ANNUAL SCIENTIFIC REPORT 2023

ROAYL CENTER FOR DISEASE CONTROL

MINISTRY OF HEALTH

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I. National Notifiable Diseases Surveillance through NEWARS, 2023

National Disease Surveillance & Epidemiology Unit, RCDC

1. Background:

Royal Centers for Disease Control (RCDC) has been a focal point for National Disease Surveillance and Outbreak investigation since 2009. RCDC, thus coordinating and conducting national notifiable disease surveillance (NNDS) since 2010. In 2014, the NNDS was revised to incorporate event-based surveillance (EBS) to supplement the indicator-based surveillance (IBS). The revised NNDS was renamed as National Early Warning Alert and Response Surveillance (NEWARS). The disease surveillance and response system for infectious diseases or syndromes of public health concern aims to detect and alert potential threats and contain the disease outbreak effectively and efficiently. It is a real-time reporting system through web-based and mobile Short Message Service (SMS).

The NEWARS surveillance is augmented by additional sentinel surveillance that focus on the collection of more information on specific diseases and their etiologic agents including risk factors. These systems are: Influenza-like illness (ILI) and severe acute respiratory infections (SARI); food-borne disease sentinel surveillance; acute un-differentiated febrile illness (AUFI); national diarrheal diseases surveillance; and measles and rubella surveillance.

NEWARS consists of indicator-based surveillance (IBS), immediate reporting and event-based surveillance (EBS).

- Indicator-based surveillance: IBS involves weekly reporting of cases and deaths of national priority diseases /syndromes and conditions as per pre-defined case definitions. The aggregated data is reported weekly through a web-based online reporting system or SMS through mobile phones.

- Immediate reporting: Immediately reporting any diseases/syndromes that are of major public health importance as a single case may lead to an outbreak or widespread transmission and requires immediate action.

- Event-based surveillance is ad-hoc. It involves immediate reporting of any 'unusual' events such as the unusual cluster of cases or suspected outbreak/events that are of potential risk to public health and require investigation.

The data obtained is analyzed, interpreted, and disseminated by the disease surveillance and epidemiology unit of RCDC at different levels. This report publishes recent surveillance data on diseases of public health significance with the aim of providing health centers, government agencies, and the general public with important statistical information on national notifiable diseases and conditions.

Objectives:

1. Early warning to prevent or minimize morbidity and mortality through:
    - Monitoring trends of endemic diseases
    - Detecting outbreaks/events in a timely manner
    - Providing an adequate and timely response
2. Monitor the effectiveness of control programs through:
    - Estimating the burden of different diseases in the country
    - Identifying risk groups in the community
    - Evaluating control interventions implemented by a health program
    - Prioritizing and mobilizing resources
3. Stimulate research on public health priority diseases or syndromes

2. Methodology

The NEWARS is nationwide surveillance, all the health centers across the country serving as surveillance sites. Notifiable diseases are reported from 290 health centers across the country. The NEWARS system uses series of steps for collecting, collating, verifying, and responding to diseases and events of public health concern. Currently, there are 11 weekly and 16 immediately notifiable diseases or syndromes (**Table 1).**

Table 1: List of Notifiable Diseases/ Syndromes in NEWARS

List of Notifiable diseases/Syndrome in NEWARS			
SL. No	Notifiable diseases/Disease/Syndrome	Weekly Notifiable	Immediately Notifiable
1	Acute Bloody Diarrhea	√	X
2	Acute Watery Diarrhea	√	X
3	Acute Jaundice Syndrome	√	X
4	Acute Respiratory Infection	√	X
5	Mumps	√	X
6	Fever with Rash	√	X
7	Food poisoning	√	X
8	Dengue Fever	√	X
9	Typhoid /Paratyphoid /Enteric Fever	√	X
10	Severe Acute Respiratory Infection	√	X
11	Rickettsioses	√	X
12	Anthrax (Suspected)	X	√
13	Acute Flaccid Paralysis (Suspected Poliomyelitis)	X	√
14	Acute Hemorrhagic Fever Syndrome (Suspected)	X	√
15	Avian Influenza (Suspected)	X	√
16	Bacterial Meningitis (Suspected)	X	√
17	Cholera (Suspected)	X	√
18	Malaria (Microscopy/RDT Confirmed)	X	√
19	Measles/Rubella (Suspected)	X	√
20	Pertussis (Suspected)	X	√
21	Congenital Rubella Syndrome (Suspected)	X	√
22	Rabies (Human)(Suspected)	X	√
23	Severe Dengue Fever (Suspected)	X	√
24	Neonatal Tetanus (Suspected)	X	√
25	Diphtheria (Suspected)	X	√
26	Acute Encephalitis Syndrome (Suspected)	X	√
27	Monkey-pox (suspected)	X	√

Weekly notifiable disease

To effectively monitor and respond to diseases of public health concern, 11 national priority diseases/syndromes have been identified for weekly reporting. This process involves the surveillance focal point of the health center reviewing and collating daily reports from various data sources, including the Outpatient Department (OPD) register, Inpatient Department (IPD), register of Maternal and Child Health (MCH) unit, Laboratory unit, and the emergency registers. The National Diseases Surveillance and Epidemiology unit (NDSAE) then verifies the report to ensure accuracy, completeness, and correctness for further analysis.

Immediately notifiable diseases

An efficient system has been established to monitor and respond to diseases that require immediate notification. There are 16 immediately notifiable diseases/syndromes, and any one of them has the potential to cause an outbreak or widespread transmission. When a clinician/nurse detects one of these diseases, they must report it immediately. The surveillance officer at NDSAE then verifies the information and provides recommendations. Upon verification, the health worker is then required to complete a case investigation form (CIF) for the disease and send appropriate samples to RCDC if applicable. If no cases are detected, the surveillance focal updates the Zero report on a weekly basis.

