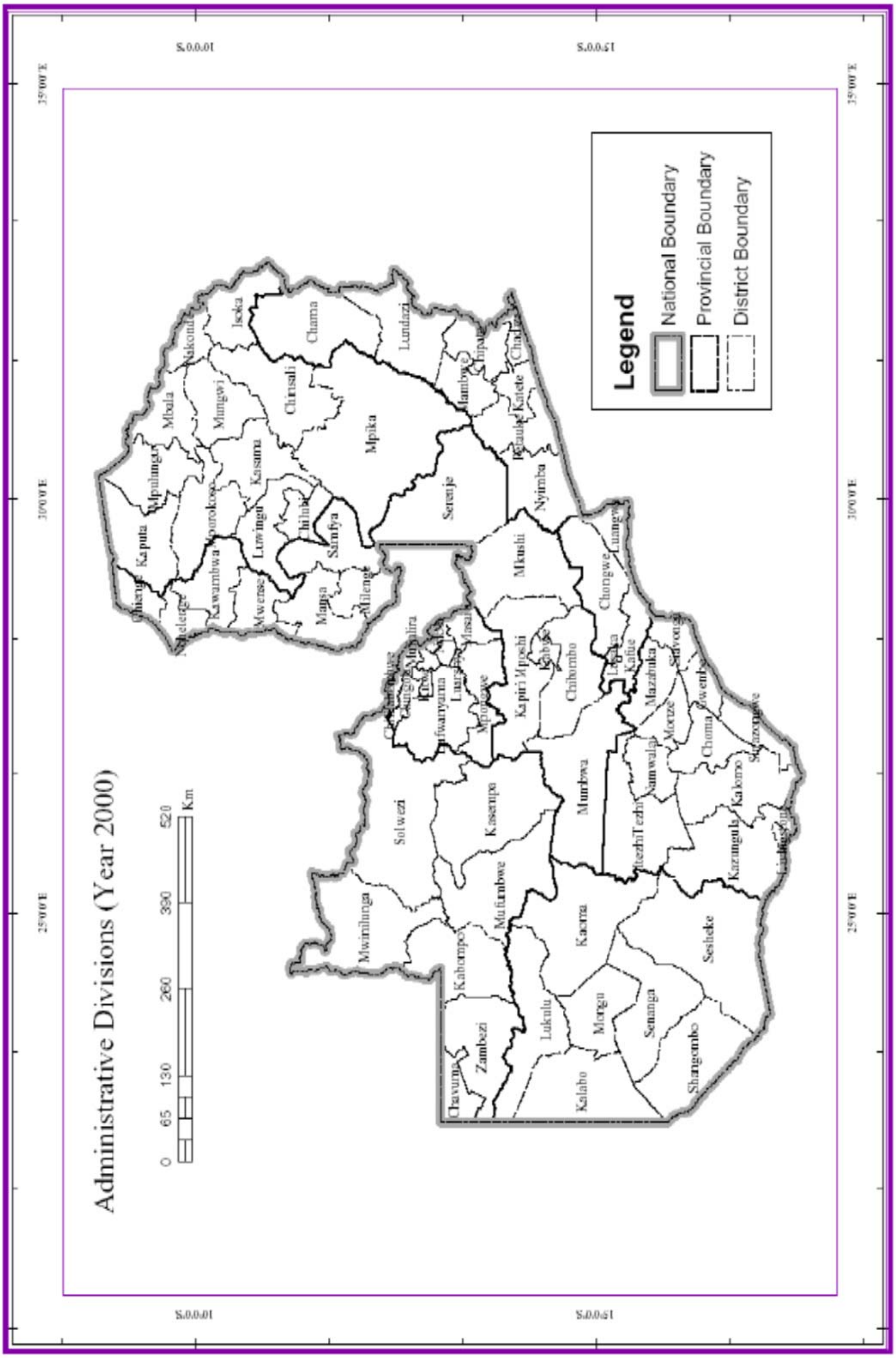

FIGURE 2.2 Zambia's Districts (Sources: CSO, 2000)

Samples were collected from the Eastern, Southern, Northern, Copperbelt and Luapula Provinces (Fig 2.1). Samples collected from districts located along a border area; with the neighboring country indicated in parentheses are Nakonde (Tanzania), Chililabombwe (Democratic Republic of Congo), Mufulira (Democratic Republic of Congo), Kazungula (Botswana) and Chipata (Malawi). Samples collected from districts located near water bodies (indicated in parentheses) are Namwala (Kafue flats-a marsh), Itezhi tezhi (Itezhi Tezhi dam in the Kafue National park), Samfya (Lake Bangweulu), Siavonga (Lake Kariba) and Mpulungu (Lake Tanganyika). Monze District is located along a major railway (Fig. 2.3 and 2.4).

FIGURE 2.3 Zambia's Neighbours (Bized, 2001)

FIGURE 2.4 Kafue National Park (www.nanzhila.com/kafue_national_park.php)

2.1.3 Laboratory Analysis

Samples were analysed using a commercial ND FlockChek ELISA kit (IDEXX Laboratories, USA) according to the recommended procedure. The laboratory work was undertaken at the Central Veterinary Research Institute (CVRI) in Lusaka, Zambia.

2.1.4 Procedure

Reagents and serum were allowed to come to room temperature (22 – 27 ^{0}C). The contents of each container were then mixed gently by inverting and swirling. The position of the controls and test samples were recorded on a sample information sheet. Antibody was added to the wells (that are pre-coated with antigen) except for the negative controls. The wells were then covered and incubated at room temperature for 30 minutes to allow a complex to form with the antibody specific to NDV. After incubation, the liquid contents of all wells were aspirated. Each well was then washed four times by hand with a wash buffer dissolved in distilled water. After washing away unbound material from the wells, the conjugate (coupled to horseradish peroxidase) was then added (which binds to any attached chicken antibody in the wells). The plates were again covered and incubated for 30 minutes, after which the liquid contents were again aspirated. Unbound conjugate washed away using the wash buffer dissolved in distilled water. Enzyme substrate was then added to the wells and incubated for 15 minutes. At the end of the 15 minutes incubation period, stop solution was dispensed into each well to stop the reaction.The subsequent colour development showed a direct relation to the amount of antibody to NDV present in the samples. An ELISA reader (Multiskan- Labsystems) was used to read the results. Absorbance values were measured at 650 nm. According to the protocol in the kit, serum samples with Sample/Positive (S/P) ratios less than or equal to 0.2 should be considered negative. S/P ratios greater than 0.2 (in this study, titres greater than 396) should be considered positive and indicates either vaccination or other exposure to NDV.

2.1.5 Data Analysis

Data were initially entered into Microsoft Excel and later transferred to SPSS (SPSS Inc. USA) for analysis. The seroprevalence of NDV exposure was calculated for each Province and District under study. Comparison in seroprevalence between the Provinces and between the Districts was done using the Fisher's exact test. Analysis of variance (ANOVA) was done to determine whether the log transformed titers from the various provinces were significantly different or not. Values were considered significant at $p \leq 0.05$.

CHAPTER THREE: RESULTS

TABLE 3.1 Seroprevalence of Antibodies to Newcastle Disease Virus in the 5 provinces sampled in descending order

S/N	Province	Number of bird sampled	Number of positive birds	Seroprevalence (%) (P)	95% Confidence Interval (CI)
1	Eastern	92	76	82.6	74.9 – 90.4
2	Southern	566	413	73.0	69.3 - 76.7
3	Northern	184	150	81.5	75.9 – 87.1
4	Copperbelt	184	95	51.6	44.4 – 58.8
5	Luapula	29	14	48.3	30.1 – 66.5

TABLE 3.2 Seroprevalence of Antibodies to Newcastle Disease Virus in each district sampled by province

Province	District	Number of birds sampled	Number of positive birds	Sero-prevalence	95% Confidence Interval
Eastern	Chipata	92	76	82.6	74.9 – 90.4
Southern	Monze	92	84	91.3	85.5 – 97.1
	Namwala	135	77	57.0	81.8 – 93.0
	Siavonga	92	71	77.2	68.6 – 85.8
	Itezhi Tezhi	63	48	76.2	65.9 – 86.7
	Kazungula	184	133	72.3	65.2 – 78.2
Northern	Mpulungu	92	84	91.3	85.5 – 97.1
	Nakonde	92	66	71.7	62.5 – 80.9
Copperbelt	Chililabombwe	92	74	80.4	72.3 – 88.5
	Mufulira	92	21	22.8	14.2 – 31.4
Luapula	Samfya	29	14	48.3	30.1 – 66.5

3.1 Results

The results of the seroprevalence of NDV in the five provinces of Zambia are shown in Table 3.1 in descending order of seroprevalence. The results show that Eastern Province had the highest seroprevalence (P = 82.6 %; CI = 74.9 to 90.4 %), followed by Southern Province (P = 80.0 %; CI = 76.7 to 83.3 %), Northern Province (P = 77.3 %; CI = 70.4 to 84.2 %), Copperbelt Province (P = 51.6 %; CI = 44.4 to 58.8 %) and Luapula Province had the least seroprevalence (P = 48.3 %; CI = 30.1 to 66.5 %). The seroprevalence of NDV among the provinces was significantly different ($p<0.001$).

The seroprevalence of NDV was also determined at district level (Table 3.2) to determine whether there was any variation in seroprevalence among the 11 districts sampled. The seroprevalence among the districts was also found to be different. Comparing the districts by province and starting with Southern Province, the results showed that Monze District (P = 91.3 %; CI = 85.5 to 97.1 %) had the highest seroprevalence, while Kazungula District had the lowest seroprevalence (P = 71.7 %; CI = 65.2 – 78.2 %). Of the two districts that were sampled in Northern Province, Mpulungu District had the highest seroprevalence (P = 87.8 %; CI = 78.6 – 97.0 %) while Nakonde District had the least (P = 71.7 %; CI = 62.5 – 80.9 %). For the two districts that were sampled in the Copperbelt Province, Chlilabombwe District had the highest seroprevalence (P = 80.4 %; CI = 72.3 – 88.5 %) while Mufulira District had the lowest (P = 22.8 %; CI = 14.2 – 31.4 %). Only one district in Eastern and Luapula Provinces was sampled and could therefore not be compared. The seroprevalence of NDV among the districts within the provinces was significantly different ($p<0.001$). Furthermore, the results also showed that the province with the highest seroprevalence of NDV did not necessarily have districts with the highest seroprevalence of NDV (see Table 3.2).

TABLE 3.3 Seroprevalence of Newcastle Disease in each district in descending order

Province	District	Number of birds sampled	Number of positive birds	Sero-prevalence	95% Confidence Interval
Southern	Monze	92	84	91.3	85.5 – 97.1
Northern	Mpulungu	92	84	91.3	85.5 – 97.1
Southern	Namwala	135	77	57.0	48.6 – 65.4
Eastern	Chipata	92	76	82.6	74.9 – 90.4
Copperbelt	Chililabombwe	92	74	80.4	72.3 – 88.5
Southern	Siavonga	92	71	77.2	68.6 – 85.8
Southern	Itezhi Tezhi	63	48	76.2	65.9 – 86.7
Southern	Kazungula	184	133	72.3	65.8 – 78.8
Northern	Nakonde	92	66	71.7	62.5 – 80.9
Luapula	Samfya	29	14	48.3	30.1 – 66.5
Copperbelt	Mufulira	92	21	22.8	14.2 – 31.4

TABLE 3.4 Mean log_{10} ELISA titre readings of NDV among the provinces

Province	N	Mean log titre	Std. Deviation	95% Confidence Interval for Mean		Range	
				Lower Boundary	Upper Boundary	Min.	Max.
Copperbelt	184	2.2352	1.07886	2.0783	2.3921	.00	4.00
Southern	566	2.9431	.62315	2.8917	2.9946	.00	4.29
Northern	141	2.8714	.55331	2.7793	2.9635	.00	4.16
Eastern	92	2.9288	.53099	2.8188	3.0387	1.08	4.21
Luapula	29	2.5398	.88462	2.2033	2.8763	.00	4.06
Total	1012	2.7915	.76861	2.7441	2.8390	.00	4.29

* The mean difference is significant at $P \leq 0.001$.

3.2 Comparison of the Titres

In order to determine whether the titre levels varied in chickens from different provinces, one-way ANOVA was carried out on the $\log_{10}$ transformed ELISA titres. Log transformation was done because the titres were positively skewed and not normally distributed (data not shown). Results of the analysis showed that there was a significant difference in the titre levels among the provinces ($p < 0.001$) with the province with the lowest seroprevalence having low titres and the one with highest seroprevalence having high titres. The results are shown in Table 3.4. Southern Province had the highest mean titres and these were significantly higher than those from Copperbelt and Luapula Provinces ($p < 0.001$). Eastern Province had the second highest mean titres and these were also significantly higher than those from Copperbelt Province, while Northern Province had the third highest mean titres and these were significantly higher than those from Copperbelt Province. Copperbelt Province had the lowest mean titres. The actual readings of individual chickens are shown in Appendix 1. The results further showed that 73.9 % of the birds sampled tested were positive for NDV antibodies.

CHAPTER FOUR: DISCUSSION

4.1 Sample Areas

The serum samples used in this study were collected from various randomly selected, apparently healthy, unvaccinated, local chicken breeds in 11 districts from five provinces of Zambia between the months of June and December, 2009 and 2010. Birds showing signs of illness were excluded from the study. The areas sampled included Chililabombwe and Mufulira Districts (Copperbelt Province), Monze, Siavonga, Itezhi Tezhi, Namwala and Kazungula Districts (Southern Province), Chipata District (Eastern Province), Mpulungu and Nakonde Districts (Northern Province) and Samfya District (Luapula Province). The sampling was done either at border areas, busy market places or near water bodies. This was done because it was anticipated that NDV may be found in chickens that are reared in or around such areas. NDV is spread among chickens when they are gathered to be sold at busy market places or are moved across borders. Some wild birds have been reported to be carriers of the NDV. In a study to determine the seroprevalence of NDV in local chickens in Central Ethiopia, Tadesse *et al.* (2005) included wild birds as one of the risk factors associated with the maintenance of NDV. Wild birds were also cited as a risk factor in the maintenance of NDV by a study conducted in Kenya by Njagi, Nyaga, Mbuthia, Bebora, Michieka, Kibe & Minga (2010). It was beyond the scope of this study to determine to which lineage or genotype the virus/es causing the seroprevalence of NDV in Zambia belongs as it is not possible to do so from sera- the sample material used in the study.

4.2 Factors affecting ND outbreaks

In Zambia, the seroprevalence of NDV among the provinces and districts studied differed and a number of factors may account for this. These include, but are not limited to, seasonal conditions, social reasons, management practices, and the number of chickens in the province / district and circulating lentogenic and / or mesogenic strains. Songolo and Katongo (2000) attributed the occurrence of ND in Zambia to seasonal and social factors.

