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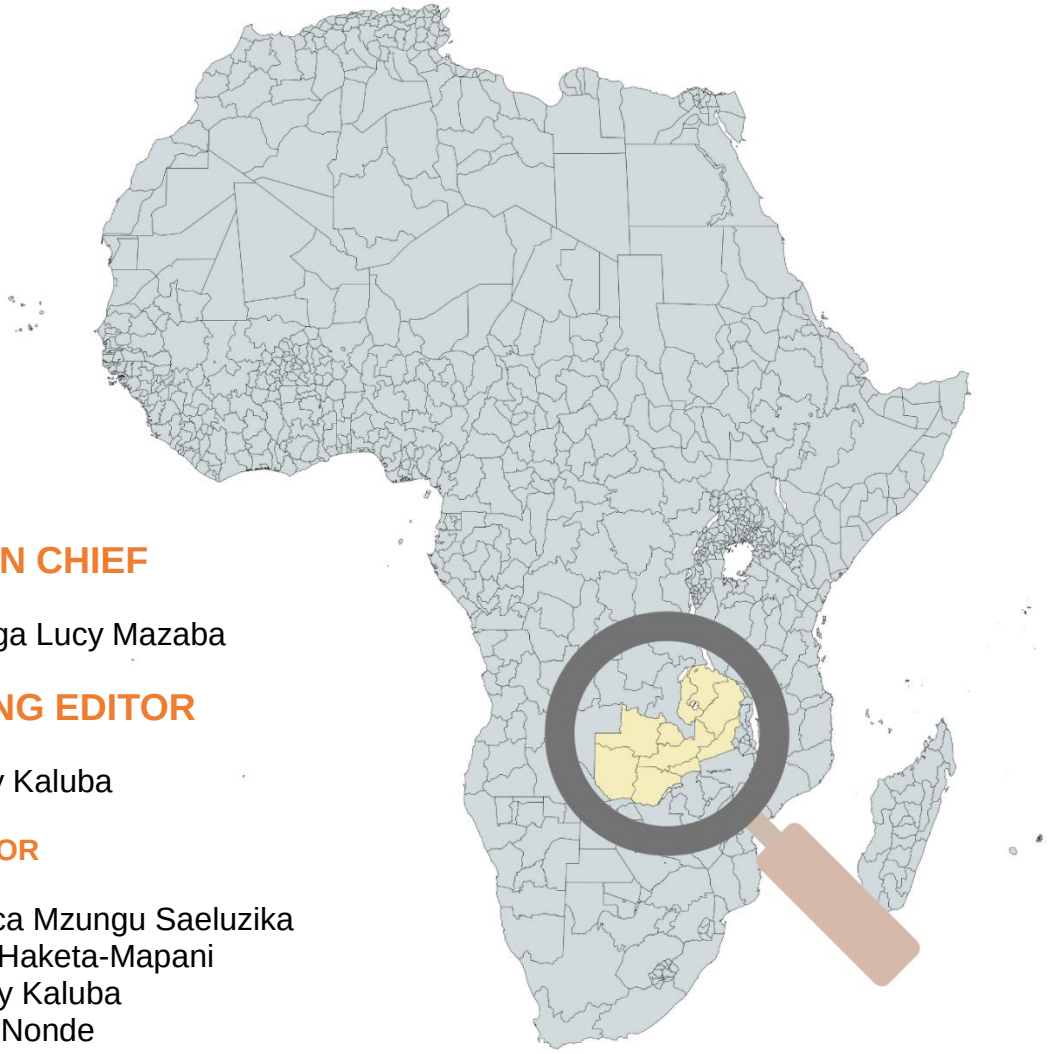

SPEAK OUT

"The journey to end cholera in Zambia has just begun"

Prof. Roma Chilengi

About The Health Press

The Health Press is an open-access and peer-reviewed public health bulletin published by Zambia National Public Health Institute (ZNPHI).

The mission of the Health Press is to provide a platform for sharing and communicating of health-related knowledge and research in Zambia and globally. It aspires to disseminate evidence that may inform policy direction on various issues, enhance national development and help secure Zambia's public health security.

An issue of the Health Press typically includes a research article, outbreak investigation, field notes and epidemiological bulletin. A new issue is published quarterly online and can be accessed at https://thp.znphi.co.zm/index.php/thehealthpress.

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FOREWORD

Dear Readers,

On behalf of the editorial team, I am pleased to share with you the first issue of the 2024 Health Press. Quarter 1 of 2024 was marked by the Cholera outbreak affecting 71 districts nationwide with Lusaka being the epicentre. We would like to thank all cooperating partners that have been working hand in hand with the Ministry of Health to combat one of the most devastating cholera outbreaks in recent history. We must not relent as Prof Chilengi puts it "the journey to end Cholera in Zambia has just begun."

This publication showcases surveillance data for priority diseases for the first quarter of 2024. Notable highlights include an editorial calling for action to end Cholera and a research article investigating the existence and genetic characterization of BLV infection in wildlife in Zambia.

I would like extend my gratitude to the editorial team, authors and the Surveillance and Disease Intelligence Cluster whose immeasurable contribution and support made this publication possible. We hope this issue inspires and informs public health action to enhance public health security in Zambia.

Dr. Mazyanga Lucy Mazaba

Editor-in-Chief, The Health Press

EDITORIAL

"SPEAK OUT" – The journey to eliminating cholera in Zambia just begun

Prof Roma Chilengi

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SPEAK OUT" – The ZNPHI says the journey to eliminating cholera in Zambia just began. Health Press Bull. 2024;08(1):3-5.

As your institute mandated to spearhead public health security, we wish to remind the Zambian public that it is time to enhance the fight against cholera towards its elimination. The fight against cholera requires a multifaceted approach that encompasses prevention, detection, treatment, and control measures. Key to winning this battle is everyone taking up their responsibility, hence the emphasis on multisectoral approaches. The fight requires all to throw targeted and strong punches. Indeed, the fight against cholera is an ongoing battle that requires sustained efforts from governments, private sector organizations, researchers, academics, communities, and individuals worldwide.