Event Reporting

Any health event that poses a potential risk to public health can be reported by health professionals through web-based or SMS reporting. The informal events report can be reported by the general public, social media, community leaders, and non-governmental organizations. The reports are verified and risks are assessed by RCDC. Response for action is recommended accordingly. Events related to the following are to be reported:

1) Unusual cluster of diseases or syndromes

2) Unusual cluster of death

3) Disease or deaths in animals

4) Contaminated food and food products

5) Environment hazards such as chemicals and radio-nuclear

The flow of information for weekly and immediate reporting is shown in **Figure 1**. The roles and responsibilities of various healthcare workers are detailed in the NEWARS guideline.

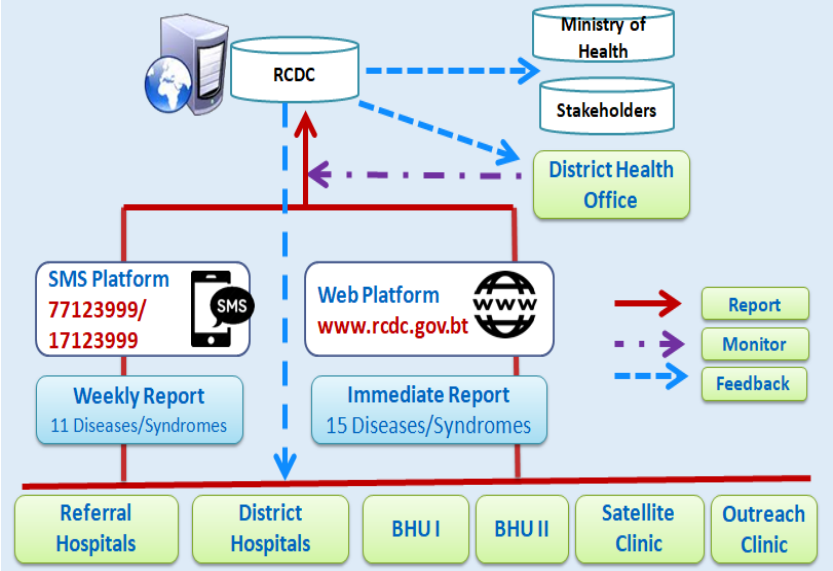

Figure 1: Flowchart for reporting weekly and immediately notifiable diseases (source: NEWARS guideline, RCDC)

Data Analysis

The data from the NEWARS system are exported in Excel and analyzed. The top three common diseases are described by time, person, and place. The QGIS software 3.16 was used to generate the data by place. The feedback is provided through monthly epidemiology reports and quarterly RCDC bulletin to all the stakeholders by uploading to RCDC Website (https://www.rcdc.gov.bt/web/) . Reports are being shared also through email and hard copy.

3. Results

In 2023, 82.0% of the health centers reported on time, 12.0% had delayed reporting and 6.0% did not submit a weekly report. The timeliness of reporting had significantly increased in 2023 compared with 2022 (82.0% vs. 61.0%) (**Figures 2 & 3).**