Seasonal outbreaks of ND have been recognized in many countries in Africa. These outbreaks have been attributed to the weather conditions prevalent at the time. In Zambia for instance, although the occurrence on ND is reported from the districts all year round, there are slight peaks in the months of January to March and September to November. January to March is cool and humid with heavy rains, while September to November is the hot and dry season with increased wind flow throughout the country. Both seasons are thought to favour the airborne transmission of the virus (Sharma *et al.* 1985), but more so the hot and dry season because of the wind. This study was conducted over a ten year period. A similar scenario was reported in Mozambique by Harun and Massango (2001).

In Nigeria, a study conducted by Musa, Abdu, Dafwang, Umoh, Sa'idu, Mera & Edache (2009), it was demonstrated that ND outbreaks occurred year round in the villages sampled, with the highest incidence of 86.6% observed from November to March (dry season) and September to October, 8.31% (pre-dry season). In this study, it was reported that ND was most common and severe at times of climatic stress. Outbreaks were often associated with change of season. Cold and hot weather had been cited as contributory factors in ND outbreaks (Abdu, Mera & Sa'idu, 1992). The disease was also reported to be more common during the dry Harmattan (November-March) and cold stress had been known to worsen the outcome of ND (Halle, Umoh, Sa'idu & Abdu, 1999; Sa'idu, Abdu, Tekdek, Umoh, Usman & Oladele, 2006). Of the two areas (LM5- lower midland 5 and LH1- lower midland 1) that were considered in a study conducted in Kenya, the findings concerning seasonal occurrence of ND agreed with the responses given by the farmers (Njangi *et al.* 2010). Previous studies associated ND with change of seasons, some reportedly associated with the start of the wet season (Thitisak, Janviriyasopak, Morris, Srihakim & Kruedener, 1988; Jintana, 1987). Cold weather has been associated with ND outbreaks in some countries (Dao & Pham, 1985), while in others it is hot weather (Bell, Kane & Le Jan, 1990); for others still, it is with both cold and hot seasons (Nyaga, Nyaga & Kariuki, 1985). In Ethiopia, poultry diseases worsened after the initiation of an Ethiopian “villagization” programme in which farmers from different areas were settled in certain localities (1984–1986). Prior to the programme, disease outbreaks occurred at the beginning of the rainy season, but after villagization, outbreaks remained a problem throughout the year (Tadesse *et al.*2005). In another study conducted by Chaka, Goutard, Bisschop & Thompson (2012) to estimate, among other poultry diseases, the seroprevalence of Newcastle disease (ND) in four live poultry markets in two districts of Eastern Shewa zone Ethiopia, they found that area and season had no significant effect on the seroprevalence of NDV indicating the widespread presence of the virus throughout the year in the study areas. As can be observed, there are diverse views regarding which season is associated with ND outbreaks. What is clear however is that although outbreaks occur at certain times of the year, the incidence of the disease and occasional deaths are reported throughout the year.

It is possible that the seasonal occurrence of ND may be as a result of other factors. Thus, although seasonal conditions are generally viewed as one of the causes of the ND outbreaks, they may be only indirectly involved (Spradbrow, 2001b). Increased movement of chickens may be the direct influence. Increased movement of sick and healthy chickens in anticipation of various festivals particularly of Sallah, Christmas and New Year festivities may have been responsible for the peaks of ND outbreaks within this period (November-March). Also, movement of infected chickens which may mix with healthy ones is probably the main source of NDV (Sa'idu *et al*. 2006; Nwanta, Umoh, Abdu, Ajogi & Alli-Balogun, 2006). In Zambia, chickens are carried during extensive travelling to visit friends and relatives for the festive season, and the need in January, for cash to meet school fees requirements (Songolo & Katongo, 2000). In addition, because of the fast cash that can be made during these periods, people tend to bring chickens together to market places and/or border areas to be sold. It is at these market places and/or border areas that village chicken trading occurs and ultimately that transmission of the disease may occur from bird to bird through direct contact with faeces and respiratory discharges or by contamination of the environment including food and water. Though this may be doubtful, the virus does survive for longer periods in faeces in the environment (Alexander, 1998). As a result this, virus laden chicken faeces left behind many days before may still be infective to susceptible chickens many days after.

The management system in traditional production may serve as a stress factor and favor infection. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, the absence of vaccination in traditionally managed chickens, and contact of chickens of different age groups from one village with those from another may facilitate the spread of NDV. The keeping of flocks of various ages can facilitate the transmission of ND (Spradbrow, 2001b). This is common in village chicken husbandry and although mortality is higher in young chickens, depending on the strain, 100% mortality may occur in adult chickens as well (Alexander, 1998).

Among the risk factors associated with the maintenance of NDV mentioned in a study conducted in Kenya was village poultry population dynamics (Njagi *et al.* 2010). This may also be a factor in the Zambian situation. In 2003, the Central Statistics Office (CSO) survey report revealed that Eastern and Northern Provinces had the highest number of chickens in the country and Copperbelt and Luapula Provinces had the least. It is well known that the virus is contagious and spreads very easily through contact. Consequently, the more chickens there are in an area, the higher the chance of exposure to the virus. When ND was first reported in Zambia in 1952, it was concentrated along the railway line where the largest population of poultry, especially chickens, occurred (Songolo & Katongo, 2000). The large population of chickens provided a sustainable reservoir for the maintenance of the virus and as a consequence could have allowed the disease to persist or could have allowed the introduction of new disease more frequently. Therefore, although Eastern and Northern Provinces are not situated along the railway line, the increase in chicken numbers could have shifted the infection to these areas. This was observed in this present study, where Eastern Province was followed by Southern and Northern Provinces in demonstrating the increase in chicken numbers, which could have lead to the subsequent increase in ND seroprevalence.

The difference in seroprevalence among the provinces may also be attributed to the percentage of birds positive for antibodies to NDV approaching 50 % or more. It is possible that the birds were exposed to a lentogenic or a mesogenic strain (Sagild & Haresnape, 1987). Although the aforementioned study was conducted in Malawi; being one of Zambia's neighbours and sharing a long border with the Eastern Province of Zambia, its conclusions can be extrapolated. Lentogenic and sometimes mesogenic strains cause mild clinical signs and up to 10 % mortality respectively in chickens. It is thus possible that similar viruses are circulating and spreading in Zambia without causing clinical disease in the flocks, yet causing increasing titres in the chickens affected.

The areas from where the samples were collected had no outbreaks, although results showed that the disease exists. Although clinically diseased chickens are the most important hosts for NDV, latently infected birds and survivors of natural infection, which still harbour the agent, may also act as reservoirs. Village chickens may be exposed naturally to virulent virus shed from recovered birds, vaccinated birds having various levels of antibodies in their blood, non – susceptible species carrying virulent virus or susceptible birds yielding virulent virus, which may have evolved from passages in birds of mesogenic viruses (Westbury, Parsons & Allan, 1984; De Leeuw, Hartog, Koch & Peeters, 2003). It is worthwhile to consider chickens vaccinated against ND as a potential factor in the occurrence of the disease. The samples had been collected from apparently healthy, unvaccinated birds suggesting that the infections were due to circulating avirulent strains, but whether these were due to vaccine strains or another source, e.g. Class I or Class II lineage 1 NDVs that are common in wild birds (Czegledi *et al*. 2006), is unknown and since a single serotype of APMV-1 exists, we were unable to distinguish between vaccine-related and field strain antibodies here. In Zambia, the lentogenic, thermolabile LaSota/46 vaccine strain is widely used in the commercial sector and at present,thermotolerant vaccines are not being used.Active vaccination of village and backyard chickens against ND is rarely practiced due to cost and problems associated with maintaining the cold chain for the heat labile live vaccine. Nevertheless, unrecorded or unreported vaccinations could be a factor that may be associated with the spread of ND among village chicken flocks in Zambia. It is also possible that some spillover of vaccine into backyard chickens occurs, especially where spent layers end up in villages. This may contribute to the high incidence of NDV seropositivity in Zambia. It has been shown that vaccines alter the epidemiology of ND to some extent since they prevent disease, but not infection. Vaccinated birds exposed to virulent virus develop no clinical signs, however, some replication of the infecting virus occurs and birds excrete virulent ND virus. This would probably not be excreted in quantities as those excreted by susceptible birds, but there would be sufficient virus to infect other birds (Mavale, 2001; Miller, King, Afonso & Suarez, 2007).

The factors stated above accounting for the different seroprevalence levels of NDV in the provinces may also account for the seroprevalence of antibodies to NDV in the districts. Table 4.1 shows the prevalence of ND from the districts in descending order and the main geographical feature in/near the district from where the samples were collected. The seroprevalence of NDV in the districts did not show a clear pattern as to whether the main geographical feature from where the samples were collected in a particular district played a significant role in the seroprevalence of NDV. The table however, shows that whether a district from where the samples were collected was located near a water body or a border area, trading of village chickens occures and high seroprevalence levels of NDV were recorded as the results show. Many villagers are aware that NDV can be spread through trade and that outbreaks of NDV may follow the introduction of a newly purchased or newly gifted chicken (Spradbrow, 2001b). Thus, trading in village chickens plays a role in the occurrence of infection, and as the study conducted by Chaka *et al.* (2012) attested, it is possible that even in Zambia, area has no significant effect on the seroprevalence of NDV.

TABLE 4.1 Important local geographical feature in the district sampled

District	Province	Seroprevalence	Important feature in the district
Monze	Southern	91.3	Line of rail/ trading
Namwala	Southern	57.0	Water body/ trading
Siavonga	Southern	77.2	Water body/ trading
Itezhi tezhi	Southern	76.2	Water body/ trading
Kazungula	Southern	72.3	Border/ trading
Mpulungu	Northern	87.8	Water body/ trading
Nakonde	Northern	71.7	Border/ trading
Chililabombwe	Copperbelt	80.4	Border/ trading
Mufulira	Copperbelt	22.8	Border/ trading
Chipata	Eastern	82.6	Border/ trading
Samfya	Luapula	48.3	Water body/trading

CHAPTER FIVE: CONCLUSION

The results of this ELISA-based study showed that the seroprevalence of NDV in Zambia's provinces has significantly increased since the previous survey conducted by Alders et al. (1994), that was based on HI. In the current study, 1012 samples were analysed using a commercial ND ELISA. Other studies (De Wit, Davelaar & Braunius, 1992; De Sousa, Montassier & Pinto, 2000) have reported a good correlation between HI and ELISA for the detection of ND antibodies in chickens. Thus, the current results should be comparable to those of the previous survey conducted in 1994.

Seroprevalence for all but one of the provinces sampled was above the 50 % mark; the highest being Eastern Province with 82.3 % and the lowest being Luapula Province with 48.3 %. For the districts, Monze District in Southern Province had the highest prevalence of 91.3 %, while Mufulira District in the Copperbelt Province had the least with 22.8 %. Though Luapula Province had the lowest seroprevalence in this study, it was 40 % higher than the lowest level reported by Alders et al. in 1994. In the 1994 study, seroprevalence levels among the provinces varied from 29.2 % in Northern Province to 51.3 % in the Copperbelt Province.

Although unidentified NDV strains are apparently widespread in Zambia, little official attention has been given to the control of the disease in chickens since the Government ceased subsidized ND vaccinations in the 1980's (Sharma *et al.* 1985). The significant increase in seropositivity to antibodies to ND coupled with reports of the disease from the districts in the country throughout the year, could suggest endemicity of an NDV strain/s- the origins of which are at present unknown. This, therefore, highlights the ease with which the infection can spread. Incursion of a highly virulent ND strain to which the poultry population has insufficient immunity could have devastating consequences. However, these could be mitigated by a good vaccination strategy. Improved small-scale poultry farming would make an important contribution to the national economy and this could motivate the re-establishment of an official subsidized ND vaccination programme in Zambia. It would also be important to remember that other factors (such as village poultry management and other poultry diseases) other than ND have an impact on poultry production in Zambia. Therefore, the establishment of improved management packages is also recommended so that preventive and control programmes can be formulated. Thus, a vaccination programme in village and backyard chickens and effective extension messages designed for poultry farmers would limit mortalities, improve flock numbers and ultimately improve food security and generate much-needed income for many poor households in the country. Since the last study to isolate and characterize NDV strains causing the losses in the Zambian poultry sector was published almost 30 years ago (Hussein, Sharma, Ando and Chizyuka, 1984), there is a need to isolate and genetically characterize currently circulating strains affecting poultry in Zambia, so that appropriate control decisions may be taken.

REFERENCES

ABDU, P.A., MERA, U.M. & SA'IDU, L. 1992. A study on chicken mortality in Zaria. Nigeria. In: Proceedings.19th World's Poultry Congress, Amsterdam, The Netherlands, 20-24th Sept. 2:151.

ABOLNIK, C. 2007. Newcastle Disease. *Onderstepoort Journal of Veterinary Research,* 70:157-160 (2004).
Accessible at http://www.upetd.up.ac.za/thesis/available/etd-06202007.../01chapter1.pdf

ALDERS, R.G., INOUE, S. & KATONGO, J.C. 1994. Prevalence and evaluation of Hitchner B 1 and V4 vaccines for the control of Newcastle disease in village chickens in Zambia. *Preventive Veterinary Medicine* 21, 125-132.

ALDERS, R.G., MUDENDA, S.J., KATONGO, J.C. & INOUE, S. 1994. Preliminary investigations into the control of Newcastle Disease in village chickens in Zambia. Paper presented at village Animal Health Care workshop. Nairobi, Kenya.

ALDERS, R.G. (2001). Sustainable Control of Newcastle disease in Rural Areas. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N° 103, 80-87.

ACIAR, accessible at http://www.aciar.gov.au

ALDERS, R.G AND SPRADBROW, P.B. 2000. Newcastle Disease in Village chickens. A field manual. Australian Centre for International Agricultural Research, Canberra.

ALDERS, R.G. & SPRADBROW, P. B. 2001. "Controlling Newcastle Disease in Village Chickens: A Field Manual", Australian Centre for International Agricultural Research, Version 14.03. 7

ALDOUS, E. W., MYNN, J. K., BANKS, J. & ALEXANDER, D. J. 2003. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathology*, 32 (3), 239-257.

ALEXANDER, D. J. 1991. Newcastle disease and other paramyxovirus infections. Edited by B. W. Calnek, H. J. Barnes, W. M. Reid & H. W. Yoder. *Diseases of Poultry,* 9th ed, 496-519. (London, Wolfe).