Cholera is an ancient disease associated with lack of access to safe drinking water. Failure to provide safe water is nearly always associated with poor sanitation. These factors are really two sides of the same coin and characteristic of "under development". Unfortunately, our current context in Zambia presents these faces everywhere one looks, especially the urban areas – without exception. *Urbanisation* in our country, and indeed the wider developing world carries with it large populations typically living in high-density unplanned or inadequately planned areas where social services are grossly lacking.

The result is always the same: poor housing, which is unfit for habitation, lack of safe water supply, poorly managed waste, high population density and often the label "*cholera hot spot"*.

Sadly, our developmental agenda seemingly skips this category of our community ignoring the continued expansion of the unfit for habitation situation. Political rhetoric of discomfort to unsettle the masses largely relied upon for political mileage is not helping. These communities are considered highly only during political activities, obviously because of their numbers. Despite the pronouncements at the highest political realm to deal with and resolve the poor state of these communities, we have been slow to act. Outside political election activities, the only mention of these communities is exclusively in association with problems such as extreme poverty, lawless criminality and disease outbreaks. And when the outbreak hits, we scamper along to ensure that we limit the number of lives lost and sometimes we are even "surprised".

Zambia holds a clear agenda to control and finally eliminate cholera, visibly articulated in the Multisectoral Cholera Elimination Plan. The plan outlines the roles and responsibilities of each

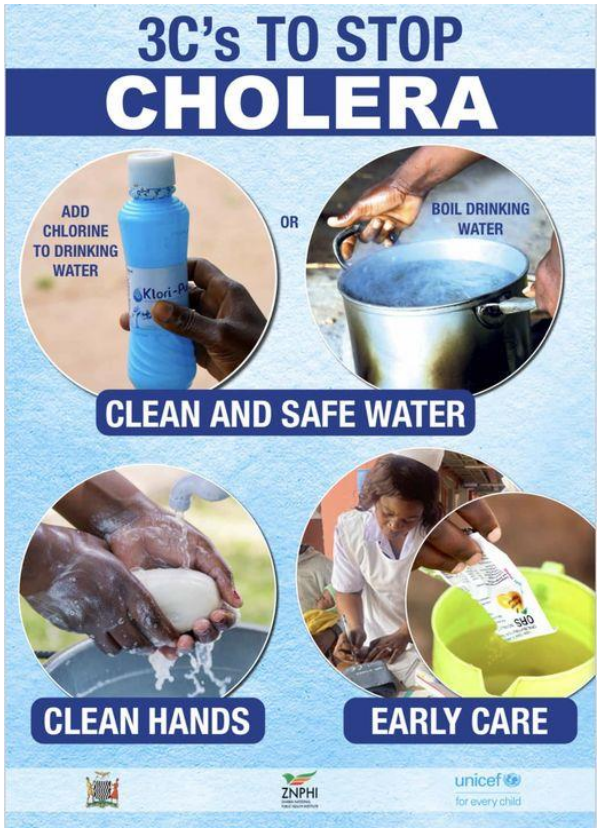
sector pushing a proactive approach and yet we are not paced adequately to implement the planned actions with "lack of resources" being the blame.

When people die in masses, resources to proportions one could never imagine possible - then cholera treatment centres are equipped, water bankers show up, trucks collecting garbage are seen, and excitement ensues when the uniformed men and women engage to clean up - become available. This approach has cost us more than it would, to have prevented the scourge.
The most affected communities on the other hand, somehow seem unable to understand that their living conditions predispose them to many public health dangers, among which cholera is just one. Despite community engagement activities focusing on health education, the adoption of the social and environmental public health measures remains a huge gap. The wanton behaviour generally oblivious to the reality that basic hygiene is the beginning of all personal, and indeed public health. Common beliefs which result in people dying at home are reminiscent of ancient history.

"In the 1840s, one of the prominent health theories of the time – the Miasma Theory – suggested that bad smells and bad air, especially at night, led to people contracting diseases like cholera and the Black Death.
It is really disturbing that in 2024, affected people of Zambia would attribute cholera in their homes as being due to **"*Chimpepo*" ("bad air").**

The reactive approach of bombardment of public places and water sources with intense chlorination, temporary supply of "imported" water by bowsers, forced burying of shallow wells, and in some cases a cholera vaccine, is not a sustainable strategy for public health.

The Zambia National Public Health Institute (ZNPHI) – your disease intelligence wing – is deeply concerned by the current drop in guard and return to business and politics as usual. The Call by His Excellency the President of the Republic of Zambia Mr Hakainde Hichilema, who is also the Global and SADC Champion for cholera elimination, to transition into medium- and long-term actions requires all Zambians to rally behind, get to work and sustain the cholera fight.
The **real fight against cholera** must be staged **NOW**. This requires consideration by all line Ministries and stakeholders who showed their contributions on camera to get into the serious work of addressing underlying factors that predispose communities to cholera. What is needed now is work on sustainable provision of safe water and meaningful sanitation in all these communities. In the "selfish interest" of public health, the ZNPHI here advocates that the Constituency Development Fund (CDF) be tweaked to largely address water and sanitation as a primary target. For what good will it be to construct buildings when there is no safe water to drink or a sanitary place to manage human excrement?



We must also put a stop to the rampant so-called residential development activities which hoodwink the public to own small pieces of land for housing without a thought to where the water will come from, and much less where the sewer will go. An end to these actions requires ONLY the will and stamina to stop what has not yet been done and will cost no money!

A clarion call is here made publicly that unless serious attention is given to these matters, we risk shutting down the country and economy because there will again be so much cholera – it's only a matter of time.

The tough work then must be how to deal with the already existing high-risk communities in unplanned peri-urban areas and fishing camps across the country.
The ZNPHI, while acknowledging the support rendered so far, calls on all stakeholders to recognise that a more deliberate effort in significantly moving the cholera control and elimination agenda is urgently needed. As a country, and individually, we need to make up our minds and work towards preventing cholera NOW! *If we do not, we must as well be preparing to again open Heroes Stadium, not for football matches, but for managing cholera cases*!