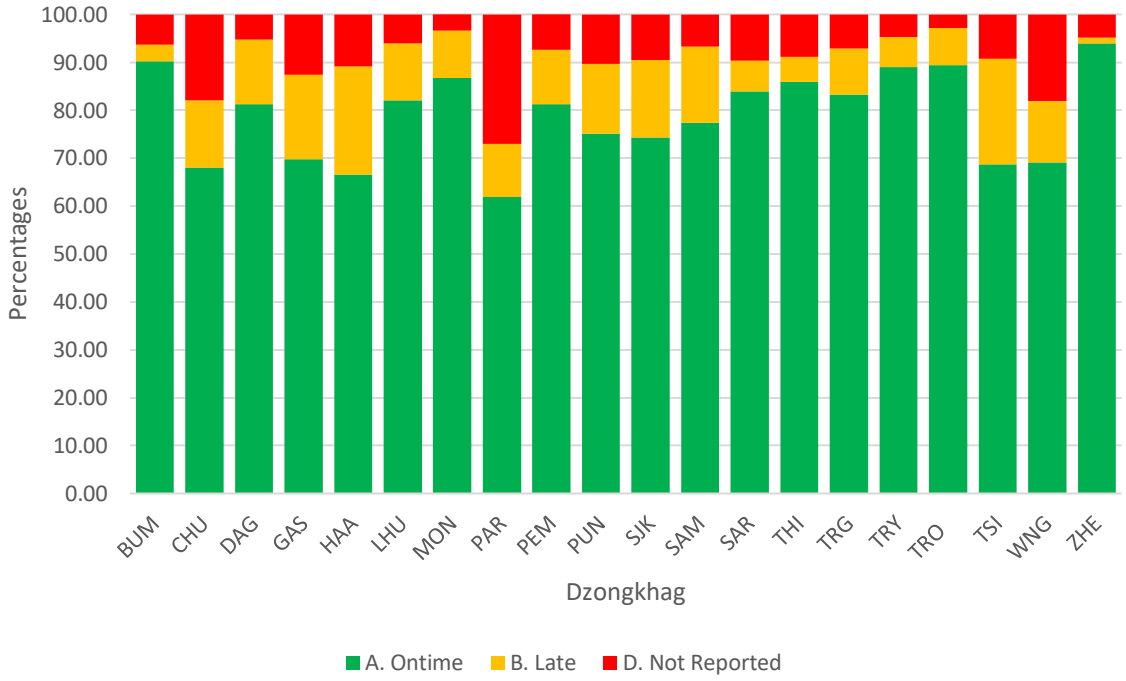

Figure 2: Status of weekly notifiable disease reporting by district 2023

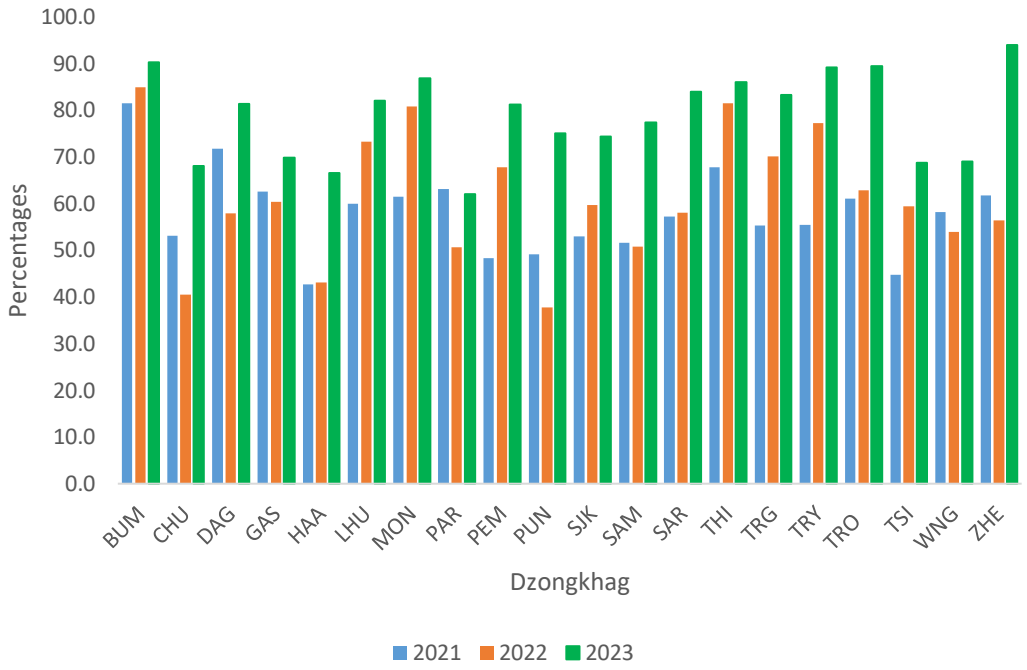

Figure 3 Timely reporting of weekly notifiable disease 2021-2023

Weekly Notifiable Diseases

In 2023, the weekly reporting system recorded 219518 notified cases. Of the 11 weekly reportable diseases/syndromes, respiratory infections and acute diarrheal diseases were the most frequently reported, accounting for 85.0% (n=186633) and 13.0% (n=29195) of notified cases respectively (**Table 2).**

Table 2: Notifiable diseases/syndromes reported by Dzongkhags 2023

Dzongkhag	ABD	AWD	AJS	ARI	DGF	MUM	FWR	FDP	TPF	SAR	RKS
BUM	15	410	15	3118	0	0	3	0	0	20	0
CHU	238	2301	28	18839	246	10	212	35	48	259	55
DAG	100	1557	50	10502	135	6	19	7	16	101	82
GAS	5	230	2	303	0	0	0	0	0	1	0
HAA	11	431	0	1958	1	1	2	23	2	13	1
LHU	51	384	21	4158	32	0	0	2	2	1	0
MON	262	1426	24	9642	9	0	15	26	2	138	24
PAR	187	1397	12	4402	0	2	5	0	0	1	0
PEM	38	943	6	6372	7	2	0	6	21	19	44
PUN	171	1209	27	4626	1	5	46	5	0	99	0
SJK	125	1470	28	12386	6	2	22	62	2	323	3
SAM	98	2282	63	14939	19	6	87	13	1	222	8
SAR	151	2387	37	21096	54	14	242	5	24	698	67
THI	201	3582	2	27630	84	86	41	3	1	465	0
TRG	153	1950	74	6195	4	1	160	30	57	245	40
TRY	97	939	27	5230	0	2	12	12	0	61	13
TRO	39	912	34	6750	2	3	34	6	0	32	1
TSI	83	545	71	4521	11	0	4	1	52	128	0
WNG	319	1915	72	12447	15	28	152	12	1	116	3
ZHE	69	482	19	8428	8	4	5	12	34	24	46
Total	**2419**	**26776**	**612**	**183667**	**634**	**172**	**1061**	**260**	**264**	**2966**	**387**

Abbreviations: ABD (Acute Bloody Diarrhea), AWD (Acute Watery Diarrhea), AJS (Acute Jaundice Syndrome), ARI (Acute Respiratory Infection), MUM (Mumps), FWR (Fever with Rash), FDP (Food borne Illness), TPF (Typhoid/Paratyphoid fever), SARI (Severe Acute Respiratory Infection), RKS (Rickettsioses).

Descriptive analysis of commonly reported notifiable diseases

Respiratory illnesses

A total of 186,633 cases of respiratory illness were reported (ARI-98.4% and SARI-1.6%) in 2023. The trend of ARI was higher than that of the median of the last three years 2020-2022 (**Figures 4A & 4B**).