ALEXANDER, D. J. 1995. Newcastle Disease: Methods of Spread in Newcastle Disease. Ed. D. J. Alexander. Norwell, Kluwer Academic Press. 256-276

ALEXANDER, D. J., 1998, 'Newcastle disease' in S. E Aiello & A. Mays (eds), *The Merck Veterinary Manual*, pp. 1941-1942, Whitehouse Station, NJ.

ASHENAFI, H. 2000. Survey on Identification of Major Diseases of Local Chickens in Three Selected Agro-Climatic Zones in Central Ethiopia. Veterinary doctoral thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

BAGNOL, B. 2001. The Social Impact of Newcastle disease Control. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N° 103, 69-75.

BELL, J. G., KANE, M. & LE JAN, C. 1990. An investigation of the disease status of village poultry in Mauritania; *Preventive Veterinary Medicine* 8: 291 – 294.

BELL, J.G. 1992. Newcastle disease in village chickens in North, West and Central Africa. Edited by P.B Spardbrow. Newcastle disease in Village chickens, Control with Thermostable Oral Vaccines. Proceedings. International Workshop held in Kaula Lumpur: Malaysia, 6-10 October 1991, Centre for International Agriculture Research ACIAR, Canberra, 142-143.

BELL, J.G. 2001. A comparison of the different vaccines available for the control of Newcastle disease in village chickens. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6–9 March 2000. ACIAR Proceedings N° 103, 56–60.

BIZED. 2001. Map of Zambia- Provinces/ Neighbours.
Accessible at http://www.bized.co.uk › Virtual Worlds › Virtual Developing Country.

BRAY, H. & MOFFAT, B. 1990. A review of the Zambian poultry industry with special reference to village chickens. Report prepared for International Development Assistance Bureau.

CANNON, R.M. & ROE, R.T. 1982. Livestock Disease Surveys: A Field Manual For Veterinarians. Canberra: Australian Bureau of Animal Health.

CATTOLI, G., FUSARI, A., MONNE, I., MOLIA, S., LE MENACH, A., MAREGEYA, B., NCHARE, A., BANGANA, I., GARBA MAINA, A., N'GORAN KOFFI, J.N., THIAM, H., BEZEID, O.E., SALVIATO, A., NISI, R., TERREGINO, C. & CAPUA, I. 2009. Emergence of a new genetic lineage of Newcastle disease virus in West and Central Africa-implications for diagnosis and control. *Veterinary Microbiology* 19;142 (3-4) (Abstract, *Veterinary Microbiology*, 168-176).

CENTER FOR FOOD SECURITY AND PUBLIC HEALTH (CFSPH). 2005. Newcastle Disease. Iowa State University.

CENTRAL STATISTICS OFFICE. 2000. REPUBLIC OF ZAMBIA. 2000. The 2000 Census of Population and Housing.

CENTRAL STATISTICS OFFICE. 2003. REPUBLIC OF ZAMBIA. "2002/2003 Post Harvest Survey.

CENTRAL STATISTICS OFFICE. 2004. REPUBLIC OF ZAMBIA. "Living Conditions and Monitoring Survey Report (III): 2002-03".

CHAKA, H., GOUTARD, F., BISSCHOP, S. P. & THOMPSON, P. N. 2012. Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia. *Poultry Science* 91(4):862-9.

CZEGLEDI, A., UJVARI, D., SOMOGYI, E., WEHMANN, E., WERNER, O. & LOMNICZI, B. 2006. Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Research* 120: 36-48.

DAO, T. D. & PHAM, C. 1985**.** *National overviews: Vietnam.* Edited by A. J. Della–Porta. Veterinary viral diseases: their significance in south – East Asia and the Western Pacific. Academic Press, Sydney 246 – 250.

DE LEEUW, O. S., HARTOG, L., KOCH, G. & PEETERS, B. 2003. Effect of fusion protein cleavage site mutations on virulence of Newcastle disease virus: non – virulent cleavage site mutants revert to virulence after one passage in chicken brain. *Journal of General Virology* 84: 475 – 484.

DE SOUSA, R. L. M., MONTASSIER, H. J. & PINTO, A. A. 2000. Detection and Quantification of Antibodies to Newcastle Disease Virus in Ostrich and Rhea Sera Using a Liquid Phase Blocking Enzyme-Linked Immunosorbent Assay. Clinical and vaccine immunology, 7:6, 940-944 http://intl-cvi.asm.org/content/7/6/940.full

DE WIT, J. J., DAVELAAR, F. G. & BRAUNIUS, W. W. 1992. Comparison of the enzyme linked immunosorbent assay, the haemagglutination inhibition test and the agar gel precipitation test for the detection of antibodies against infectious bronchitis and Newcastle disease in commercial broilers. *Avian Pathology*, 21:4, 651-658
http://dx.doi.org/10.1080/03079459208418886

DIELEMAN, E. F. 2001. VETAID field experiences with Newcastle Disease vaccinations in Mozambique. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N^{o} 103.

BELL, J.G. 2001. A comparison of the different vaccines available for the control of Newcastle disease in village chickens. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6–9 March 2000. ACIAR Proceedings N^{o} 103, 56–60.

ESKOLI, C.D. 1984. Prevalence of disease virus antibodies in local and exotic chickens under different management systems in Nigeria. Bulletin of Animal Health and Production in Africa. 32:253–257.

EZEOKOLI, C.D., UMOH, J. U., ADESIYUN, A. A. & ABDU, P. A. 1984. Prevalence of Newcastle disease virus antibodies in local and exotic chickens under different management systems in Nigeria. Bulletin of Animal Health and Prodution in Africa 32: 253- 257.

HAFEZ, H.M. 2005. Governmental regulations and concept behind eradication and control of some important poultry diseases. *World's Poultry Science Journal* 61. 573.

HALLE, P.D., UMOH, J.U., SA'IDU, L. & ABDU, P. A. 1999. Prevalence and seasonality of Newcastle disease in Zaria, Nigeria. *Tropical Veterinarian*, 17: 53-62.

HARUN, M. & MASSANGO, F. A. 2001. Village Poultry Production in Mozambique: Farming Systems and Ethnoveterinary Knowledge in Angonia and Tsangano Districts, Tete Province. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N° 103, 76-79.

HUSSEIN, N.A., SHARMA, R.N., ANDO, R. & CHIZYUKA, H. G. B. 1984. A study on Newcastle disease virus pathotypes in Zambia. *Revue Scientifique et Technique, Office International des Epizooties*, 3: 383-389.

Images for Map of Africa.

Accessible at http://www.worldatlas.com

JACKSON, R. 2010. The chicken Vet talks about Newcastle Disease.

Accessible at http://www.poullrtykeeper.com/…/the chicken Vet talks about Newcastle Disease Health.html

JINTANA, D. 1987. Thailand: poultry production. Edited by J.W. Copland. Newcastle disease in poultry. A new food pellet vaccine. ACIAR monograph No.5. ACIAR, Canberra,108 – 109.

LOMNICZI, B., WEHMANN, E., HERCZEG, J., BALLAGI-PORDANY, A., KALETA, E.F., WERNER, O., MEULEMANS,G., JORGENSEN, P.H., MANTE, A.P., GIELKENS, A.L.J., CAPUA, I. & DAMOSER, J. 1998. Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). *Archives of Virology* 143, 49-64.

MAVALE, A. P. 2001. Country Report: Mozambique. Edited by R.G. Alders and P.B. Spradbrow. 2001. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N° 103, 20-25.

MILLER, P.J., KING, D.J., AFONSO, C.L. & SUAREZ, D.L. 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. *Vaccine* 25: 7238-7246.

MILLER, P.J., AFONSO, C.L., SPACKMAN, E., SCOTT, M.A., PEDERSEN, J.C., SENNE, D.A., BROWN, J.D., FULLER, C.M., UHART, M.M., KARESH, W.B., BROWN, I.H., ALEXANDER, D.J.A. & SWAYNE, D.E. (2010). Evidence for a New Avian Paramyxovirus Serotype 10

Detected in Rockhopper Penguins from the Falkland Islands. (Abstract, *Journal of Virology* 84:21).

MINGA, U.M., KATULE, A., MAEDA, T. & MUSASA, J. 1989. Potential and problems of the traditional chicken industry in Tanzania. Proceedings. 7thTanzania Veterinary Association Scientific Conference, held in Arusha International Conference center, December 1989. Tanzania Veterinary Association 7: 207-215.

MUSA, U., ABDU, P.A., DAFWANG, I.I., UMOH, J.U., SA'IDU, L., MERA, U.M. & EDACHE, J.A. 2009. Seroprevalence, Seasonal Occurrence and Clinical Manifestation of Newcastle Disease in Rural Household Chickens in Plateau State, Nigeria. *International Journal of Poultry Science* 8 (2): 200-204, 2009.

NJAGI, L.W., NYAGA, P.N., MBUTHIA, P.G., BEBORA, L.C., MICHIEKA, J.N., KIBE, J.K. & MINGA, U.M. 2010. Prevalence of Newcastle disease virus in village indigenous chickens in varied agro-ecological zones in Kenya. Livestock Research for Rural Development 22 (5).

nmcc.org.zm. 2009. Images for map of Zambia- districts, viewed 20 November, 2012. http://www.nmcc.org.zm/report-map.htm .

NWANTA, J.A., UMOH, J. U., ABDU, P. A., AJOGI, I. & ALLI- BALOGUN, J. K. 2006. Management of losses and Newcastle disease in rural poultry in Kaduna State, Nigeria. *Nigerian Journal Animal Production* 33: 274-285.

NYAGA, J.M., NYAGA, P.N. & KARIUKI, D.P. 1985. Epidemiology of Newcastle disease in Kenya. Bulletin of Animal Health and Production in Africa 33: 249-251.

OIE 2010. “Newcastle disease”. Manual of standards for diagnostic tests and vaccines: Manualof Diagnostic Tests and Vaccines for Terrestrial Animals.
Accessible at http://www.oie.int/eng/normes/mmanual/a_summry.htm

ORAJAKA, L.J.E., ADENE, D.F., ANENE, B. & ONUOHA, E.A. 1999. Seroprevalence of Newcastle disease in local chickens from southeast derived Savannah zone of Nigeria. Rvued'Elevage et de Medicine Veterinaries des Pays Tropicaux 52: 185-188.

OYEWOLA, K.A., OGUNDIPE, G.A.T. & DUROJAIYE, O.A. 1996. Seroprevalence of Gumboro and Newcastle disease in local chickens in Ibadan, Nigeria. Bulletin of Animal Health and Production in Africa 34: 57-59.

PERTTULA, L. 2009. Epidemiology and Characterization of Newcastle Disease in Smallholder Poultry in Mozambique.
Accessible at http://epsilon.slu.se

SAGILD, I.K. & HARESNAPE, J.M. 1987. The status of Newcastle disease and the use of V4 vaccine in Malawi. *Avian Pathology* 16:165-176.

SA'IDU, L., ABDU, P. A., TEKDEK, L. B., UMOH, J. U., USMAN, M & OLADELE, S. B. 2006. Newcastle disease in Nigeria. *Nigerian Veterinary Journal* 27: 23-32.

SHARMA, R.N., HUSSEIN, N.A., PANDEY, G.S. AND SHADOMO, M.N. 1985. A study of Newcastle disease outbreaks in Zambia, 1975–1984. Revue Scientifique et Technique, Office International des Epizooties 5 (1): 5–14.

SOLOMON, P., ABOLNIK, C., JOANNIS, T. M. & BISSCHOP, B. 2011. Virulent Newcastle disease virus in Nigeria: identification of a new clade of sub-lineage 5f from livebird markets. *Virus genes,* 44(1):98-103.

SONGOLO, A. & KATONGO, J.C. (2000). Country Report: Zambia. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March, 2000. ACIAR Proceedings N° 103. 43-45.

SNOECK, C. J., DUCATEZ, M. F., OWOADE, A. A., FALEKE, O. O., ALKALI, B. R., TAHITA, M. C., TARNAGDA, Z., OUEDRAOGO, J. B., MAIKANO, I., MBAH, P. O., KREMER, J. R. & MULLER, C. P. 2009. Newcastle disease virus in West Africa: new virulent strains identified in non-commercial farms. *Archives of Virology*, 154(1) (Abstract, *Archives of Virology*, 47-54).

SPRADBROW, P.B. (1993). Newcastle disease in village chickens. *Poultry Science Review* 5: 57-96.

SPRADBROW, P. B. (2001a). Thermostable Newcastle Disease Vaccines. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N° 103, 61-66.

SPRADBROW, P. B. (2001b). The Epidemiology of Newcastle Disease in Village Chickens. Edited by R.G. Alders and P.B. Spradbrow. SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings. International Workshop, Maputo, Mozambique, 6-9 March 2000. ACIAR Proceedings N° 103, 53-55.

TADESSE, S., ASHENAFI, H. & ASCHALEW, Z. 2005. Seroprevalence Study of Newcastle Disease in Local Chickens in Central Ethiopia. *International Journal of Applied Research in Veterinary Medicine* 3 (1).

THITISAK, W., JANVIRIYASOPAK, O., MORRIS, R. S., SRIHAKIM, S. & KRUEDENER, R. V. 1988. Causes of death found in an epidemiological study of native chickens in Thai villages. *Acta Veterinarian Scandinavica*, Supplement, 84: 200-202.

WAMBURA, P. N., KAPAGA, A. M. AND HYERA, J. M. 2000. Experimental trials with a thermostable Newcastle disease virus (strain I-2) in commercial and village chickens in Tanzania; *Preventive Veterinary Medicine*, 43 (2): 75-83.

WESTBURY, H. A., PARSONS, G. & ALLAN, H. 1984. Duration of excretion of virulent Newcastle disease virus following challenge of chickens with different titres of serum antibody to the virus; *Australian Veterinary Journal* 61: 44 – 46.

XIUFAN, L. & YONGZHONG, C. 2005. Newcastle disease virus evolution: multiple genetic lineages and virulence variation with adaptation in different host species.
Accessible at http://*www.veterinertavukculuk.org/uploadimg/dosyalar/2005_istanbul/.../liu.pdf*

YONGOLO, M.G.S. 1996. Epidemiology of Newcastle disease in village chickens in Tanzania. MSc Dissertation, Sokoine University of Agriculture, Morogoro, Tanzania.

www.nanzhila.com/kafue_national_park.php. 2012. Kafue National Park, viewed 11 December, 2012.