The control and eventual elimination of cholera requires a coordinated approach focused on prevention through water, sanitation, and hygiene (wash) interventions; vaccination campaigns; continuous surveillance and early detection; treatment and case management; health education and community engagement aimed at social behavioural change; strengthened coordination and collaboration.

Furthermore, cholera control and elimination necessitate that we address the underlying social determinants and vulnerability factors as well as support research initiatives focused on development of new tools, technologies, and strategies for cholera prevention, diagnosis, and treatment.

A clarion call is here made publicly that unless serious attention is given to these matters, we risk shutting down the country and economy because there will again be so much cholera – it's only a matter of time.

RESEARCH ARTICLE

Molecular Investigation of Bovine Leukemia Virus Infection in Wildlife in Zambia

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ABSTRACT

Bovine Leukemia Virus (BLV), a member of the genus *Deltaretroviruses* of the family *Retroviridae* is the etiological agent for Enzootic Bovine Leukosis (EBL), a disease of cattle. The most common manifestation of EBL is persistent lymphocytosis, which is characterized by a lymphocyte count above 7500/µL with about 5-10% of cases progressing to lymphosarcomas leading to death of infected animals. While epidemiological studies have shown that BLV infection is present in dairy and beef cattle in Zambia, there has been no studies to detect BLV in wildlife. In this study, 172 archived wildlife blood samples from Buffalo Buffalo *(Bubalus bubalis)*, Impala *(Aepyceros melampus)* and Hartebeest *(Alcelaphus buselaphus)* were tested for BLV proviral DNA. By nested PCR, BLV was detected in 5.7% of the total wildlife samples and 9.1% of the total Impala samples. Sequencing and phylogenetic analysis of the *env* gene revealed that, the strain detected in Impala belonged to genotype 4 and closely related to genotype 4 strains detected in cattle in Zambia, Russia and Poland. For the first time, this study demonstrated that BLV is present in impala in Zambia.

INTRODUCTION

Bovine Leukemia Virus (BLV) infection is associated with considerable economic losses in the cattle industry globally (Ma et al., 2016). BLV is the most prevalent oncogenic virus of cattle and is closely related to Human T Cell Leukemia Virus (HTLV-1) as these viruses share structural homologies (Willems et al., 2004).

Significant economic losses in BLV infected animals result from reduced milk production in dairy cattle and poor reproductive efficiency (USDA, 2008). Producers can further incur economic losses from death of infected animals, increased veterinary costs and the ineligibility to export beef, live cattle, semen and ova (APHIS, 2008). Consequently, Enzootic Bovine Leukosis (EBL) is listed by the World Organization for Animal Health (OIE) as a disease of importance to international trade (Kobayashi et al., 2010).

Studies have shown that BLV could infect wildlife within the *Bovidae* family. A study by Meas *et al* (2000) found 0.8% of water buffaloes to be positive for anti-BLV antibodies

in Pakistan, while De Oliveira *et al* (2016) found complete absence of BLV infection in buffaloes from the Amazon basin in Brazil. On the other hand, BLV was detected in 27.6% of swamp- and riverine-type water buffaloes in the Phillipines (Mingala et al. 2009). Moreover, BLV was recently detected (14.8-18.9% prevalence rates) in domestic yaks in China (Ma et al. 2016).

There is very little information on BLV prevalence in Africa (OIE, 2020). In Zambia, BLV infection has been reported in traditional and dairy cattle (Mundia et al., 2019; Pandey et al., 2016; Meas et al., 2004). Blood and lymph-node biopsy samples obtained from 2 three-year-old Holstein Friesian cows for clinical, hematological, pathological and molecular analyses confirmed existence of EBL in dairy cattle (Pandey et al., 2016). BLV was detected using PCR in traditional beef cattle with an estimated pooled prevalence of 2.1% (Mundia et al., 2019). Using serological surveillance, 5% of 262 analyzed sera were found positive for anti-BLV gp51 antibodies as detected by immunodiffusion test (Meas et al., 2004).

Various studies have shown that, risk factors associated with the widespread distribution of BLV genotypes within and between distant geographical locations may be driven by the spread of the virus through the movement of live animal populations because of human migration and animal domestication, as well as with viral transmission during close contact between individual animals (Polat et al., 2017; Kobayashi et al., 2010). Further, BLV is transmitted through blood of infected cattle, particularly during iatrogenic procedures such as the use of infected needles and the re-use of infected surgical examination gloves (Juliarena et al., 2017). Fomites such as restraint devices may also transmit the virus between adult animals. Transmission to calves may be transplacental, during delivery or ingestion of milk or colostrum with high viral loads Kobayashi et al., 2010; Spretcher et al., 1991).).

Hematophagous flies are also possible vectors in the transmission and spread of BLV (Juliarena et al., 2017).

While there has been studies confirming BLV infection in both traditional and dairy cattle, no attempts have been made to detect and characterize the virus from wildlife in Zambia. Surveillance of infectious diseases in wildlife is important for conservation purposes and prevention of disease transmission at the wildlife-domestic interface. This study was therefore aimed at establishing the existence and genetic characterization of BLV infection in wildlife in order to fully understand the epidemiology of BLV in Zambia.

METHODS

Study Design and Sample Collection

The study tested the presence of BLV in 172 archived wildlife samples of whole blood stored at -80^{o}C in the Virology Laboratory, Department of Disease Control, School of Veterinary Medicine at the University of Zambia. Whole blood samples were collected as described by Squarre et al., (2020) from Moi-oa-Tunya National Park in Livingstone in Southern province, Kafue National Park in Mumbwa Central province and Nchila game reserve in Ikelenge, North Western province between June and August 2016. Whole blood samples were collected by employing aseptic techniques using 5ml syringes and sterile 18G or 21G needles from jugular veins. Samples collected in EDTA tubes were stored in a portable refrigerator at 4^{o}C before being transported to the virology laboratory. The blood samples consisted 29 from Buffaloes *(Syncerus Caffer)*, 106 from Impala *(Aepyceros melampus)* and 37 from Hartebeest *(Alcelaphus buselaphus).* The 172 samples were pooled in 18 pools for molecular analysis.