The most commonly affected age group for ARI & SARI were pediatric age of ≤ 5 years respectively (**Figure 4C**). By district, Thimphu, Chhukha Samtse, and Sarpang reported the maximum number of ARI & SARI cases **(Figure 4D).**

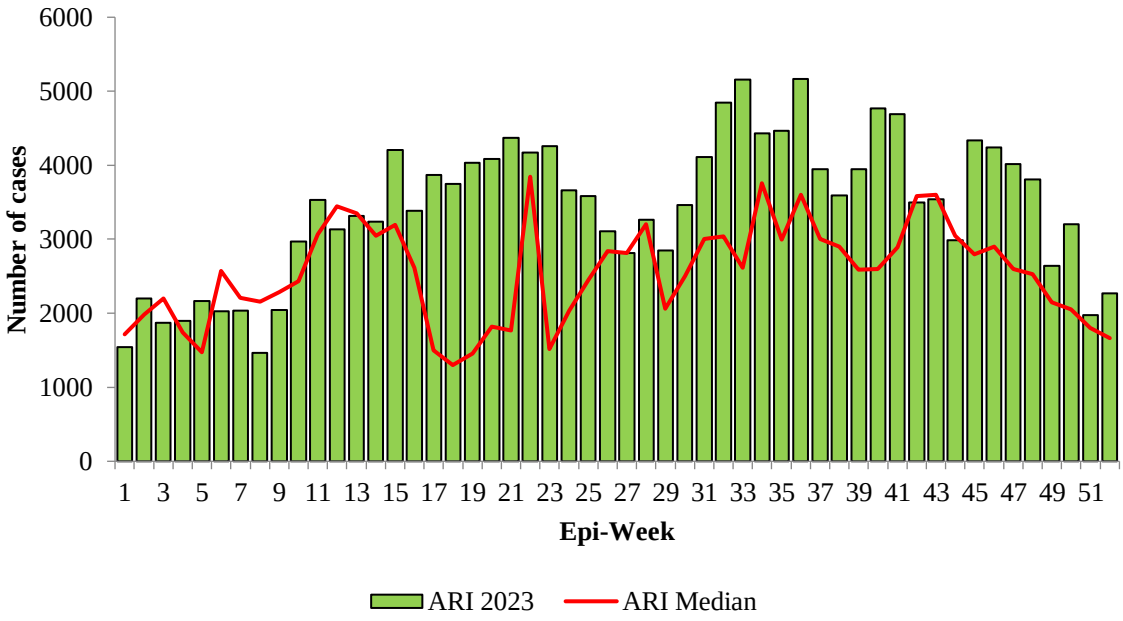

Figure 4A: Incidence of Respiratory illness by epidemiological week 2023

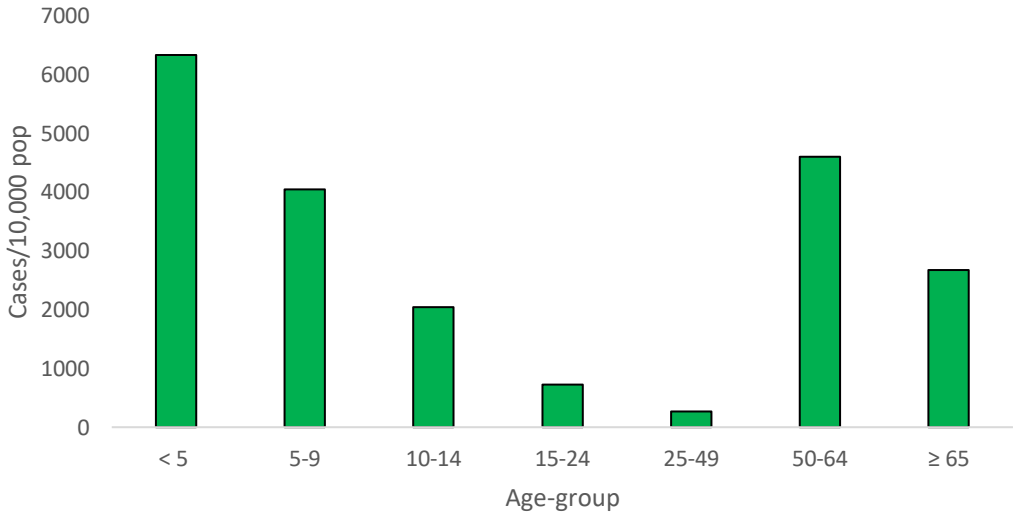

Figure 4B: Distribution of cases of Respiratory illness by age group

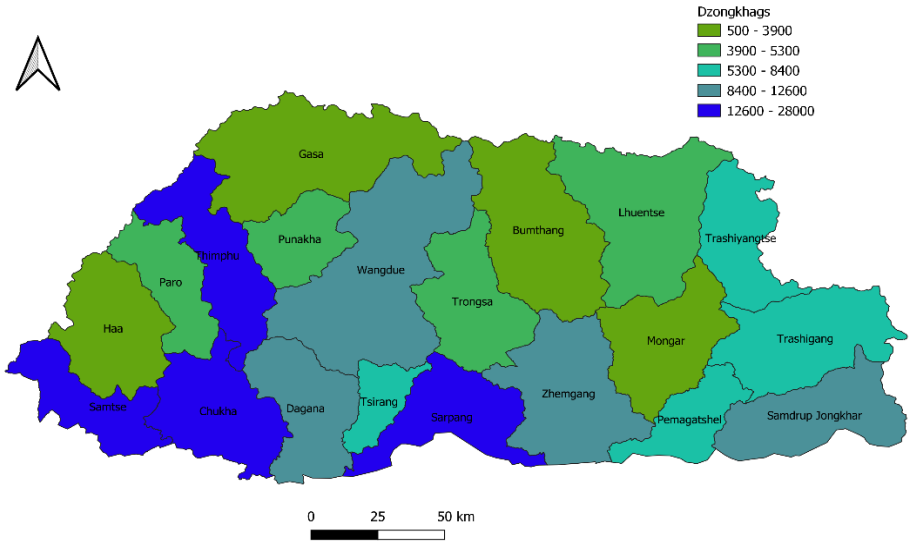

Figure 4C: Distribution of cases of Respiratory illness by district

Diarrheal diseases

The second highest incidence of reportable diseases was diarrheal diseases. A total of 24656 cases (AWD-91.4% & ABD-8.6%) were reported in 2023. The trend of AWD was in consistence with that of the median of the last three years (**Figure 5A**). The most commonly affected age-group for AWD and ABD was below nine year-old (**Figure 5B**). By district, Thimphu, Chhukha & Sarpand reported the maximum number of AWD **(Figure 5C).**