APPENDIX 1

Sample data analysis table

Sample id	Province	District	O.D	S/P	Titre	Grp	Result
1	Copperbelt	Chililabombwe	0.122	0.271	552	1	Pos
2	Copperbelt	Chililabombwe	0.137	0.322	666	1	Pos
3	Copperbelt	Chililabombwe	0.127	0.292	599	1	Pos
4	Copperbelt	Chililabombwe	0.094	0.176	345	0	Neg
5	Copperbelt	Chililabombwe	0.08	0.129	246	0	Neg
6	Copperbelt	Chililabombwe	0.467	1.444	3419	4	Pos
7	Copperbelt	Chililabombwe	0.236	0.658	1452	2	Pos
8	Copperbelt	Chililabombwe	0.055	0.044	76	0	Neg
9	Copperbelt	Chililabombwe	0.093	0.176	345	0	Neg
10	Copperbelt	Chililabombwe	0.126	0.285	583	1	Pos
11	Copperbelt	Chililabombwe	0.208	0.566	1232	2	Pos
12	Copperbelt	Chililabombwe	0.497	1.546	3683	4	Pos
13	Copperbelt	Chililabombwe	0.256	0.729	1623	2	Pos
14	Copperbelt	Chililabombwe	0.174	0.447	952	1	Pos
15	Copperbelt	Chililabombwe	0.481	1.492	3543	4	Pos
16	Copperbelt	Chililabombwe	0.165	0.42	890	1	Pos
17	Copperbelt	Chililabombwe	0.076	0.115	217	0	Neg
18	Copperbelt	Chililabombwe	0.232	0.644	1418	2	Pos
19	Copperbelt	Chililabombwe	0.092	0.169	330	0	Neg
20	Copperbelt	Chililabombwe	0.156	0.39	821	1	Pos
21	Copperbelt	Chililabombwe	0.152	0.373	782	1	Pos
22	Copperbelt	Chililabombwe	0.114	0.244	492	1	Pos
23	Copperbelt	Chililabombwe	0.131	0.302	621	1	Pos
24	Copperbelt	Chililabombwe	0.046	0.014	22	0	Neg
25	Copperbelt	Chililabombwe	0.084	0.142	273	0	Neg
26	Copperbelt	Chililabombwe	0.145	0.349	727	1	Pos
27	Copperbelt	Chililabombwe	0.095	0.18	353	0	Neg
28	Copperbelt	Chililabombwe	0.121	0.268	545	1	Pos
29	Copperbelt	Chililabombwe	0.163	0.414	876	1	Pos
30	Copperbelt	Chililabombwe	0.074	0.108	203	0	Neg
31	Copperbelt	Chililabombwe	0.194	0.519	1121	2	Pos
32	Copperbelt	Chililabombwe	0.171	0.441	939	1	Pos

33	Copperbelt	Chililabombwe	0.137	0.322	666	1	Pos
34	Copperbelt	Chililabombwe	0.184	0.481	1032	2	Pos
35	Copperbelt	Chililabombwe	0.271	0.776	1738	2	Pos
36	Copperbelt	Chililabombwe	0.092	0.169	330	0	Neg
37	Copperbelt	Chililabombwe	0.092	0.169	330	0	Neg
38	Copperbelt	Chililabombwe	0.101	0.203	403	1	Pos
39	Copperbelt	Chililabombwe	0.153	0.376	789	1	Pos
40	Copperbelt	Chililabombwe	0.192	0.508	1095	2	Pos
41	Copperbelt	Chililabombwe	0.149	0.366	766	1	Pos
42	Copperbelt	Chililabombwe	0.16	0.4	844	1	Pos
43	Copperbelt	Chililabombwe	0.057	0.051	89	0	Neg
44	Copperbelt	Chililabombwe	0.175	0.451	962	1	Pos
45	Copperbelt	Chililabombwe	0.154	0.38	798	1	Pos
46	Copperbelt	Chililabombwe	0.13	0.298	612	1	Pos
47	Copperbelt	Chililabombwe	0.257	0.729	1623	2	Pos
48	Copperbelt	Chililabombwe	0.086	0.153	296	0	Neg
49	Copperbelt	Chililabombwe	0.193	0.515	1111	2	Pos
50	Copperbelt	Chililabombwe	0.165	0.42	890	1	Pos
51	Copperbelt	Chililabombwe	0.493	1.529	3639	4	Pos
52	Copperbelt	Chililabombwe	0.131	0.302	621	1	Pos
53	Copperbelt	Chililabombwe	0.314	0.925	2104	3	Pos
54	Copperbelt	Chililabombwe	0.055	0.044	76	0	Neg
55	Copperbelt	Chililabombwe	0.112	0.241	486	1	Pos
56	Copperbelt	Chililabombwe	0.546	1.712	4116	5	Pos
57	Copperbelt	Chililabombwe	0.421	1.288	3019	4	Pos
58	Copperbelt	Chililabombwe	0.193	0.515	1111	2	Pos
59	Copperbelt	Chililabombwe	0.102	0.203	403	1	Pos
60	Copperbelt	Chililabombwe	0.244	0.688	1524	2	Pos
61	Copperbelt	Chililabombwe	1.19	3.895	10084	9	Pos
62	Copperbelt	Chililabombwe	0.165	0.42	890	1	Pos
63	Copperbelt	Chililabombwe	0.119	0.264	536	1	Pos
64	Copperbelt	Chililabombwe	0.158	0.397	837	1	Pos
65	Copperbelt	Chililabombwe	0.241	0.675	1493	2	Pos
66	Copperbelt	Chililabombwe	0.343	1.02	2341	3	Pos
67	Copperbelt	Chililabombwe	0.112	0.241	486	1	Pos

68	Copperbelt	Chililabombwe	0.181	0.471	1008	2	Pos
69	Copperbelt	Chililabombwe	0.11	0.231	464	1	Pos
70	Copperbelt	Chililabombwe	0.329	0.973	2224	3	Pos
71	Copperbelt	Chililabombwe	0.18	0.471	1008	2	Pos
72	Copperbelt	Chililabombwe	0.134	0.315	650	1	Pos
73	Copperbelt	Chililabombwe	0.17	0.434	922	1	Pos
74	Copperbelt	Chililabombwe	0.149	0.366	766	1	Pos
75	Copperbelt	Chililabombwe	0.177	0.458	978	1	Pos
76	Copperbelt	Chililabombwe	0.315	0.925	2104	3	Pos
77	Copperbelt	Chililabombwe	0.116	0.251	508	1	Pos
78	Copperbelt	Chililabombwe	0.196	0.522	1128	2	Pos
79	Copperbelt	Chililabombwe	0.323	0.956	2181	3	Pos
80	Copperbelt	Chililabombwe	0.105	0.214	427	1	Pos
81	Copperbelt	Chililabombwe	0.087	0.153	296	0	Neg
82	Copperbelt	Chililabombwe	0.138	0.325	673	1	Pos
83	Copperbelt	Chililabombwe	0.74	2.369	5865	6	Pos
84	Copperbelt	Chililabombwe	0.779	2.498	6214	7	Pos
85	Copperbelt	Chililabombwe	0.081	0.132	252	0	Neg
86	Copperbelt	Chililabombwe	0.153	0.376	789	1	Pos
87	Copperbelt	Chililabombwe	0.401	1.217	2838	3	Pos
88	Copperbelt	Chililabombwe	0.119	0.264	536	1	Pos
89	Copperbelt	Chililabombwe	0.099	0.193	381	0	Neg
90	Copperbelt	Chililabombwe	0.202	0.546	1185	2	Pos
91	Copperbelt	Chililabombwe	0.112	0.241	486	1	Pos
92	Copperbelt	Chililabombwe	0.11	0.231	464	1	Pos
	Copperbelt	Mufulira					
1	Copperbelt	Mufulira	0.419	2.033	4964	5	Pos
2	Copperbelt	Mufulira	0.083	0.177	347	0	Neg
3	Copperbelt	Mufulira	0.067	0.088	162	0	Neg
4	Copperbelt	Mufulira	0.082	0.171	334	0	Neg
5	Copperbelt	Mufulira	0.068	0.094	174	0	Neg
6	Copperbelt	Mufulira	0.064	0.072	130	0	Neg
7	Copperbelt	Mufulira	0.085	0.188	371	0	Neg
8	Copperbelt	Mufulira	0.076	0.138	265	0	Neg
9	Copperbelt	Mufulira	0.054	0.017	27	0	Neg

10	Copperbelt	Mufulira	0.047	0	1	0	Neg
11	Copperbelt	Mufulira	0.054	0.017	27	0	Neg
12	Copperbelt	Mufulira	0.055	0.022	36	0	Neg
13	Copperbelt	Mufulira	0.093	0.232	466	1	Pos
14	Copperbelt	Mufulira	0.046	0	1	0	Neg
15	Copperbelt	Mufulira	0.058	0.039	67	0	Neg
16	Copperbelt	Mufulira	0.057	0.033	56	0	Neg
17	Copperbelt	Mufulira	0.048	0	1	0	Neg
18	Copperbelt	Mufulira	0.056	0.028	46	0	Neg
19	Copperbelt	Mufulira	0.053	0.011	17	0	Neg
20	Copperbelt	Mufulira	0.053	0.011	17	0	Neg
21	Copperbelt	Mufulira	0.043	0	1	0	Neg
22	Copperbelt	Mufulira	0.069	0.099	184	0	Neg
23	Copperbelt	Mufulira	0.075	0.133	254	0	Neg
24	Copperbelt	Mufulira	0.05	0	1	0	Neg
25	Copperbelt	Mufulira	0.04	0	1	0	Neg
26	Copperbelt	Mufulira	0.047	0	1	0	Neg
27	Copperbelt	Mufulira	0.047	0	1	0	Neg
28	Copperbelt	Mufulira	0.046	0	1	0	Neg
29	Copperbelt	Mufulira	0.096	0.249	503	1	Pos
30	Copperbelt	Mufulira	0.066	0.083	152	0	Neg
31	Copperbelt	Mufulira	0.043	0	1	0	Neg
32	Copperbelt	Mufulira	0.056	0.028	46	0	Neg
33	Copperbelt	Mufulira	0.067	0.088	162	0	Neg
34	Copperbelt	Mufulira	0.065	0.077	140	0	Neg
35	Copperbelt	Mufulira	0.091	0.221	442	1	Pos
36	Copperbelt	Mufulira	0.046	0	1	0	Neg
37	Copperbelt	Mufulira	0.047	0	1	0	Neg
38	Copperbelt	Mufulira	0.073	0.122	231	0	Neg
39	Copperbelt	Mufulira	0.058	0.039	67	0	Neg
40	Copperbelt	Mufulira	0.046	0	1	0	Neg
41	Copperbelt	Mufulira	0.056	0.028	46	0	Neg
42	Copperbelt	Mufulira	0.052	0.006	9	0	Neg
43	Copperbelt	Mufulira	0.068	0.094	174	0	Neg
44	Copperbelt	Mufulira	0.052	0.006	9	0	Neg

45	Copperbelt	Mufulira	0.052	0.006	9	0	Neg
46	Copperbelt	Mufulira	0.12	0.381	800	1	Pos
47	Copperbelt	Mufulira	0.044	0	1	0	Neg
48	Copperbelt	Mufulira	0.058	0.039	67	0	Neg
49	Copperbelt	Mufulira	0.093	0.232	466	1	Pos
50	Copperbelt	Mufulira	0.056	0.028	46	0	Neg
51	Copperbelt	Mufulira	0.054	0.017	27	0	Neg
52	Copperbelt	Mufulira	0.046	0	1	0	Neg
53	Copperbelt	Mufulira	0.115	0.354	739	1	Pos
54	Copperbelt	Mufulira	0.056	0.028	46	0	Neg
55	Copperbelt	Mufulira	0.1	0.271	552	1	Pos
56	Copperbelt	Mufulira	0.233	1.006	2306	3	Pos
57	Copperbelt	Mufulira	0.131	0.442	941	1	Pos
58	Copperbelt	Mufulira	0.079	0.155	300	0	Neg
59	Copperbelt	Mufulira	0.067	0.088	162	0	Neg
60	Copperbelt	Mufulira	0.045	0	1	0	Neg
61	Copperbelt	Mufulira	0.062	0.061	109	0	Neg
62	Copperbelt	Mufulira	0.055	0.022	36	0	Neg
63	Copperbelt	Mufulira	0.048	0	1	0	Neg
64	Copperbelt	Mufulira	0.109	0.32	662	1	Pos
65	Copperbelt	Mufulira	0.118	0.37	775	1	Pos
66	Copperbelt	Mufulira	0.058	0.039	67	0	Neg
67	Copperbelt	Mufulira	0.048	0	1	0	Neg
68	Copperbelt	Mufulira	0.052	0.006	9	0	Neg
69	Copperbelt	Mufulira	0.053	0.011	17	0	Neg
70	Copperbelt	Mufulira	0.063	0.066	118	0	Neg
71	Copperbelt	Mufulira	0.05	0	1	0	Neg
72	Copperbelt	Mufulira	0.065	0.077	140	0	Neg
73	Copperbelt	Mufulira	0.113	0.343	714	1	Pos
74	Copperbelt	Mufulira	0.097	0.254	514	1	Pos
75	Copperbelt	Mufulira	0.058	0.039	67	0	Neg
76	Copperbelt	Mufulira	0.043	0	1	0	Neg
77	Copperbelt	Mufulira	0.037	0	1	0	Neg
78	Copperbelt	Mufulira	0.04	0	1	0	Neg
79	Copperbelt	Mufulira	0.069	0.099	184	0	Neg

80	Copperbelt	Mufulira	0.093	0.232	466	1	Pos
81	Copperbelt	Mufulira	0.114	0.348	725	1	Pos
82	Copperbelt	Mufulira	0.161	0.608	1332	2	Pos
83	Copperbelt	Mufulira	0.045	0	1	0	Neg
84	Copperbelt	Mufulira	0.041	0	1	0	Neg
85	Copperbelt	Mufulira	0.045	0	1	0	Neg
86	Copperbelt	Mufulira	0.047	0	1	0	Neg
87	Copperbelt	Mufulira	0.267	1.193	2777	3	Pos
88	Copperbelt	Mufulira	0.176	0.691	1531	2	Pos
89	Copperbelt	Mufulira	0.11	0.326	675	1	Pos
90	Copperbelt	Mufulira	0.059	0.044	76	0	Neg
91	Copperbelt	Mufulira	0.101	0.276	563	1	Pos
92	Copperbelt	Mufulira	0.077	0.144	277	0	Neg
	Southern	Kazungula 1					
1	Southern	Kazungula 1	0.092	0.178	349	0	Neg
2	Southern	Kazungula 1	0.132	0.347	723	1	Pos
3	Southern	Kazungula 1	0.077	0.114	215	0	Neg
4	Southern	Kazungula 1	0.113	0.267	543	1	Pos
5	Southern	Kazungula 1	0.136	0.364	761	1	Pos
6	Southern	Kazungula 1	0.113	0.267	543	1	Pos
7	Southern	Kazungula 1	0.186	0.576	1256	2	Pos
8	Southern	Kazungula 1	0.092	0.178	349	0	Neg
9	Southern	Kazungula 1	0.232	0.771	1725	2	Pos
10	Southern	Kazungula 1	0.37	1.356	3193	4	Pos
11	Southern	Kazungula 1	0.097	0.199	394	0	Neg
12	Southern	Kazungula 1	0.088	0.161	313	0	Neg
13	Southern	Kazungula 1	0.286	1	2291	3	Pos
14	Southern	Kazungula 1	0.593	2.301	5682	6	Pos
15	Southern	Kazungula 1	0.265	0.911	2070	3	Pos
16	Southern	Kazungula 1	0.139	0.377	791	1	Pos
17	Southern	Kazungula 1	0.092	0.178	349	0	Neg
18	Southern	Kazungula 1	0.143	0.394	830	1	Pos
19	Southern	Kazungula 1	0.108	0.246	497	1	Pos
20	Southern	Kazungula 1	0.213	0.691	1531	2	Pos
21	Southern	Kazungula 1	0.101	0.216	431	1	Pos