DNA Extraction and Amplification of the *env* gene

DNA was extracted from all pooled blood samples using DNeasy® Blood and Tissue Kit (QIAGEN, Hilden Germany) following the manufacturers protocol. Nested PCR was used to amplify the BLV *env* gene using One Taq DNA Polymerase (Takara Bio Inc., Otsu, Japan). Two primer pairs were used, env5032/env5608r which amplify a 598bp fragment in the first round and *env*5099/*env*5521r which amplifies a 444bp fragment in the second round. PCR products were purified using a Wizard® SV Gel and PCR clean-up system kit (Promega, Wisconsin, USA) using the manufacturer's protocol. DNA sequencing was performed on 3500 series genetic analyzer. Editing was performed using Genetyx ATGC v12 while alignment was done using Clustal-X version 2.1.

Phylogenetic Analysis

Phylogenetic analysis was done using Molecular Evolutionary Genetics Analysis (MEGA), version 10.1. Phylogenetic trees were constructed using the maximum likelihood algorithm with the K2+G model of nucleotide substitution.

RESULTS.

Amplification of BLV *env* gene

Using nested PCR, a 444bp BLV *env* gene fragment was successfully amplified in pooled blood sample number 13 which contained blood samples from Impala. (Figure 3.1). This represented an overall estimated pooled prevalence of 5.7% and a 9.1% estimated pooled prevalence of the total Impala samples tested for BLV.

BLAST Analysis of the Amplified 444bp Gene Fragment

Analysis of the BLV amplified gene fragment using the Basic Local Alignment and Search Tool revealed that the *env* gene detected in Impala was 100% identical at nucleotide and amino acid level to a genotype 4 virus GeneBank accession number LC193462 detected in dairy cattle in Zambia in 2016.

Phylogenetic Analysis of BLV env Gene

The BLV *env* gene nucleotide sequence detected in impala in Zambia was aligned together with 48 other BLV reference sequences representing genotypes G1-G10. The impala strain clustered with other genotype 4 sequences of Zambian, Polish and Russian origin as shown in the phylogenetic tree in figure 3.2.

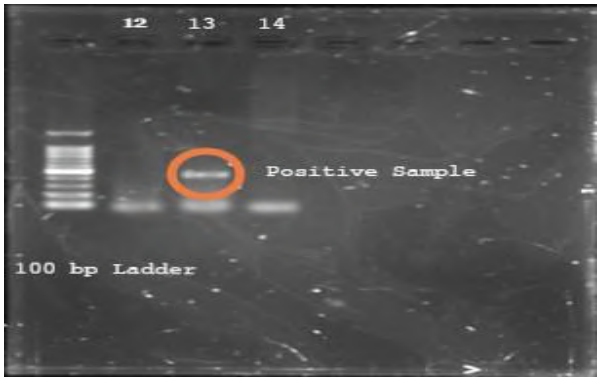

Figure 3.1 PCR Results viewed under UV light on Agarose Gel.

DISCUSSION

In this study, 18 pooled samples of whole blood from 3 wildlife species namely; Buffalo, Impala and Hartebeest were tested for BLV using PCR. Results showed that 9.1% of the total Impala samples were positive for BLV. The total samples positive for BLV in Impala represented an estimated pooled prevalence of 5.7% of the total wildlife samples tested for BLV *env* gene. These results provide evidence that BLV is present in Impala in Zambia.

While BLV is commonly a disease of cattle, studies have shown that BLV can also infect wildlife species of the *Bovidae* family including Buffalo, Yaks and Bison (Mingala et al, 2009; Wang et al, 2017; Taylor et al., 1997). In Zambia, BLV has only been previously detected in dairy and beef cattle (Mudia et al., 2019; Pandey et al., 2016; Meas et al., 2004). For the first time, this study demonstrated that BLV can infect and is present in Impala.