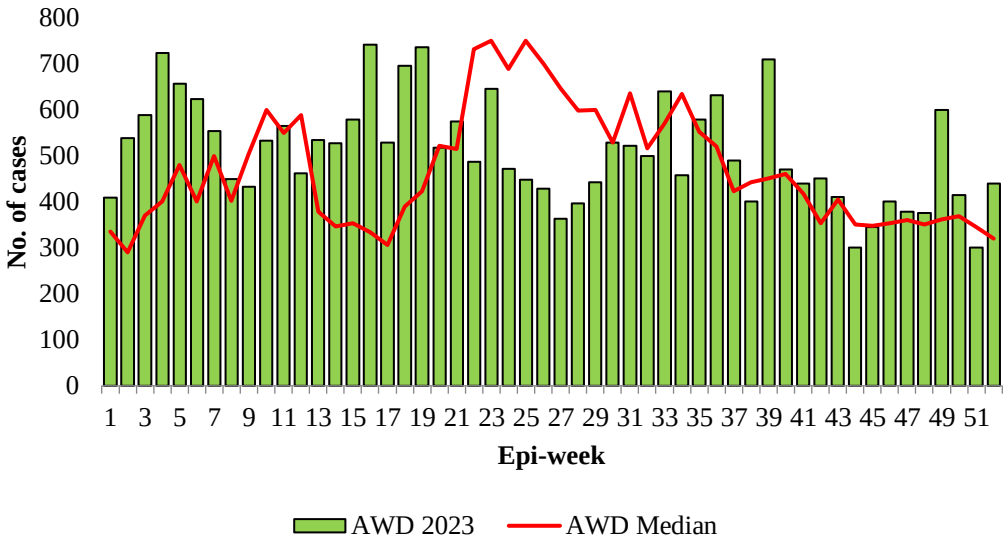

Figure 5A: Incidence of acute watery diarrhea diseases by epidemiological week, 2023

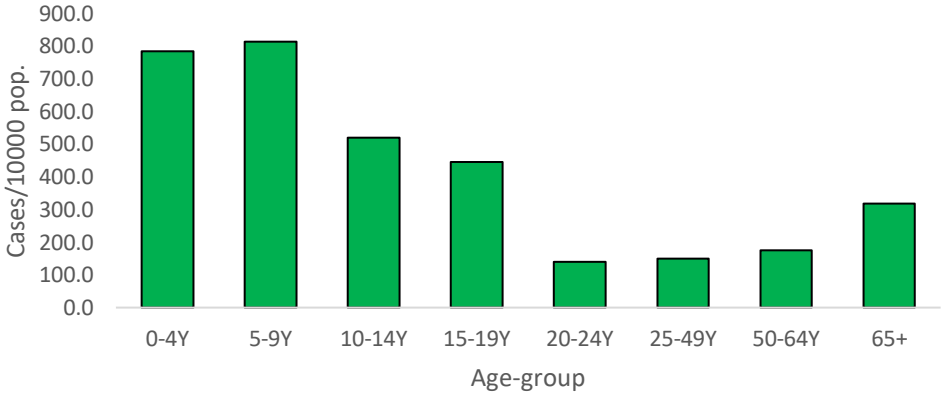

Figure 5B: Distribution of diarrheal diseases by age group

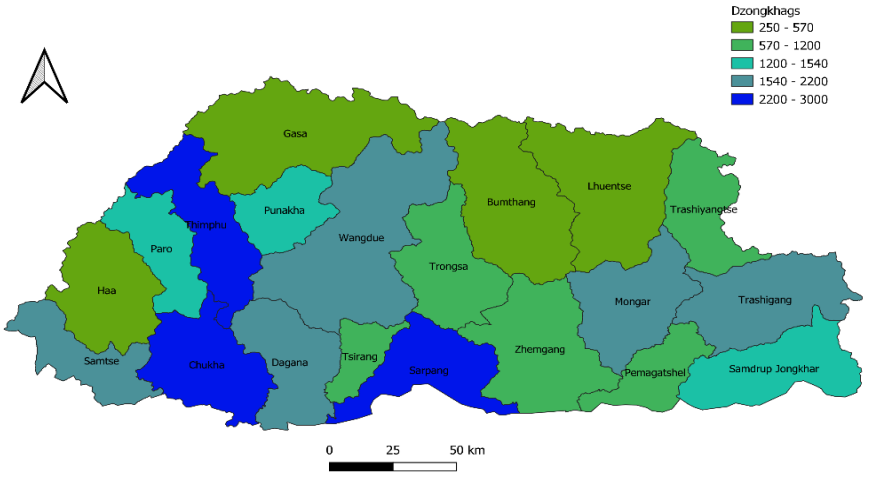

Figure 5C: Distribution of cases of diarrheal diseases by Dzongkhag

Fever with Rashes syndrome:

A total of 1061 cases of fever with rash (FWR) syndrome were reported in the year (**Figure 6A**). The trend of FWR was found higher compared with the previous year. The majority of FWR cases occurred among children 5-9 years (**Figure 6B**). By district Chhukha, Paro and Samdrup Jongkhar have reported the highest number of cases (**Figure 6C**). Other weekly reportable diseases/syndromes reported in 2023 were Mumps (260), Typhoid fever (285), acute jaundice syndrome (501) and Rickettsiosis (114). All the diseases/syndrome that are in the weekly notifiable list were detected by the health centers.