22	Southern	Kazungula 1	0.143	0.394	830	1	Pos
23	Southern	Kazungula 1	0.078	0.119	225	0	Neg
24	Southern	Kazungula 1	0.175	0.53	1147	2	Pos
25	Southern	Kazungula 1	0.122	0.305	628	1	Pos
26	Southern	Kazungula 1	0.333	1.199	2792	3	Pos
27	Southern	Kazungula 1	0.097	0.199	394	0	Neg
28	Southern	Kazungula 1	0.246	0.831	1872	2	Pos
29	Southern	Kazungula 1	0.082	0.136	260	0	Neg
30	Southern	Kazungula 1	0.11	0.254	514	1	Pos
31	Southern	Kazungula 1	0.274	0.949	2164	3	Pos
32	Southern	Kazungula 1	0.783	3.106	7880	7	Pos
33	Southern	Kazungula 1	0.388	1.432	3388	4	Pos
34	Southern	Kazungula 1	0.163	0.479	1027	2	Pos
35	Southern	Kazungula 1	0.061	0.047	82	0	Neg
36	Southern	Kazungula 1	0.17	0.508	1095	2	Pos
37	Southern	Kazungula 1	0.231	0.767	1716	2	Pos
38	Southern	Kazungula 1	0.089	0.165	321	0	Neg
39	Southern	Kazungula 1	0.123	0.309	637	1	Pos
40	Southern	Kazungula 1	0.234	0.78	1747	2	Pos
41	Southern	Kazungula 1	0.214	0.695	1541	2	Pos
42	Southern	Kazungula 1	0.279	0.97	2216	3	Pos
43	Southern	Kazungula 1	0.125	0.318	657	1	Pos
44	Southern	Kazungula 1	0.09	0.169	330	0	Neg
45	Southern	Kazungula 1	0.306	1.085	2504	3	Pos
46	Southern	Kazungula 1	0.242	0.814	1831	2	Pos
47	Southern	Kazungula 1	0.109	0.25	506	1	Pos
48	Southern	Kazungula 1	0.07	0.085	156	0	Neg
49	Southern	Kazungula 1	0.177	0.538	1166	2	Pos
50	Southern	Kazungula 1	0.095	0.191	377	0	Neg
51	Southern	Kazungula 1	0.319	1.14	2643	3	Pos
52	Southern	Kazungula 1	0.078	0.119	225	0	Neg
53	Southern	Kazungula 1	0.087	0.157	304	0	Neg
54	Southern	Kazungula 1	0.303	1.072	2471	3	Pos
55	Southern	Kazungula 1	0.108	0.246	497	1	Pos
56	Southern	Kazungula 1	0.065	0.064	114	0	Neg

57	Southern	Kazungula 1	0.048	0	1	0	Neg
58	Southern	Kazungula 1	0.062	0.051	89	0	Neg
59	Southern	Kazungula 1	0.078	0.119	225	0	Neg
60	Southern	Kazungula 1	0.162	0.475	1018	2	Pos
61	Southern	Kazungula 1	0.133	0.352	734	1	Pos
62	Southern	Kazungula 1	0.258	0.881	1995	2	Pos
63	Southern	Kazungula 1	0.18	0.551	1196	2	Pos
64	Southern	Kazungula 1	0.246	0.831	1872	2	Pos
65	Southern	Kazungula 1	0.211	0.682	1509	2	Pos
66	Southern	Kazungula 1	0.122	0.305	628	1	Pos
67	Southern	Kazungula 1	0.087	0.157	304	0	Neg
68	Southern	Kazungula 1	0.115	0.275	561	1	Pos
69	Southern	Kazungula 1	0.191	0.597	1306	2	Pos
70	Southern	Kazungula 1	0.122	0.305	628	1	Pos
71	Southern	Kazungula 1	0.263	0.903	2050	3	Pos
72	Southern	Kazungula 1	0.297	1.047	2408	3	Pos
73	Southern	Kazungula 1	0.113	0.267	543	1	Pos
74	Southern	Kazungula 1	0.089	0.165	321	0	Neg
75	Southern	Kazungula 1	0.237	0.792	1777	2	Pos
76	Southern	Kazungula 1	0.254	0.864	1953	2	Pos
77	Southern	Kazungula 1	0.202	0.644	1418	2	Pos
78	Southern	Kazungula 1	0.143	0.394	830	1	Pos
79	Southern	Kazungula 1	0.112	0.263	534	1	Pos
80	Southern	Kazungula 1	0.1	0.212	422	1	Pos
81	Southern	Kazungula 1	0.097	0.199	394	0	Neg
82	Southern	Kazungula 1	0.138	0.373	782	1	Pos
83	Southern	Kazungula 1	0.101	0.216	431	1	Pos
84	Southern	Kazungula 1	0.203	0.648	1428	2	Pos
85	Southern	Kazungula 1	0.17	0.508	1095	2	Pos
86	Southern	Kazungula 1	0.144	0.398	839	1	Pos
87	Southern	Kazungula 1	0.152	0.432	918	1	Pos
88	Southern	Kazungula 1	0.109	0.25	506	1	Pos
89	Southern	Kazungula 1	0.074	0.102	190	0	Neg
90	Southern	Kazungula 1	0.078	0.119	225	0	Neg
91	Southern	Kazungula 1	0.128	0.331	686	1	Pos!

92	Southern	Kazungula 1	0.071	0.089	164	0	Neg
	Southern	Kazungula 2					
1	Southern	Kazungula 2	0.265	0.63	1384	2	Pos
2	Southern	Kazungula 2	0.147	0.281	574	1	Pos
3	Southern	Kazungula 2	0.143	0.269	548	1	Pos
4	Southern	Kazungula 2	0.114	0.182	358	0	Neg
5	Southern	Kazungula 2	0.257	0.609	1334	2	Pos
6	Southern	Kazungula 2	0.162	0.325	673	1	Pos
7	Southern	Kazungula 2	0.21	0.466	997	1	Pos
8	Southern	Kazungula 2	0.34	0.857	1936	2	Pos
9	Southern	Kazungula 2	0.113	0.179	351	0	Neg
10	Southern	Kazungula 2	0.377	0.967	2209	3	Pos
11	Southern	Kazungula 2	0.386	0.994	2276	3	Pos
12	Southern	Kazungula 2	0.247	0.576	1256	2	Pos
13	Southern	Kazungula 2	0.151	0.29	594	1	Pos
14	Southern	Kazungula 2	0.182	0.382	803	1	Pos
15	Southern	Kazungula 2	0.349	0.884	2003	3	Pos
16	Southern	Kazungula 2	0.226	0.513	1107	2	Pos
17	Southern	Kazungula 2	0.091	0.113	213	0	Neg
18	Southern	Kazungula 2	0.245	0.573	1248	2	Pos
19	Southern	Kazungula 2	0.2	0.439	934	1	Pos
20	Southern	Kazungula 2	0.264	0.63	1384	2	Pos
21	Southern	Kazungula 2	0.513	1.373	3236	4	Pos
22	Southern	Kazungula 2	0.145	0.272	554	1	Pos
23	Southern	Kazungula 2	0.284	0.69	1529	2	Pos
24	Southern	Kazungula 2	0.121	0.203	403	1	Pos
25	Southern	Kazungula 2	0.382	0.982	2246	3	Pos
26	Southern	Kazungula 2	0.056	0.009	13	0	Neg
27	Southern	Kazungula 2	0.261	0.621	1363	2	Pos
28	Southern	Kazungula 2	0.284	0.69	1529	2	Pos
29	Southern	Kazungula 2	0.154	0.301	619	1	Pos
30	Southern	Kazungula 2	0.229	0.525	1135	2	Pos
31	Southern	Kazungula 2	0.147	0.281	574	1	Pos
32	Southern	Kazungula 2	0.321	0.8	1796	2	Pos
33	Southern	Kazungula 2	0.088	0.104	194	0	Neg

34	Southern	Kazungula 2	0.179	0.376	789	1	Pos
35	Southern	Kazungula 2	0.147	0.281	574	1	Pos
36	Southern	Kazungula 2	0.371	0.946	2156	3	Pos
37	Southern	Kazungula 2	0.111	0.173	338	0	Neg
38	Southern	Kazungula 2	0.294	0.719	1599	2	Pos
39	Southern	Kazungula 2	0.13	0.227	455	1	Pos
40	Southern	Kazungula 2	0.168	0.343	714	1	Pos
41	Southern	Kazungula 2	0.13	0.227	455	1	Pos
42	Southern	Kazungula 2	0.062	0.027	45	0	Neg
43	Southern	Kazungula 2	0.077	0.072	130	0	Neg
44	Southern	Kazungula 2	0.225	0.51	1100	2	Pos
45	Southern	Kazungula 2	0.517	1.385	3267	4	Pos
46	Southern	Kazungula 2	0.302	0.74	1650	2	Pos
47	Southern	Kazungula 2	0.158	0.313	646	1	Pos
48	Southern	Kazungula 2	0.131	0.233	468	1	Pos
49	Southern	Kazungula 2	0.315	0.782	1752	2	Pos
50	Southern	Kazungula 2	0.098	0.134	256	0	Neg
51	Southern	Kazungula 2	0.096	0.128	244	0	Neg
52	Southern	Kazungula 2	0.101	0.143	275	0	Neg
53	Southern	Kazungula 2	0.387	0.994	2276	3	Pos
54	Southern	Kazungula 2	0.209	0.466	997	1	Pos
55	Southern	Kazungula 2	0.136	0.248	501	1	Pos
56	Southern	Kazungula 2	0.123	0.209	416	1	Pos
57	Southern	Kazungula 2	0.108	0.164	319	0	Neg
58	Southern	Kazungula 2	0.085	0.096	178	0	Neg
59	Southern	Kazungula 2	0.073	0.06	107	0	Neg
60	Southern	Kazungula 2	0.188	0.4	844	1	Pos
61	Southern	Kazungula 2	0.393	1.015	2328	3	Pos
62	Southern	Kazungula 2	0.083	0.09	166	0	Neg
63	Southern	Kazungula 2	0.09	0.11	207	0	Neg
64	Southern	Kazungula 2	0.072	0.057	101	0	Neg
65	Southern	Kazungula 2	0.363	0.925	2104	3	Pos
66	Southern	Kazungula 2	0.181	0.379	796	1	Pos
67	Southern	Kazungula 2	0.16	0.316	653	1	Pos
68	Southern	Kazungula 2	0.198	0.433	920	1	Pos

69	Southern	Kazungula 2	0.191	0.412	871	1	Pos
70	Southern	Kazungula 2	0.134	0.242	488	1	Pos
71	Southern	Kazungula 2	0.134	0.242	488	1	Pos
72	Southern	Kazungula 2	0.154	0.301	619	1	Pos
73	Southern	Kazungula 2	0.757	2.101	5146	6	Pos
74	Southern	Kazungula 2	0.11	0.17	332	0	Neg
75	Southern	Kazungula 2	0.579	1.567	3738	4	Pos
76	Southern	Kazungula 2	0.121	0.203	403	1	Pos
77	Southern	Kazungula 2	0.1	0.14	269	0	Neg
78	Southern	Kazungula 2	0.254	0.6	1313	2	Pos
79	Southern	Kazungula 2	0.223	0.507	1093	2	Pos
80	Southern	Kazungula 2	0.086	0.099	184	0	Neg
81	Southern	Kazungula 2	0.278	0.672	1485	2	Pos
82	Southern	Kazungula 2	0.085	0.096	178	0	Neg
83	Southern	Kazungula 2	0.099	0.137	262	0	Neg
84	Southern	Kazungula 2	0.174	0.358	748	1	Pos
85	Southern	Kazungula 2	0.111	0.173	338	0	Neg
86	Southern	Kazungula 2	0.113	0.179	351	0	Neg
87	Southern	Kazungula 2	0.134	0.242	488	1	Pos
88	Southern	Kazungula 2	0.186	0.397	837	1	Pos
89	Southern	Kazungula 2	0.287	0.699	1551	2	Pos
90	Southern	Kazungula 2	0.284	0.69	1529	2	Pos
91	Southern	Kazungula 2	0.129	0.224	449	1	Pos
92	Southern	Kazungula 2	0.112	0.176	345	0	Neg
	Southern	Monze					
1	Southern	Monze	0.779	2.893	7292	7	Pos
2	Southern	Monze	0.239	0.75	1674	2	Pos
3	Southern	Monze	0.089	0.159	309	0	Neg
4	Southern	Monze	0.104	0.218	435	1	Pos
5	Southern	Monze	1.185	4.504	11815	9	Pos
6	Southern	Monze	0.096	0.187	368	0	Neg
7	Southern	Monze	0.333	1.127	2610	3	Pos
8	Southern	Monze	0.175	0.496	1067	2	Pos
9	Southern	Monze	0.229	0.71	1577	2	Pos
10	Southern	Monze	0.141	0.361	755	1	Pos