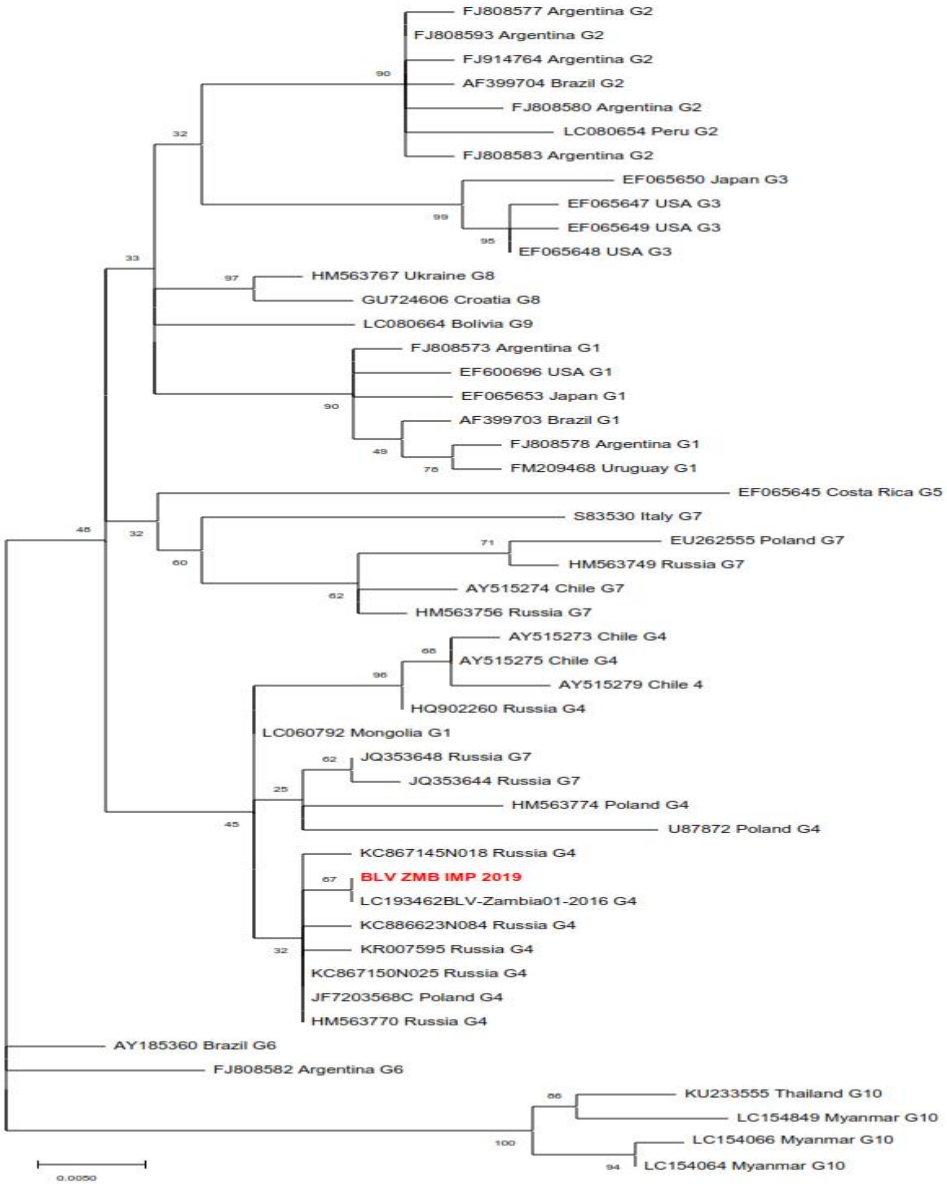

Figure 3.2 Phylogenetic relationships of nucleotide sequences of BLV env gene.

Phylogenetic relationships of nucleotide sequences of BLV *env* gene detected from Impala in Zambia. Evolutionary history was inferred using the maximum likelihood method and the K2+G parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.3694)). Phylogenetic analysis was performed in MEGA version 10.2. The BLV gene detected in this study is shown in red bold text. The reference sequences included in the analysis are shown by gene bank accession numbers, country of origin and genotype.

This is plausible as Impala also belongs to the *Bovidae* family for which BLV is known to infect. Although the results of this study were negative for BLV using PCR in all the 29 Buffalo samples tested, BLV was recently detected in Buffalo in Egypt with a seroprevalence of 10% (Selim et al., 2019). In the Philippines, a study based on blood samples from 445 water buffaloes from different places in the country found 27.6% prevalence for BLV infection (Mingala et al., 2009). Based on results from these studies, there is need to conduct Seroepidemiological studies to investigate the presence of antibodies against BLV in Zambian wildlife to fully understand the epidemiology of BLV in the country.

Interestingly, BLV genotype 4 which was detected in this study clustered with other genotype 4 viruses of Russian origin and aligned 100% with genotype 4 detected in dairy cattle in Central Province of Zambia (Pandey et al., 2016). It is important to note that the positive blood samples from Impala were collected from Kafue National Park in Mumbwa in Central Province.

While the study by Pandey et al (2016), suggests that BLV genotype 4 detected in dairy cattle in Central province may have been introduced in the country through importation of exotic cattle breeds from Europe and South Africa, this study seems to fuel the idea of interspecies transmission of BLV genotype 4 between wildlife and domestic cattle in the country. The movement of cattle between Provinces and Districts because of trade and the movement of wild animals in search of fresh water bodies and grazing land may contribute to the spread of BLV and interspecies transmission. This may also help to explain the detection of BLV genotype-4 in both cattle and wildlife.

Studies have shown that of the many species of game held in captivity in Zambia, Impala are the most abundant (Phiri et al., 2016). This has the possibility of influencing dynamics of disease spill over into domestic animals such as cattle. As the *env* gene of BLV genotype 4 detected in Impala was 100% similar at the nucleotide and amino acid level to that detected in dairy cattle in Zambia, it is possible that cattle-wildlife interaction may have resulted in interspecies transmission. Studies have shown that a bi-modal transmission of diseases between cattle and captive Impala in Zambia is possible (Nalubamba and Mudenda, 2012; Nalubamba et al., 2012). Captive impala may be kept in close range with cattle thereby creating opportunities for transmission.

At amino acid level, the BLV wildlife Impala strain had two Q to R substitutions observed. These substitutions were observed in the second neutralizing domain and the E-epitope region. The mutations observed in this study were similar to those reported by Pandey et al (2016). The substitutions observed in this study confirms that the *env* gene exhibits high levels of conservation among different strains (Mamoun et al., 1990). The regions of observed substitutions is consistent with other findings which have shown that most amino acid which have shown that most amino acid substitutions occur within epitopes rather than random locations in the surface subunit (Pluta et al., 2017). Mutations at amino acid level by substitutions are capable of influencing virulence and may result in BLV strains to circumvent immune response.

CONCLUSION

For the first time, this study demonstrated that BLV is present in impala in Zambia. With new information emerging from this study detecting BLV in Impala in Zambia, there is need to conduct more epidemiological studies in wildlife and cattle that should include molecular and serosurveys to better understand the epidemiology of BLV in the country. Such studies would help in the design of control strategies that could avert disease spill over from wildlife to domestic animals.