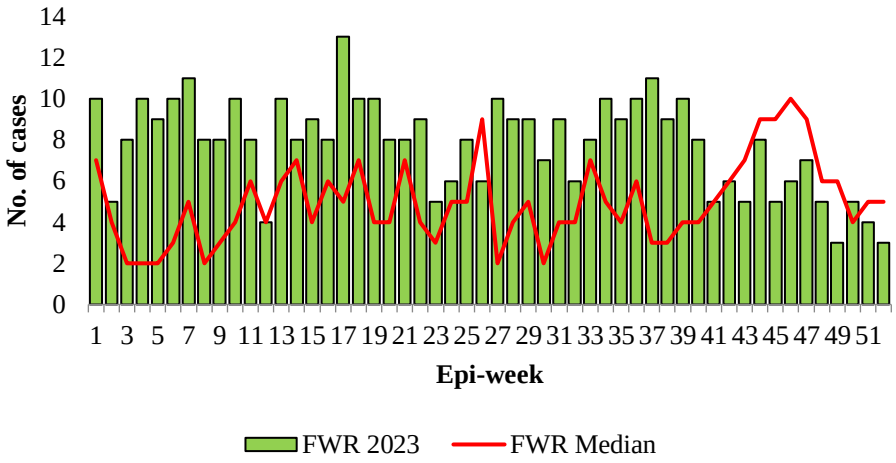

Figure 6A: Incidence of Fever with rashes by Epi-week, 2023

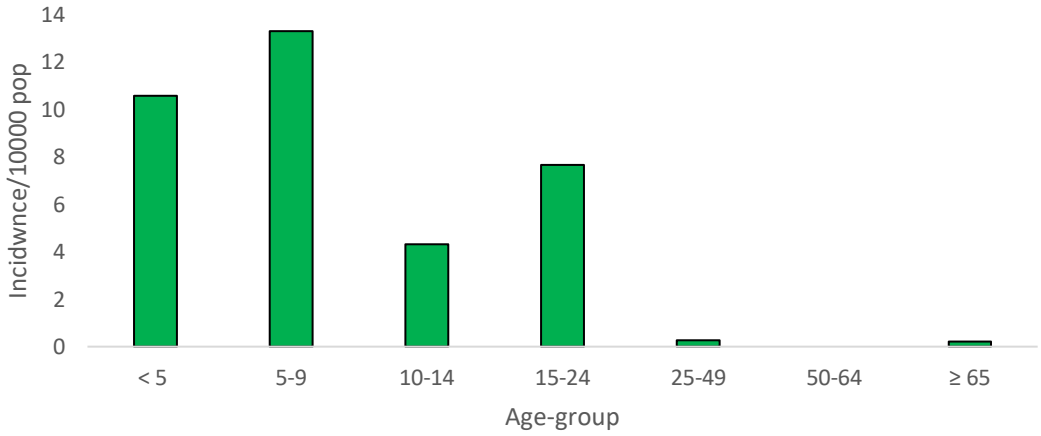

Figure 6B: Distribution of Fever with rashes by age-group

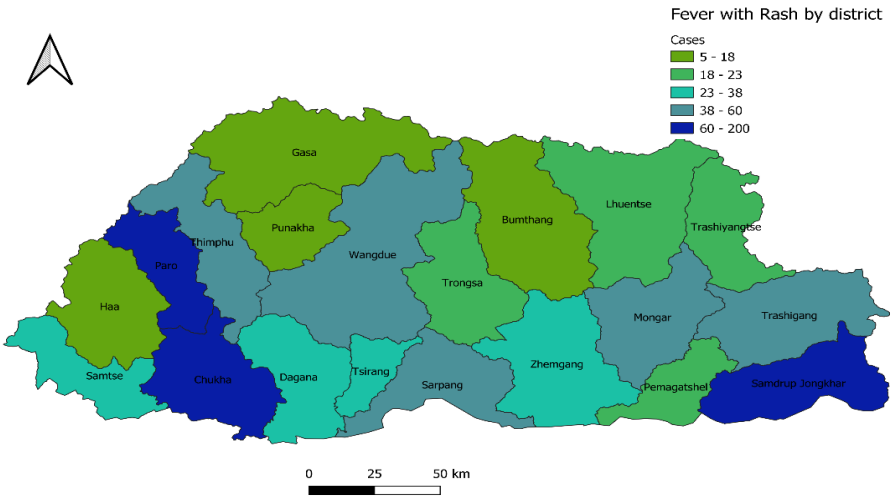

Figure 6C: Distribution of Fever with rashes by Dzongkhag

Immediately Notifiable Diseases

There are 16 diseases or syndromes that require immediate notification. Of these 10 were detected by the reporting centers in 2023 **(Figure 7)**. Suspected measles, Dengue fever, and bacterial meningitis, Malaria and pertussis were the most commonly immediately notifiable diseases or syndromes. Only Sarpang reported malaria cases, while the other districts had none. Three suspected cases of Acute Flaccid Paralysis (AFP) were reported to NEWARS but tested negative for poliovirus at a reference laboratory in Thailand.