11	Southern	Monze	0.282	0.925	2104	3	Pos
12	Southern	Monze	0.133	0.329	682	1	Pos
13	Southern	Monze	0.131	0.321	664	1	Pos
14	Southern	Monze	0.138	0.349	727	1	Pos
15	Southern	Monze	0.113	0.254	514	1	Pos
16	Southern	Monze	0.451	1.595	3811	4	Pos
17	Southern	Monze	0.206	0.619	1358	2	Pos
18	Southern	Monze	1.003	3.786	9777	8	Pos
19	Southern	Monze	1.407	5.385	14355	11	Pos
20	Southern	Monze	0.297	0.98	2241	3	Pos
21	Southern	Monze	0.818	3.052	7730	7	Pos
22	Southern	Monze	0.942	3.54	9087	8	Pos
23	Southern	Monze	0.722	2.671	6685	7	Pos
24	Southern	Monze	0.474	1.687	4051	5	Pos
25	Southern	Monze	0.998	3.766	9721	8	Pos
26	Southern	Monze	0.31	1.036	2381	3	Pos
27	Southern	Monze	0.116	0.262	532	1	Pos
28	Southern	Monze	0.143	0.373	782	1	Pos
29	Southern	Monze	0.106	0.222	444	1	Pos
30	Southern	Monze	0.167	0.464	992	1	Pos
31	Southern	Monze	0.413	1.44	3409	4	Pos
32	Southern	Monze	0.133	0.329	682	1	Pos
33	Southern	Monze	0.343	1.167	2711	3	Pos
34	Southern	Monze	0.047	0	1	0	Neg
35	Southern	Monze	1.226	4.667	12281	10	Pos
36	Southern	Monze	0.182	0.524	1133	2	Pos
37	Southern	Monze	0.159	0.433	920	1	Pos
38	Southern	Monze	0.465	1.651	3957	4	Pos
39	Southern	Monze	0.335	1.135	2630	3	Pos
40	Southern	Monze	0.108	0.234	470	1	Pos
41	Southern	Monze	0.365	1.254	2932	3	Pos
42	Southern	Monze	0.245	0.778	1742	2	Pos
43	Southern	Monze	0.27	0.877	1985	2	Pos
44	Southern	Monze	0.201	0.603	1320	2	Pos
45	Southern	Monze	0.212	0.643	1416	2	Pos

46	Southern	Monze	0.299	0.988	2261	3	Pos
47	Southern	Monze	0.176	0.5	1076	2	Pos
48	Southern	Monze	0.326	1.099	2539	3	Pos
49	Southern	Monze	0.091	0.163	317	0	Neg
50	Southern	Monze	0.172	0.488	1048	2	Pos
51	Southern	Monze	0.059	0.04	69	0	Neg
52	Southern	Monze	1.077	4.079	10605	9	Pos
53	Southern	Monze	1.393	5.333	14204	11	Pos
54	Southern	Monze	0.215	0.659	1454	2	Pos
55	Southern	Monze	0.371	1.278	2993	3	Pos
56	Southern	Monze	0.163	0.448	955	1	Pos
57	Southern	Monze	0.245	0.778	1742	2	Pos
58	Southern	Monze	0.259	0.829	1867	2	Pos
59	Southern	Monze	0.313	1.048	2411	3	Pos
60	Southern	Monze	0.313	1.048	2411	3	Pos
61	Southern	Monze	1.19	4.528	11883	9	Pos
62	Southern	Monze	0.596	2.171	5333	6	Pos
63	Southern	Monze	0.232	0.722	1606	2	Pos
64	Southern	Monze	0.168	0.468	1001	2	Pos
65	Southern	Monze	0.279	0.913	2074	3	Pos
66	Southern	Monze	0.45	1.587	3790	4	Pos
67	Southern	Monze	1.299	4.96	13124	10	Pos
68	Southern	Monze	0.934	3.512	9009	8	Pos
69	Southern	Monze	0.139	0.353	736	1	Pos
70	Southern	Monze	0.05	0.004	6	0	Neg
71	Southern	Monze	0.057	0.032	54	0	Neg
72	Southern	Monze	0.251	0.798	1791	2	Pos
73	Southern	Monze	0.38	1.313	3083	4	Pos
74	Southern	Monze	0.154	0.413	874	1	Pos
75	Southern	Monze	0.225	0.694	1538	2	Pos
76	Southern	Monze	0.665	2.444	6068	7	Pos
77	Southern	Monze	0.163	0.448	955	1	Pos
78	Southern	Monze	0.181	0.52	1123	2	Pos
79	Southern	Monze	0.201	0.603	1320	2	Pos
80	Southern	Monze	0.552	1.992	4855	5	Pos

81	Southern	Monze	0.721	2.667	6674	7	Pos
82	Southern	Monze	0.065	0.06	107	0	Neg
83	Southern	Monze	0.512	1.837	4445	5	Pos
84	Southern	Monze	0.102	0.206	409	1	Pos
85	Southern	Monze	0.258	0.825	1858	2	Pos
86	Southern	Monze	0.217	0.663	1464	2	Pos
87	Southern	Monze	0.131	0.321	664	1	Pos
88	Southern	Monze	0.199	0.591	1291	2	Pos
89	Southern	Monze	0.149	0.397	837	1	Pos
90	Southern	Monze	0.155	0.417	883	1	Pos
91	Southern	Monze	0.368	1.266	2962	3	Pos
92	Southern	Monze	1.041	3.937	10203	9	Pos
	Southern	Namwala					
1	Southern	Namwala	0.391	1.124	2602	3	Pos
2	Southern	Namwala	0.272	0.729	1623	2	Pos
3	Southern	Namwala	0.409	1.184	2754	3	Pos
4	Southern	Namwala	0.425	1.241	2899	3	Pos
5	Southern	Namwala	0.44	1.291	3026	4	Pos
6	Southern	Namwala	0.111	0.191	377	0	Neg
7	Southern	Namwala	0.143	0.298	612	1	Pos
8	Southern	Namwala	0.104	0.167	326	0	Neg
9	Southern	Namwala	0.315	0.873	1976	2	Pos
10	Southern	Namwala	1.195	3.816	9862	8	Pos
11	Southern	Namwala	0.352	0.997	2283	3	Pos
12	Southern	Namwala	0.127	0.244	492	1	Pos
13	Southern	Namwala	1.451	4.669	12287	10	Pos
14	Southern	Namwala	0.15	0.321	664	1	Pos
15	Southern	Namwala	0.546	1.645	3941	4	Pos
16	Southern	Namwala	0.798	2.485	6179	7	Pos
17	Southern	Namwala	0.176	0.408	862	1	Pos
18	Southern	Namwala	0.09	0.12	227	0	Neg
19	Southern	Namwala	0.209	0.518	1118	2	Pos
20	Southern	Namwala	0.326	0.906	2057	3	Pos
21	Southern	Namwala	0.196	0.472	1011	2	Pos
22	Southern	Namwala	0.142	0.294	603	1	Pos

23	Southern	Namwala	0.167	0.375	786	1	Pos
24	Southern	Namwala	0.348	0.983	2248	3	Pos
25	Southern	Namwala	0.302	0.826	1860	2	Pos
26	Southern	Namwala	0.23	0.589	1287	2	Pos
27	Southern	Namwala	0.296	0.809	1818	2	Pos
28	Southern	Namwala	0.117	0.207	412	1	Pos
29	Southern	Namwala	0.108	0.181	356	0	Neg
30	Southern	Namwala	0.14	0.288	590	1	Pos
31	Southern	Namwala	0.169	0.385	809	1	Pos
32	Southern	Namwala	0.053	0	1	0	Neg
33	Southern	Namwala	0.156	0.341	709	1	Pos
34	Southern	Namwala	0.417	1.214	2830	3	Pos
35	Southern	Namwala	0.211	0.522	1128	2	Pos
36	Southern	Namwala	0.094	0.13	248	0	Neg
37	Southern	Namwala	0.122	0.227	455	1	Pos
38	Southern	Namwala	0.216	0.542	1175	2	Pos
39	Southern	Namwala	0.263	0.699	1551	2	Pos
40	Southern	Namwala	0.142	0.294	603	1	Pos
41	Southern	Namwala	0.362	1.03	2366	3	Pos
42	Southern	Namwala	0.391	1.124	2602	3	Pos
43	Southern	Namwala	0.333	0.933	2124	3	Pos
44	Southern	Namwala	0.309	0.849	1917	2	Pos
45	Southern	Namwala	0.401	1.157	2686	3	Pos
46	Southern	Namwala	0.124	0.231	464	1	Pos
47	Southern	Namwala	0.336	0.94	2141	3	Pos
48	Southern	Namwala	0.138	0.278	568	1	Pos
49	Southern	Namwala	0.231	0.592	1294	2	Pos
50	Southern	Namwala	0.247	0.642	1413	2	Pos
51	Southern	Namwala	0.164	0.368	771	1	Pos
52	Southern	Namwala	0.215	0.538	1166	2	Pos
53	Southern	Namwala	0.098	0.144	277	0	Neg
54	Southern	Namwala	0.392	1.127	2610	3	Pos
55	Southern	Namwala	0.166	0.371	777	1	Pos
56	Southern	Namwala	0.363	1.03	2366	3	Pos
57	Southern	Namwala	0.194	0.468	1001	2	Pos

58	Southern	Namwala	0.095	0.137	262	0	Neg
59	Southern	Namwala	0.071	0.057	101	0	Neg
60	Southern	Namwala	0.057	0.01	15	0	Neg
61	Southern	Namwala	0.096	0.14	269	0	Neg
62	Southern	Namwala	0.235	0.605	1325	2	Pos
63	Southern	Namwala	0.338	0.95	2166	3	Pos
64	Southern	Namwala	0.252	0.662	1461	2	Pos
65	Southern	Namwala	0.131	0.254	514	1	Pos
66	Southern	Namwala	0.264	0.699	1551	2	Pos
67	Southern	Namwala	0.096	0.14	269	0	Neg
68	Southern	Namwala	0.093	0.13	248	0	Neg
69	Southern	Namwala	0.156	0.341	709	1	Pos
70	Southern	Namwala	0.315	0.873	1976	2	Pos
71	Southern	Namwala	0.551	1.662	3986	4	Pos
72	Southern	Namwala	0.58	1.756	4232	5	Pos
73	Southern	Namwala	0.368	1.047	2408	3	Pos
74	Southern	Namwala	0.364	1.033	2373	3	Pos
75	Southern	Namwala	0.314	0.87	1968	2	Pos
76	Southern	Namwala	0.377	1.077	2484	3	Pos
77	Southern	Namwala	0.343	0.967	2209	3	Pos
78	Southern	Namwala	0.363	1.03	2366	3	Pos
79	Southern	Namwala	0.083	0.094	174	0	Neg
80	Southern	Namwala	0.107	0.177	347	0	Neg
81	Southern	Namwala	0.371	1.06	2441	3	Pos
82	Southern	Namwala	0.327	0.91	2067	3	Pos
83	Southern	Namwala	0.32	0.89	2018	3	Pos
84	Southern	Namwala	0.321	0.89	2018	3	Pos
85	Southern	Namwala	0.224	0.569	1239	2	Pos
86	Southern	Namwala	0.427	1.247	2914	3	Pos
87	Southern	Namwala	0.21	0.518	1118	2	Pos
88	Southern	Namwala	0.191	0.458	978	1	Pos
89	Southern	Namwala	0.646	1.98	4824	5	Pos
90	Southern	Namwala	0.189	0.448	955	1	Pos
91	Southern	Namwala	1.472	4.742	12497	10	Pos
92	Southern	Namwala	0.174	0.398	839	1	Pos

	Southern	Siavonga					
1	Southern	Siavonga	0.218	0.554	1203	2	Pos
2	Southern	Siavonga	0.081	0.106	198	0	Neg
3	Southern	Siavonga	0.216	0.551	1196	2	Pos
4	Southern	Siavonga	1.296	4.116	10710	9	Pos
5	Southern	Siavonga	0.136	0.287	588	1	Pos
6	Southern	Siavonga	0.348	0.987	2258	3	Pos
7	Southern	Siavonga	0.108	0.195	386	0	Neg
8	Southern	Siavonga	0.753	2.323	5741	6	Pos
9	Southern	Siavonga	0.116	0.218	435	1	Pos
10	Southern	Siavonga	0.159	0.36	752	1	Pos
11	Southern	Siavonga	0.137	0.287	588	1	Pos
12	Southern	Siavonga	0.135	0.284	581	1	Pos
13	Southern	Siavonga	0.186	0.452	964	1	Pos
14	Southern	Siavonga	0.092	0.142	273	0	Neg
15	Southern	Siavonga	0.164	0.38	798	1	Pos
16	Southern	Siavonga	0.117	0.221	442	1	Pos
17	Southern	Siavonga	0.049	0	1	0	Neg
18	Southern	Siavonga	0.082	0.109	205	0	Neg
19	Southern	Siavonga	0.303	0.838	1889	2	Pos
20	Southern	Siavonga	0.138	0.29	594	1	Pos
21	Southern	Siavonga	0.046	0	1	0	Neg
22	Southern	Siavonga	0.37	1.059	2439	3	Pos
23	Southern	Siavonga	0.118	0.224	449	1	Pos
24	Southern	Siavonga	0.215	0.548	1189	2	Pos
25	Southern	Siavonga	0.077	0.089	164	0	Neg
26	Southern	Siavonga	0.197	0.485	1041	2	Pos
27	Southern	Siavonga	0.105	0.182	358	0	Neg
28	Southern	Siavonga	0.133	0.274	559	1	Pos
29	Southern	Siavonga	0.051	0.003	4	0	Neg
30	Southern	Siavonga	0.05	0.003	4	0	Neg
31	Southern	Siavonga	0.117	0.221	442	1	Pos
32	Southern	Siavonga	0.094	0.145	279	0	Neg
33	Southern	Siavonga	0.15	0.333	691	1	Pos
34	Southern	Siavonga	0.157	0.356	743	1	Pos

35	Southern	Siavonga	0.095	0.149	288	0	Neg
36	Southern	Siavonga	0.05	0.003	4	0	Neg
37	Southern	Siavonga	0.639	1.947	4736	5	Pos
38	Southern	Siavonga	0.296	0.815	1833	2	Pos
39	Southern	Siavonga	0.044	0	1	0	Neg
40	Southern	Siavonga	0.144	0.31	639	1	Pos
41	Southern	Siavonga	0.046	0	1	0	Neg
42	Southern	Siavonga	0.261	0.7	1553	2	Pos
43	Southern	Siavonga	0.394	1.139	2640	3	Pos
44	Southern	Siavonga	1.815	5.828	15646	11	Pos
45	Southern	Siavonga	0.07	0.069	124	0	Neg
46	Southern	Siavonga	0.372	1.066	2456	3	Pos
47	Southern	Siavonga	0.635	1.934	4702	5	Pos
48	Southern	Siavonga	0.281	0.762	1703	2	Pos
49	Southern	Siavonga	0.181	0.432	918	1	Pos
50	Southern	Siavonga	0.232	0.601	1315	2	Pos
51	Southern	Siavonga	0.24	0.627	1377	2	Pos
52	Southern	Siavonga	0.183	0.439	934	1	Pos
53	Southern	Siavonga	0.238	0.624	1370	2	Pos
54	Southern	Siavonga	0.601	1.822	4406	5	Pos
55	Southern	Siavonga	0.375	1.076	2481	3	Pos
56	Southern	Siavonga	0.215	0.548	1189	2	Pos
57	Southern	Siavonga	0.383	1.099	2539	3	Pos
58	Southern	Siavonga	0.331	0.931	2119	3	Pos
59	Southern	Siavonga	0.217	0.551	1196	2	Pos
60	Southern	Siavonga	0.256	0.683	1512	2	Pos
61	Southern	Siavonga	0.27	0.729	1623	2	Pos
62	Southern	Siavonga	0.209	0.528	1142	2	Pos
63	Southern	Siavonga	0.265	0.71	1577	2	Pos
64	Southern	Siavonga	0.305	0.845	1907	2	Pos
65	Southern	Siavonga	0.213	0.538	1166	2	Pos
66	Southern	Siavonga	0.399	1.155	2680	3	Pos
67	Southern	Siavonga	0.207	0.518	1118	2	Pos
68	Southern	Siavonga	0.07	0.069	124	0	Neg
69	Southern	Siavonga	0.169	0.393	828	1	Pos