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Q1 **Public Health Bulletin** 1st Jan – 31st March

Summary of outbreaks

Cholera

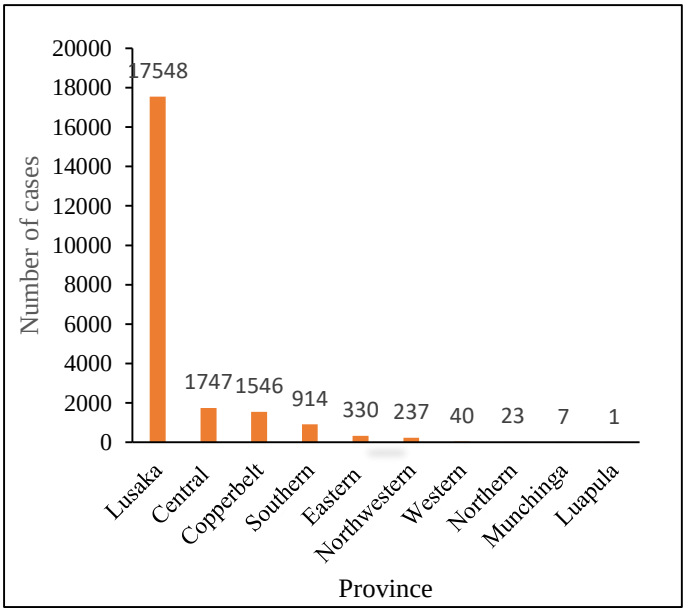

Figure 1 Cumulative Cholera cases per province as at 31st March, 2024

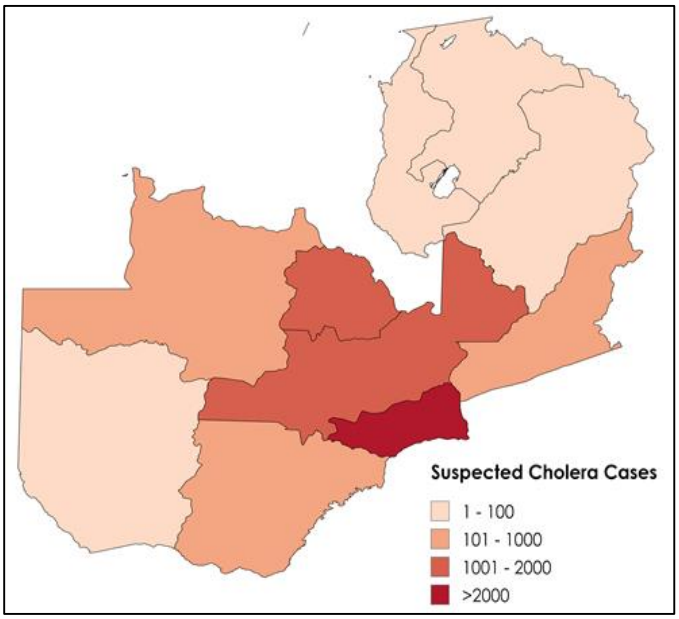

Figure 2 Map showing distribution of cholera cases per province

Since October 2023, the cholera epidemic in Zambia has been progressing, with cases reported across all 10 provinces and spanning 71 districts. In the first quarter of 2024, a total of 19,202 suspected cholera cases were recorded, bringing the cumulative total to 22,481 cases. This was a dramatic increase from the 2269 suspected cases reported in the fourth quarter of 2023. Lusaka Province reported the highest number of cumulative cases (17,548), followed by Central Province (1,747) and Copperbelt Province (1,546). A total of 724 deaths were reported with a case fatality rate (CFR) of 3.24%. Initial analysis indicated that more deaths occurred among males (57.1%) compared to females (42.9%), and notably, 59% of deaths occurred within the community. A total of 21,700 recoveries have been reported. See cholera situational reports here for more information.

Typhoid

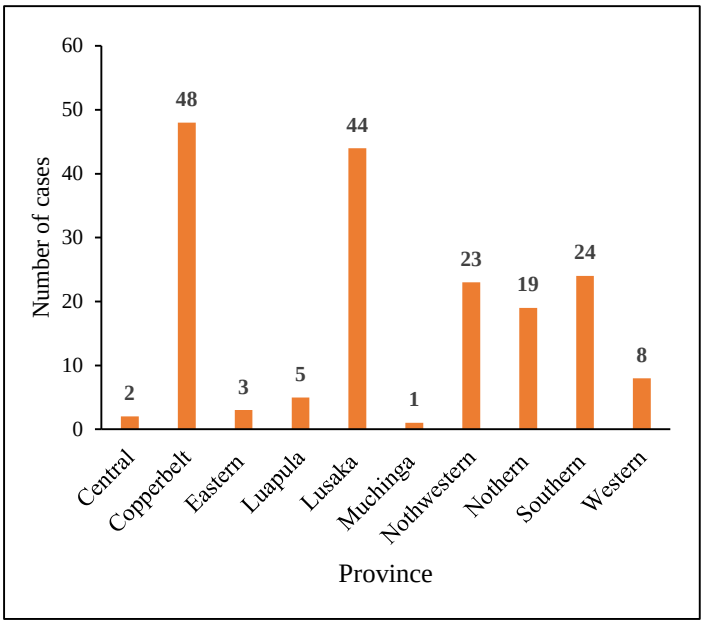

Figure 3 Quarter 1 Typhoid suspected cases per province

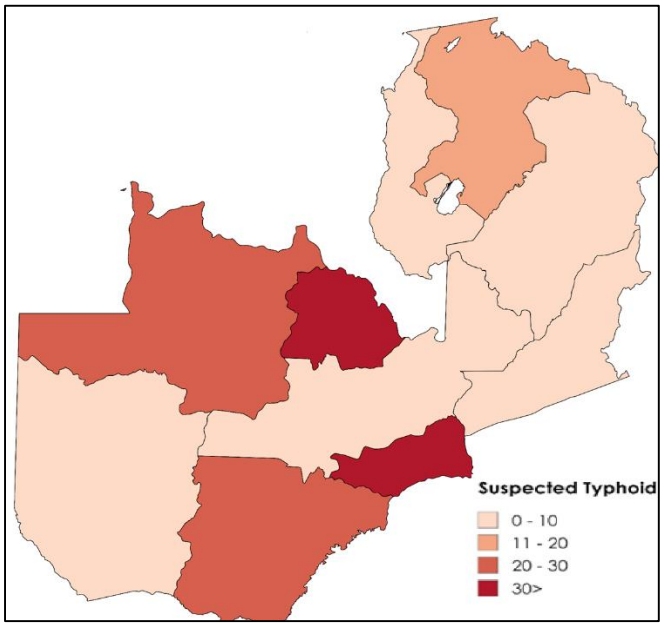

Figure 4 Map showing distribution of Typhoid cases per province

A total of 194 suspected typhoid cases were recorded in the first three months of 2024. Lusaka (48) and Copperbelt (44) provinces reported the highest numbers of suspected cases. The lowest numbers were recorded in Muchinga (1), Central (2), and Eastern provinces. Recent data from the first quarter of 2024 raises concerns about a potential resurgence of the disease in the country.