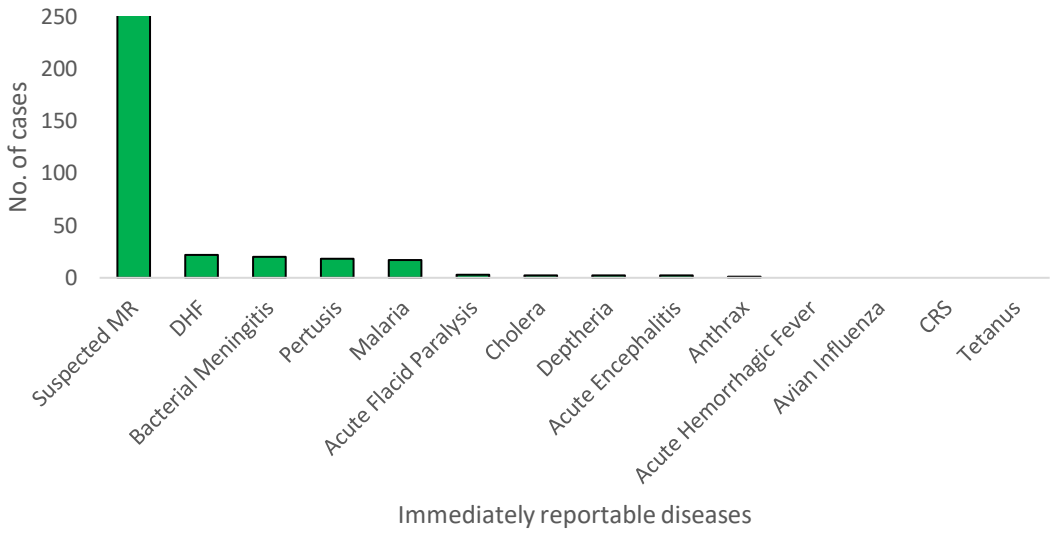

Figure 7: Number of immediately reportable cases reported in 2023

Events

During the year, a total of 117 disease events/outbreaks were reported. Among them 26 were the outbreak of Influenza-like Illness (ILI), 54 of conjunctivitis, nine were of Glossitis, Five were of Acute-gastroenteritis (AGE), 13 Chicken pox, One rabies, one dengue, and rest others**.** All outbreaks were responded to by the respective health centers and the District Health Rapid Response Team (DHRRT) upon the recommendations of the RCDC. The brief description of key events are described as follows.

Description of Key events

Influenza-like Illness:

A cluster of ILI cases in a particular locality at a given point of time is defined as an outbreak of influenza-like illness. ILI outbreaks were reported from Health Centers to RCDC through the NEWARSIS online system. Representative samples of 5-10 throat swabs from clinically symptomatic suspected cases were collected and shipped to RCDC or nearby COVID-19 testing laboratories for Influenza confirmation by RT-PCR assay.

In 2023, a total of 29 ILI events/outbreaks of Influenza-like Illness were reported. The cumulative number of cases affected was about 2500 with a majority being students. The cases were reported mostly from schools. The most outbreaks occurred in August (12), May (4), September (4) and October (4). The highest number of cases were reported from Sherubtse College and the community (732 cases), followed by Kuenzaling Central School (161 cases) (**Figure 8**). No mortality related with ILI had been reported.

A total of 222 ARI outbreak samples were received by National Influenza Centre (NIC) for confirmation, RCDC. All samples were tested for Influenza and SARS-CoV-2 and results were disseminated to respective health centers within 24 hours after testing. Almost all the outbreak samples were positive for Influenza subtype Flu A/H3 (31.5%), Flu/H1pdm09 (6.2%), Flu B/Vic (13.8%), and 2.3% were positive for SARS-CoV-2 **(Table 3**). A significant decrease in the number of ILI outbreak cases was observed, as compared to those reported in 2022 (32 vs.17). During this reporting period, all most all events were reported from Schools in 13 dzongkhag.

Table 3: ARI outbreak samples received and RT-PCR result

Health centers/ Community/ School	Flu A/H3	Flu A/ Pdm09	Flu A/ Not Subtyped	Flu B/ Victoria	Flu B/Lineage Not Determined	SARS-CoV-2	Neg	Grand Total
Dewathang Hospital				9			1	10
Gelephu CRRH	4						11	15
Gyalposhing BHU I		2		5		1	3	10
Haa Hospital	2						8	10
Kanglung BHU I			5					5
Khamdang BHU I			9				1	10
Lhamoyzingkha BHU I		6					4	10
Paro Hospital							5	5
Punakha Hospital	10			1	2	2	21	34
Samdrup Jongkhar Hospital				1				1
Silambi Subpost	9						4	13
Trashi Yangtse Hospital			11			1	2	13
Trashigang Hospital			30		6	1	28	64
Yebilaptsa Hospital	9			2			1	12
Zhemgang BHU I	7						3	10
Grand Total	**41**	**8**	**55**	**18**	**8**	**5**	**92**	**222**