70	Southern	Siavonga	0.143	0.31	639	1	Pos
71	Southern	Siavonga	0.216	0.551	1196	2	Pos
72	Southern	Siavonga	0.149	0.33	684	1	Pos
73	Southern	Siavonga	0.079	0.099	184	0	Neg
74	Southern	Siavonga	0.081	0.106	198	0	Neg
75	Southern	Siavonga	0.09	0.135	258	0	Neg
76	Southern	Siavonga	0.207	0.518	1118	2	Pos
77	Southern	Siavonga	0.111	0.205	407	1	Pos
78	Southern	Siavonga	0.279	0.759	1696	2	Pos
79	Southern	Siavonga	0.144	0.31	639	1	Pos
80	Southern	Siavonga	0.223	0.574	1251	2	Pos
81	Southern	Siavonga	0.316	0.881	1995	2	Pos
82	Southern	Siavonga	0.256	0.683	1512	2	Pos
83	Southern	Siavonga	0.082	0.109	205	0	Neg
84	Southern	Siavonga	0.122	0.241	486	1	Pos
85	Southern	Siavonga	0.321	0.898	2037	3	Pos
86	Southern	Siavonga	0.21	0.528	1142	2	Pos
87	Southern	Siavonga	0.608	1.842	4458	5	Pos
88	Southern	Siavonga	0.126	0.251	508	1	Pos
89	Southern	Siavonga	0.19	0.462	987	1	Pos
90	Southern	Siavonga	0.179	0.429	911	1	Pos
91	Southern	Siavonga	0.13	0.264	536	1	Pos
92	Southern	Siavonga	0.926	2.894	7295	7	Pos
	Southern	Itezhi tezhi					
1	Southern	Itezhi tezhi	0.053	0.01	15	0	Neg
2	Southern	Itezhi tezhi	0.169	0.378	793	1	Pos
3	Southern	Itezhi tezhi	0.121	0.229	459	1	Pos
4	Southern	Itezhi tezhi	0.296	0.784	1757	2	Pos
5	Southern	Itezhi tezhi	0.089	0.127	242	0	Neg
6	Southern	Itezhi tezhi	0.121	0.229	459	1	Pos
7	Southern	Itezhi tezhi	0.117	0.213	425	1	Pos
8	Southern	Itezhi tezhi	0.187	0.438	932	1	Pos
9	Southern	Itezhi tezhi	0.048	0	1	0	Neg
10	Southern	Itezhi tezhi	0.155	0.333	691	1	Pos
11	Southern	Itezhi tezhi	0.321	0.863	1951	2	Pos

12	Southern	Itezhi tezhi	0.102	0.165	321	0	Neg
13	Southern	Itezhi tezhi	0.298	0.79	1772	2	Pos
14	Southern	Itezhi tezhi	0.129	0.251	508	1	Pos
15	Southern	Itezhi tezhi	0.069	0.06	107	0	Neg
16	Southern	Itezhi tezhi	0.267	0.689	1526	2	Pos
17	Southern	Itezhi tezhi	0.37	1.019	2338	3	Pos
18	Southern	Itezhi tezhi	0.188	0.438	932	1	Pos
19	Southern	Itezhi tezhi	*2.304	7.156	19570	13	Pos
20	Southern	Itezhi tezhi	0.159	0.346	720	1	Pos
21	Southern	Itezhi tezhi	0.328	0.886	2008	3	Pos
22	Southern	Itezhi tezhi	0.083	0.105	196	0	Neg
23	Southern	Itezhi tezhi	0.183	0.422	895	1	Pos
24	Southern	Itezhi tezhi	0.874	2.619	6543	7	Pos
25	Southern	Itezhi tezhi	0.201	0.483	1036	2	Pos
26	Southern	Itezhi tezhi	0.125	0.238	479	1	Pos
27	Southern	Itezhi tezhi	0.128	0.251	508	1	Pos
28	Southern	Itezhi tezhi	0.058	0.025	41	0	Neg
29	Southern	Itezhi tezhi	1.725	5.321	14169	11	Pos
30	Southern	Itezhi tezhi	0.132	0.26	528	1	Pos
31	Southern	Itezhi tezhi	0.173	0.39	821	1	Pos
32	Southern	Itezhi tezhi	0.075	0.083	152	0	Neg
33	Southern	Itezhi tezhi	0.145	0.302	621	1	Pos
34	Southern	Itezhi tezhi	0.589	1.711	4114	5	Pos
35	Southern	Itezhi tezhi	1.391	4.26	11119	9	Pos
36	Southern	Itezhi tezhi	0.156	0.337	700	1	Pos
37	Southern	Itezhi tezhi	0.179	0.413	874	1	Pos
38	Southern	Itezhi tezhi	0.28	0.73	1626	2	Pos
39	Southern	Itezhi tezhi	0.094	0.14	269	0	Neg
40	Southern	Itezhi tezhi	0.12	0.225	451	1	Pos
41	Southern	Itezhi tezhi	0.09	0.13	248	0	Neg
42	Southern	Itezhi tezhi	0.119	0.219	438	1	Pos
43	Southern	Itezhi tezhi	0.197	0.467	999	1	Pos
44	Southern	Itezhi tezhi	0.438	1.235	2883	3	Pos
45	Southern	Itezhi tezhi	0.105	0.175	343	0	Neg
46	Southern	Itezhi tezhi	0.083	0.105	196	0	Neg

47	Southern	Itezhi tezhi	0.108	0.187	368	0	Neg
48	Southern	Itezhi tezhi	0.262	0.673	1488	2	Pos
49	Southern	Itezhi tezhi	0.126	0.241	486	1	Pos
50	Southern	Itezhi tezhi	0.135	0.273	556	1	Pos
51	Southern	Itezhi tezhi	0.856	2.562	6388	7	Pos
52	Southern	Itezhi tezhi	0.104	0.175	343	0	Neg
53	Southern	Itezhi tezhi	0.244	0.616	1351	2	Pos
54	Southern	Itezhi tezhi	0.157	0.343	714	1	Pos
55	Southern	Itezhi tezhi	0.264	0.679	1502	2	Pos
56	Southern	Itezhi tezhi	0.14	0.286	585	1	Pos
57	Southern	Itezhi tezhi	0.12	0.225	451	1	Pos
58	Southern	Itezhi tezhi	0.093	0.14	269	0	Neg
59	Southern	Itezhi tezhi	0.216	0.53	1147	2	Pos
60	Southern	Itezhi tezhi	0.118	0.216	431	1	Pos
61	Southern	Itezhi tezhi	0.154	0.33	684	1	Pos
62	Southern	Itezhi tezhi	0.174	0.394	830	1	Pos
63	Southern	Itezhi tezhi	0.129	0.251	508	1	Pos
	Northern	Mpulungu					
1	Northern	Mpulungu	0.191	0.466	997	1	Pos
2	Northern	Mpulungu	0.082	0.113	213	0	Neg
3	Northern	Mpulungu	0.087	0.126	240	0	Neg
4	Northern	Mpulungu	0.145	0.314	648	1	Pos
5	Northern	Mpulungu	0.148	0.327	677	1	Pos
6	Northern	Mpulungu	0.128	0.262	532	1	Pos
7	Northern	Mpulungu	0.169	0.395	832	1	Pos
8	Northern	Mpulungu	0.109	0.197	390	0	Neg
9	Northern	Mpulungu	0.251	0.657	1449	2	Pos
10	Northern	Mpulungu	0.104	0.184	362	0	Neg
11	Northern	Mpulungu	0.27	0.722	1606	2	Pos
12	Northern	Mpulungu	0.455	1.32	3100	4	Pos
13	Northern	Mpulungu	0.316	0.871	1971	2	Pos
14	Northern	Mpulungu	0.212	0.531	1149	2	Pos
15	Northern	Mpulungu	0.116	0.22	440	1	Pos
16	Northern	Mpulungu	0.084	0.117	221	0	Neg
17	Northern	Mpulungu	0.314	0.861	1946	2	Pos

18	Northern	Mpulungu	0.056	0.029	48	0	Neg
19	Northern	Mpulungu	0.781	2.375	5881	6	Pos
20	Northern	Mpulungu	0.607	1.809	4371	5	Pos
21	Northern	Mpulungu	0.227	0.579	1263	2	Pos
22	Northern	Mpulungu	0.176	0.414	876	1	Pos
23	Northern	Mpulungu	0.221	0.563	1225	2	Pos
24	Northern	Mpulungu	0.338	0.939	2139	3	Pos
25	Northern	Mpulungu	0.15	0.333	691	1	Pos
26	Northern	Mpulungu	0.181	0.43	913	1	Pos
27	Northern	Mpulungu	0.12	0.233	468	1	Pos
28	Northern	Mpulungu	0.14	0.301	619	1	Pos
29	Northern	Mpulungu	0.945	2.903	7320	7	Pos
30	Northern	Mpulungu	0.287	0.777	1740	2	Pos
31	Northern	Mpulungu	0.123	0.246	497	1	Pos
32	Northern	Mpulungu	0.177	0.421	892	1	Pos
33	Northern	Mpulungu	0.893	2.735	6859	7	Pos
34	Northern	Mpulungu	0.306	0.835	1882	2	Pos
35	Northern	Mpulungu	1.716	5.398	14392	11	Pos
36	Northern	Mpulungu	0.166	0.382	803	1	Pos
37	Northern	Mpulungu	0.214	0.54	1170	2	Pos
38	Northern	Mpulungu	0.19	0.46	983	1	Pos
39	Northern	Mpulungu	0.113	0.21	418	1	Pos
40	Northern	Mpulungu	0.167	0.385	809	1	Pos
41	Northern	Mpulungu	0.12	0.233	468	1	Pos
42	Northern	Mpulungu	0.23	0.592	1294	2	Pos
43	Northern	Mpulungu	0.197	0.482	1034	2	Pos
44	Northern	Mpulungu	0.509	1.492	3543	4	Pos
45	Northern	Mpulungu	0.301	0.819	1843	2	Pos
46	Northern	Mpulungu	0.182	0.434	922	1	Pos
47	Northern	Mpulungu	0.134	0.282	576	1	Pos
48	Northern	Mpulungu	0.171	0.401	846	1	Pos
49	Northern	Mpulungu	0.197	0.482	1034	2	Pos
50	Northern	Mpulungu	0.517	1.521	3618	4	Pos
51	Northern	Mpulungu	0.06	0.039	67	0	Neg
52	Northern	Mpulungu	0.176	0.414	876	1	Pos

53	Northern	Mpulungu	0.198	0.485	1041	2	Pos
54	Northern	Mpulungu	0.574	1.702	4090	5	Pos
55	Northern	Mpulungu	0.445	1.285	3011	4	Pos
56	Northern	Mpulungu	0.5	1.463	3468	4	Pos
57	Northern	Mpulungu	0.316	0.871	1971	2	Pos
58	Northern	Mpulungu	0.434	1.252	2927	3	Pos
59	Northern	Mpulungu	0.391	1.113	2574	3	Pos
60	Northern	Mpulungu	0.575	1.706	4101	5	Pos
61	Northern	Mpulungu	0.607	1.809	4371	5	Pos
62	Northern	Mpulungu	0.27	0.722	1606	2	Pos
63	Northern	Mpulungu	0.358	1.006	2306	3	Pos
64	Northern	Mpulungu	0.549	1.625	3889	4	Pos
65	Northern	Mpulungu	0.287	0.777	1740	2	Pos
66	Northern	Mpulungu	0.336	0.935	2129	3	Pos
67	Northern	Mpulungu	0.219	0.553	1201	2	Pos
68	Northern	Mpulungu	0.466	1.356	3193	4	Pos
69	Northern	Mpulungu	0.184	0.443	943	1	Pos
70	Northern	Mpulungu	0.292	0.79	1772	2	Pos
71	Northern	Mpulungu	0.276	0.738	1645	2	Pos
72	Northern	Mpulungu	0.156	0.353	736	1	Pos
73	Northern	Mpulungu	0.13	0.265	539	1	Pos
74	Northern	Mpulungu	0.052	0.016	25	0	Neg
75	Northern	Mpulungu	0.449	1.298	3044	4	Pos
76	Northern	Mpulungu	0.162	0.372	780	1	Pos
77	Northern	Mpulungu	0.484	1.414	3342	4	Pos
78	Northern	Mpulungu	0.309	0.848	1914	2	Pos
79	Northern	Mpulungu	0.385	1.091	2519	3	Pos
80	Northern	Mpulungu	0.247	0.644	1418	2	Pos
81	Northern	Mpulungu	0.202	0.502	1081	2	Pos
82	Northern	Mpulungu	0.183	0.437	929	1	Pos
83	Northern	Mpulungu	0.391	1.113	2574	3	Pos
84	Northern	Mpulungu	0.571	1.693	4067	5	Pos
85	Northern	Mpulungu	0.282	0.761	1701	2	Pos
86	Northern	Mpulungu	0.299	0.816	1835	2	Pos
87	Northern	Mpulungu	0.241	0.625	1372	2	Pos