To effectively combat this potential rise in typhoid fever, a multi-pronged approach is crucial. Heightened surveillance systems are the first line of defense. Additionally, there is a need for improved case reporting, laboratory diagnostics, and outbreak investigation capabilities, including the early detection of antimicrobial resistance (AMR) among organisms.

Maternal Mortality

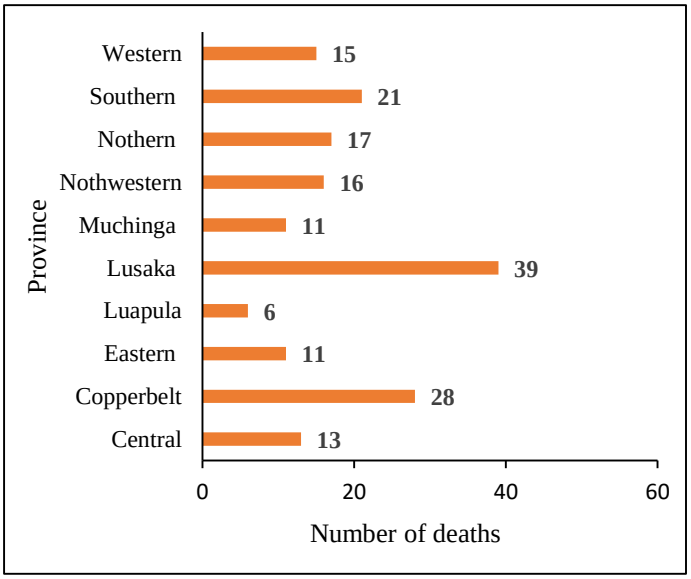

Figure 6 Quarter 1 reported maternal deaths per province

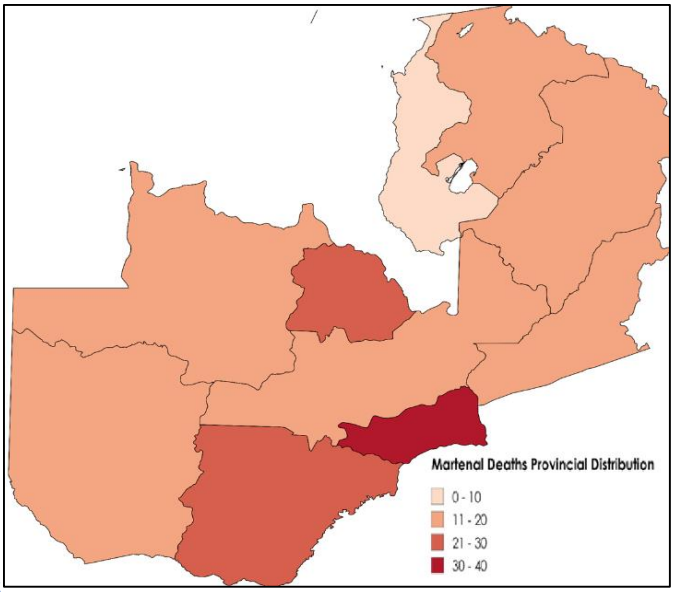

Figure 5 Map showing distribution of maternal deaths per province

Despite significant progress in combating maternal mortality, Zambia continues to record deaths. In the first quarter of 2024, a total of 177 maternal deaths were reported, an increase from the 162 deaths reported in the fourth quarter of 2023. Lusaka province had the highest number of deaths with 39, followed by Copperbelt Province (28) and Southern province (21). Conversely, Luapula province with 6 deaths, recorded the least deaths.

The leading cause of maternal mortalities in Q1 2024 continues to be obstetric hemorrhage, highlighting the critical need for more available blood products in labor wards across the country. Educating communities about antenatal care, recognizing danger signs during pregnancy, and ensuring skilled birth attendance can empower women and families to make informed healthcare decisions. By implementing these strategies, investigating the Q1 2024 deaths in detail, and consistently analyzing data, Zambia can strive towards a future where childbirth is safer for all mothers.