88	Northern	Mpulungu	0.242	0.628	1380	2	Pos
89	Northern	Mpulungu	0.363	1.023	2348	3	Pos
90	Northern	Mpulungu	0.324	0.893	2025	3	Pos
91	Northern	Mpulungu	0.448	1.294	3034	4	Pos
92	Northern	Mpulungu	0.157	0.356	743	1	Pos
	Northern	Nakonde					
1	Northern	Nakonde	0.13	0.268	545	1	Pos
2	Northern	Nakonde	0.275	0.789	1769	2	Pos
3	Northern	Nakonde	0.251	0.7	1553	2	Pos
4	Northern	Nakonde	0.141	0.311	641	1	Pos
5	Northern	Nakonde	0.158	0.371	777	1	Pos
6	Northern	Nakonde	0.079	0.089	164	0	Neg
7	Northern	Nakonde	0.071	0.061	109	0	Neg
8	Northern	Nakonde	0.106	0.186	366	0	Neg
9	Northern	Nakonde	0.106	0.186	366	0	Neg
10	Northern	Nakonde	0.141	0.311	641	1	Pos
11	Northern	Nakonde	0.12	0.232	466	1	Pos
12	Northern	Nakonde	0.055	0.004	6	0	Neg
13	Northern	Nakonde	0.172	0.421	892	1	Pos
14	Northern	Nakonde	0.12	0.232	466	1	Pos
15	Northern	Nakonde	0.093	0.139	267	0	Neg
16	Northern	Nakonde	0.166	0.396	835	1	Pos
17	Northern	Nakonde	0.114	0.214	427	1	Pos
18	Northern	Nakonde	0.303	0.889	2015	3	Pos
19	Northern	Nakonde	0.202	0.529	1144	2	Pos
20	Northern	Nakonde	0.118	0.229	459	1	Pos
21	Northern	Nakonde	0.185	0.468	1001	2	Pos
22	Northern	Nakonde	0.244	0.679	1502	2	Pos
23	Northern	Nakonde	0.17	0.414	876	1	Pos
24	Northern	Nakonde	0.086	0.114	215	0	Neg
25	Northern	Nakonde	0.198	0.514	1109	2	Pos
26	Northern	Nakonde	0.075	0.075	136	0	Neg
27	Northern	Nakonde	0.108	0.193	381	0	Neg
28	Northern	Nakonde	0.142	0.314	648	1	Pos
29	Northern	Nakonde	0.245	0.682	1509	2	Pos

30	Northern	Nakonde	0.106	0.186	366	0	Neg
31	Northern	Nakonde	0.263	0.746	1665	2	Pos
32	Northern	Nakonde	0.143	0.318	657	1	Pos
33	Northern	Nakonde	0.111	0.204	405	1	Pos
34	Northern	Nakonde	0.117	0.221	442	1	Pos
35	Northern	Nakonde	0.655	2.143	5258	6	Pos
36	Northern	Nakonde	0.146	0.325	673	1	Pos
37	Northern	Nakonde	0.603	1.961	4773	5	Pos
38	Northern	Nakonde	0.15	0.343	714	1	Pos
39	Northern	Nakonde	0.478	1.514	3600	4	Pos
40	Northern	Nakonde	0.262	0.739	1647	2	Pos
41	Northern	Nakonde	0.129	0.264	536	1	Pos
42	Northern	Nakonde	0.154	0.357	745	1	Pos
43	Northern	Nakonde	0.963	3.246	8267	8	Pos
44	Northern	Nakonde	0.239	0.657	1449	2	Pos
45	Northern	Nakonde	0.133	0.282	576	1	Pos
46	Northern	Nakonde	0.127	0.261	530	1	Pos
47	Northern	Nakonde	1.014	3.429	8777	8	Pos
48	Northern	Nakonde	0.124	0.246	497	1	Pos
49	Northern	Nakonde	0.475	1.5	3564	4	Pos
50	Northern	Nakonde	0.098	0.154	298	0	Neg
51	Northern	Nakonde	0.098	0.154	298	0	Neg
52	Northern	Nakonde	1.229	4.193	10928	9	Pos
53	Northern	Nakonde	0.093	0.139	267	0	Neg
54	Northern	Nakonde	0.089	0.125	237	0	Neg
55	Northern	Nakonde	0.306	0.9	2042	3	Pos
56	Northern	Nakonde	0.197	0.507	1093	2	Pos
57	Northern	Nakonde	0.104	0.179	351	0	Neg
58	Northern	Nakonde	0.128	0.264	536	1	Pos
59	Northern	Nakonde	0.227	0.614	1346	2	Pos
60	Northern	Nakonde	0.06	0.018	29	0	Neg
61	Northern	Nakonde	0.084	0.107	200	0	Neg
62	Northern	Nakonde	0.774	2.571	6412	7	Pos
63	Northern	Nakonde	0.219	0.586	1279	2	Pos
64	Northern	Nakonde	0.103	0.175	343	0	Neg

65	Northern	Nakonde	0.119	0.232	466	1	Pos
66	Northern	Nakonde	0.124	0.246	497	1	Pos
67	Northern	Nakonde	0.142	0.314	648	1	Pos
68	Northern	Nakonde	0.142	0.314	648	1	Pos
69	Northern	Nakonde	0.107	0.189	373	0	Neg
70	Northern	Nakonde	0.226	0.611	1339	2	Pos
71	Northern	Nakonde	0.146	0.325	673	1	Pos
72	Northern	Nakonde	0.182	0.454	969	1	Pos
73	Northern	Nakonde	0.17	0.414	876	1	Pos
74	Northern	Nakonde	0.071	0.061	109	0	Neg
75	Northern	Nakonde	0.117	0.221	442	1	Pos
76	Northern	Nakonde	0.102	0.168	328	0	Neg
77	Northern	Nakonde	0.321	0.95	2166	3	Pos
78	Northern	Nakonde	0.495	1.575	3759	4	Pos
79	Northern	Nakonde	1.192	4.064	10562	9	Pos
80	Northern	Nakonde	0.073	0.068	122	0	Neg
81	Northern	Nakonde	0.124	0.246	497	1	Pos
82	Northern	Nakonde	0.089	0.125	237	0	Neg
83	Northern	Nakonde	0.077	0.082	150	0	Neg
84	Northern	Nakonde	0.136	0.293	601	1	Pos
85	Northern	Nakonde	0.238	0.657	1449	2	Pos
86	Northern	Nakonde	0.053	0	1	0	Neg
87	Northern	Nakonde	0.269	0.764	1708	2	Pos
88	Northern	Nakonde	0.133	0.282	576	1	Pos
89	Northern	Nakonde	0.123	0.246	497	1	Pos
90	Northern	Nakonde	0.234	0.639	1406	2	Pos
91	Northern	Nakonde	0.149	0.339	705	1	Pos
92	Northern	Nakonde	0.068	0.05	87	0	Neg
	Eastern	Chipata					
1	Eastern	Chipata	0.08	0.09	166	0	Neg
2	Eastern	Chipata	0.253	0.579	1263	2	Pos
3	Eastern	Chipata	0.115	0.192	379	0	Neg
4	Eastern	Chipata	0.237	0.537	1163	2	Pos
5	Eastern	Chipata	0.221	0.492	1057	2	Pos
6	Eastern	Chipata	0.329	0.794	1782	2	Pos

7	Eastern	Chipata	0.214	0.472	1011	2	Pos
8	Eastern	Chipata	*2.161	5.972	16068	12	Pos
9	Eastern	Chipata	0.147	0.282	576	1	Pos
10	Eastern	Chipata	*2.181	6.028	16233	12	Pos
11	Eastern	Chipata	0.374	0.921	2094	3	Pos
12	Eastern	Chipata	0.531	1.367	3221	4	Pos
13	Eastern	Chipata	0.108	0.172	336	0	Neg
14	Eastern	Chipata	0.547	1.41	3332	4	Pos
15	Eastern	Chipata	0.474	1.206	2810	3	Pos
16	Eastern	Chipata	0.423	1.062	2446	3	Pos
17	Eastern	Chipata	0.13	0.232	466	1	Pos
18	Eastern	Chipata	0.192	0.41	867	1	Pos
19	Eastern	Chipata	0.207	0.452	964	1	Pos
20	Eastern	Chipata	0.137	0.251	508	1	Pos
21	Eastern	Chipata	0.51	1.305	3062	4	Pos
22	Eastern	Chipata	0.131	0.234	470	1	Pos
23	Eastern	Chipata	0.142	0.268	545	1	Pos
24	Eastern	Chipata	0.142	0.268	545	1	Pos
25	Eastern	Chipata	0.175	0.359	750	1	Pos
26	Eastern	Chipata	0.165	0.333	691	1	Pos
27	Eastern	Chipata	0.151	0.291	597	1	Pos
28	Eastern	Chipata	0.184	0.387	814	1	Pos
29	Eastern	Chipata	0.194	0.415	878	1	Pos
30	Eastern	Chipata	0.3	0.712	1582	2	Pos
31	Eastern	Chipata	0.209	0.458	978	1	Pos
32	Eastern	Chipata	0.336	0.816	1835	2	Pos
33	Eastern	Chipata	0.345	0.842	1899	2	Pos
34	Eastern	Chipata	0.313	0.751	1677	2	Pos
35	Eastern	Chipata	0.099	0.147	283	0	Neg
36	Eastern	Chipata	0.26	0.599	1310	2	Pos
37	Eastern	Chipata	0.214	0.472	1011	2	Pos
38	Eastern	Chipata	0.33	0.799	1794	2	Pos
39	Eastern	Chipata	0.134	0.246	497	1	Pos
40	Eastern	Chipata	0.269	0.624	1370	2	Pos
41	Eastern	Chipata	0.058	0.028	46	0	Neg

42	Eastern	Chipata	0.062	0.04	69	0	Neg
43	Eastern	Chipata	0.056	0.025	41	0	Neg
44	Eastern	Chipata	0.056	0.025	41	0	Neg
45	Eastern	Chipata	0.127	0.226	453	1	Pos
46	Eastern	Chipata	0.101	0.153	296	0	Neg
47	Eastern	Chipata	0.831	2.212	5443	6	Pos
48	Eastern	Chipata	0.288	0.678	1500	2	Pos
49	Eastern	Chipata	0.213	0.469	1004	2	Pos
50	Eastern	Chipata	0.364	0.895	2030	3	Pos
51	Eastern	Chipata	0.326	0.788	1767	2	Pos
52	Eastern	Chipata	0.219	0.483	1036	2	Pos
53	Eastern	Chipata	0.057	0.028	46	0	Neg
54	Eastern	Chipata	0.217	0.477	1022	2	Pos
55	Eastern	Chipata	0.179	0.373	782	1	Pos
56	Eastern	Chipata	0.225	0.5	1076	2	Pos
57	Eastern	Chipata	0.085	0.107	200	0	Neg
58	Eastern	Chipata	0.098	0.141	271	0	Neg
59	Eastern	Chipata	0.267	0.619	1358	2	Pos
60	Eastern	Chipata	0.134	0.246	497	1	Pos
61	Eastern	Chipata	0.256	0.59	1289	2	Pos
62	Eastern	Chipata	0.334	0.808	1816	2	Pos
63	Eastern	Chipata	0.154	0.299	614	1	Pos
64	Eastern	Chipata	0.201	0.435	925	1	Pos
65	Eastern	Chipata	0.871	2.325	5746	6	Pos
66	Eastern	Chipata	0.136	0.251	508	1	Pos
67	Eastern	Chipata	0.102	0.153	296	0	Neg
68	Eastern	Chipata	0.194	0.415	878	1	Pos
69	Eastern	Chipata	0.197	0.421	892	1	Pos
70	Eastern	Chipata	0.238	0.54	1170	2	Pos
71	Eastern	Chipata	0.317	0.763	1706	2	Pos
72	Eastern	Chipata	0.261	0.605	1325	2	Pos
73	Eastern	Chipata	0.068	0.059	105	0	Neg
74	Eastern	Chipata	0.177	0.367	768	1	Pos
75	Eastern	Chipata	0.192	0.41	867	1	Pos
76	Eastern	Chipata	0.162	0.325	673	1	Pos

77	Eastern	Chipata	0.221	0.492	1057	2	Pos
78	Eastern	Chipata	0.259	0.599	1310	2	Pos
79	Eastern	Chipata	0.566	1.463	3468	4	Pos
80	Eastern	Chipata	0.466	1.184	2754	3	Pos
81	Eastern	Chipata	0.655	1.715	4124	5	Pos
82	Eastern	Chipata	0.135	0.249	503	1	Pos
83	Eastern	Chipata	0.183	0.381	800	1	Pos
84	Eastern	Chipata	0.124	0.215	429	1	Pos
85	Eastern	Chipata	0.217	0.477	1022	2	Pos
86	Eastern	Chipata	0.2	0.432	918	1	Pos
87	Eastern	Chipata	0.167	0.336	698	1	Pos
88	Eastern	Chipata	0.108	0.172	336	0	Neg
89	Eastern	Chipata	0.529	1.359	3200	4	Pos
90	Eastern	Chipata	1.801	4.952	13101	10	Pos
91	Eastern	Chipata	0.05	0.008	12	0	Neg
92	Eastern	Chipata	0.169	0.345	718	1	Pos
	Luapula	Samfya					
1	Luapula	Samfya	0.062	0.038	65	0	Neg
2	Luapula	Samfya	0.183	0.422	895	1	Pos
3	Luapula	Samfya	0.067	0.057	101	0	Neg
4	Luapula	Samfya	0.07	0.067	120	0	Neg
5	Luapula	Samfya	0.085	0.114	215	0	Neg
6	Luapula	Samfya	0.069	0.06	107	0	Neg
7	Luapula	Samfya	0.096	0.149	288	0	Neg
8	Luapula	Samfya	0.149	0.317	655	1	Pos
9	Luapula	Samfya	0.13	0.254	514	1	Pos
10	Luapula	Samfya	0.802	2.387	5914	6	Pos
11	Luapula	Samfya	0.087	0.117	221	0	Neg
12	Luapula	Samfya	0.097	0.152	294	0	Neg
13	Luapula	Samfya	0.175	0.397	837	1	Pos
14	Luapula	Samfya	0.161	0.352	734	1	Pos
15	Luapula	Samfya	0.173	0.39	821	1	Pos
16	Luapula	Samfya	0.124	0.235	473	1	Pos
17	Luapula	Samfya	0.046	0	1	0	Neg
18	Luapula	Samfya	0.045	0	1	0	Neg

19	Luapula	Samfya	0.111	0.197	390	0	Neg
20	Luapula	Samfya	0.329	0.889	2015	3	Pos
21	Luapula	Samfya	0.09	0.13	248	0	Neg
22	Luapula	Samfya	0.078	0.092	170	0	Neg
23	Luapula	Samfya	0.383	1.057	2434	3	Pos
24	Luapula	Samfya	0.41	1.146	2658	3	Pos
25	Luapula	Samfya	0.098	0.152	294	0	Neg
26	Luapula	Samfya	1.432	4.39	11489	9	Pos
27	Luapula	Samfya	0.089	0.127	242	0	Neg
28	Luapula	Samfya	0.42	1.175	2731	3	Pos
29	Luapula	Samfya	0.149	0.317	655	1	Pos

Note: Sample statuses (groups) of 1-13 designate a positive titre, while a sample status (group) of 0 designates a negative titre. Further, titre values equal and above 403 are classed as 1; equal and above 1001, as 2, equal and above 2003, as 3, equal and above 3011, as 4, equal and above 4051, as 5, equal and above 5146, as 6, equal and above 6068, as 7, equal and above 8267, as 8, equal and above 10084, as 9, equal and above 12281, as 10, equal and above 14169, as 11, equal and above 16068, as 12 and equal and above 19570, as 13.