Acute Flaccid Paralysis

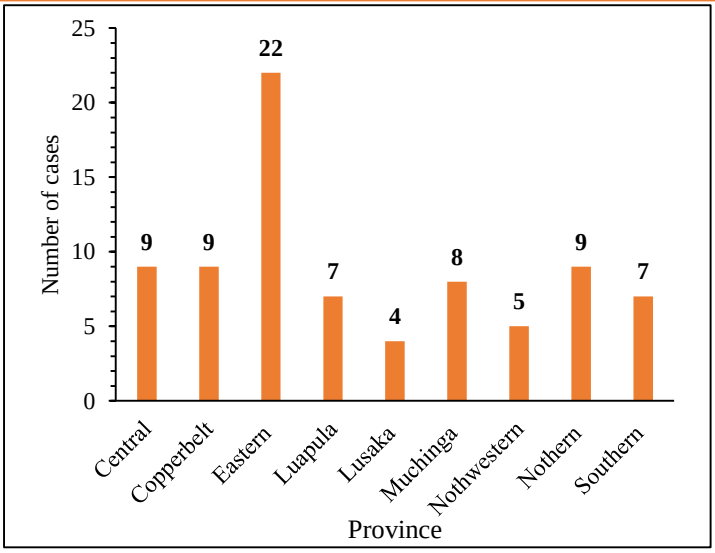

Figure 7 Quarter 1 suspected AFP cases per province

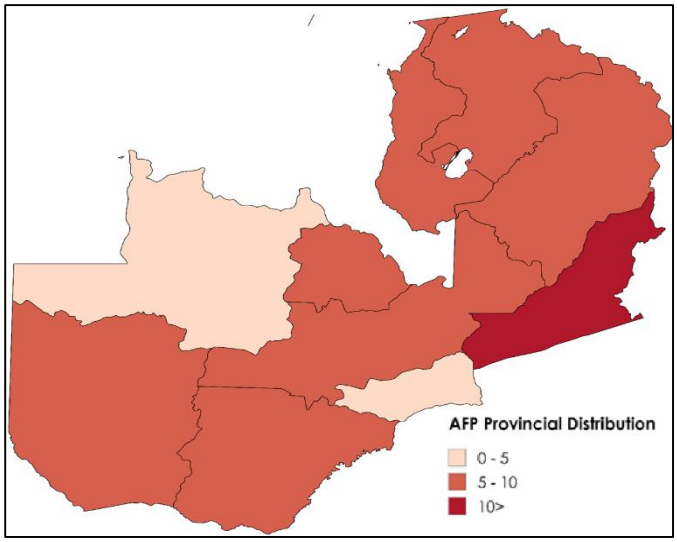

Figure 8 Map showing provincial distribution of AFP cases

AFP surveillance is a key indicator in polio eradication efforts. A total of 133 Acute Flaccid Paralysis (AFP) cases were reported across all 10 provinces in the first quarter of 2024. Eastern Province reported the highest number of AFP cases with 22, while Lusaka and Northwestern provinces reported the lowest numbers, with 4 and 5 cases respectively. The 133 suspected AFP cases reported in Q1 2024 represent an increase from the 110 cases recorded in Q4 2023. Given the geographic distribution of AFP cases, strengthening polio surveillance and laboratory testing in Eastern and Lusaka provinces is critical.

Anthrax

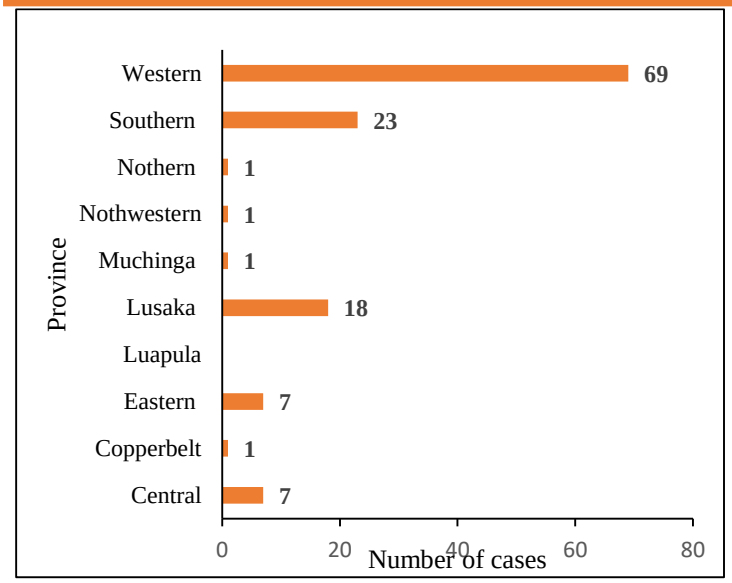

Figure 10 Quarter 1 suspected AFP cases per province

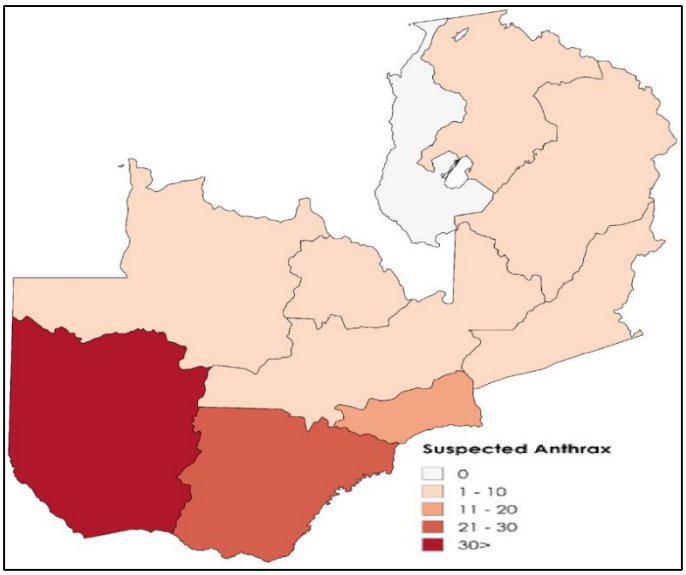

Figure 9 Map showing distribution of suspected Anthrax case per provinces

A total of 123 suspected anthrax cases were reported across nine provinces, excluding Luapula. Western province with 69 cases recorded the highest, followed by Southern (23) and Lusaka (18). There was significant decrease in reported cases from 402 reported in the fourth quarter of 2023.

The resurgence of anthrax underscores the need for heightened public awareness and education. Public health authorities should focus on educating communities about identifying sick animals and avoiding contact with carcasses. Additionally, safe handling and consumption of animal products, along with the importance of immediate reporting of suspected cases to healthcare providers, are crucial elements of public education.

Summary Report Priority Diseases, Conditions and Events

Disease/Event/Condition	Week 1 - 13		
	Suspected	Tested	Confirmed
COVID-19	29,049	27,317	4,510
HIV	676,181	648,107	16,142
Malaria	3,432,811	3,276,015	1,767,624
Non Bloody diarrhea	265,637	11,086	0
Maternal Deaths	178	0	178
Influenza	1,231	1,214	156
Dysentery	13,635	732	171
AFP	133	133	0
Cholera	19,202	3,818	40
Meningitis (Neisseria)	72	33	13
Measles	2,514	185	71
Scabies	23,001	0	0
Mumps	9,727	0	0