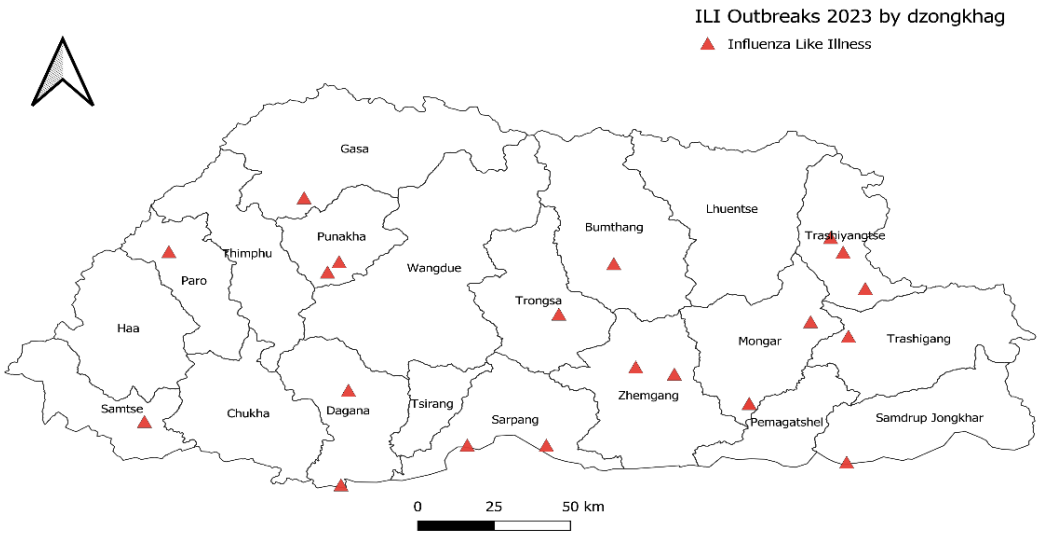

Figure 8: Distribution of Influenza-like Illness outbreak by Dzongkhag

The outbreaks were investigated by the respective Local Health centers or District Health Rapid Response Team (DHRRT) as per the "Disease Outbreak Investigation & Control Manual, First Edition 2015".

Glossitis:

A total of nine glossitis outbreaks were reported, the cumulative number of person affected was around 500: almost all were students from the different boarding schools. The samples could not be tested because of lack of reagents. There was no any complication or mortality following the outbreaks.

An increased in the number of Glossitis outbreaks was observed, as compared to those reported in 2022. It has been also observed that the trend of Glossitis cases increases during the winter, especially at the end of the School's academic year. The events were reported from Schools in six dzongkhag (Punakha, Gasa, Mongar, Pemagatshel, Trashigang and Trashiyangtshe) (**Figure 9)**.

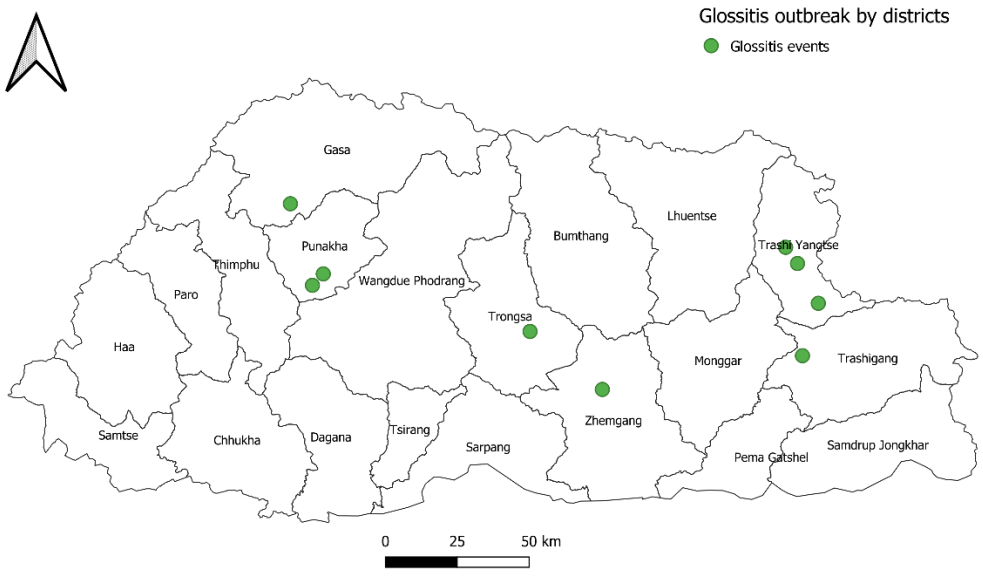

Figure 9: Distribution of Glossitis outbreak by Dzongkhag

Dengue Fever:

The first event/outbreak of Dengue fever was reported in NEWARS during the first week of November from Phuntsholing Hospital. A team comprising of the NEWARS focal person, Medical Officer, and Malaria technicians from the hospital conducted the field investigation. A total of 49 cases were detected dengue positive by RDT, all were in the age range between 17 to 74 years old. Half (50.0%) of the cases have a travel history to neighboring town Jaigoan, India. Ten cases required hospitalization and two were referred to JDWNRH due to complications. There were no dengue-related deaths. Most of the cases have been reported from the lower market and NPPF colony Phuntsholing. Among the 49 samples referred to RCDC, 42 (86.0%) were found positive for Dengue NS1 by ELISA.

A vector survey was conducted in 23 households in the area from where the cases emerged. Aedes aegypti larvae were found in three households in the lower market, four households in Toorsa, and one household in the NPPF colony.

Public Health Intervention:

- Vector survey was conducted in 23 households in the area from where the cases emerged.
- Larvicides have been applied and larvae containing water holding containers were emptied on the spot.
- The residents and the public in the locality were re-educated on measures to prevent mosquito breeding sites.

Conclusion on Dengue Outbreak

Phuentsholing is the commercial hub of Bhutan with the potential of propagating vector-borne diseases on large scale. The community is frequently affected by Dengue outbreak in the recent years. There is a need for enhanced public sensitization and re-sensitization about the disease transmission, and control measures. Vector-borne Disease and Control Program (VDCP) needs to streamline its interventions according to the risky areas or dengue hotspots in the country at the

earliest possible. For effective vector-borne disease control, it is imperative for enhanced cross-border collaboration and regular vector surveillance.

Conjunctivitis:

During the month of August 2023, a surge in Acute Hemorrhagic Conjunctivitis were reported from across the country on NEWARS. Upon the recommendation from RCDC, the local health center conducted an outbreak investigation. Conjunctival and throat swabs samples were collected from patients and shipped to RCDC to rule out the etiological agents. Samples were further shipped to AFRIMS for further investigations.

Findings:

Following the initial notification, a total of 54 events of suspected viral conjunctivitis were reported. The highest number was observed in in the third week of August 2023 (**Figure 10**).

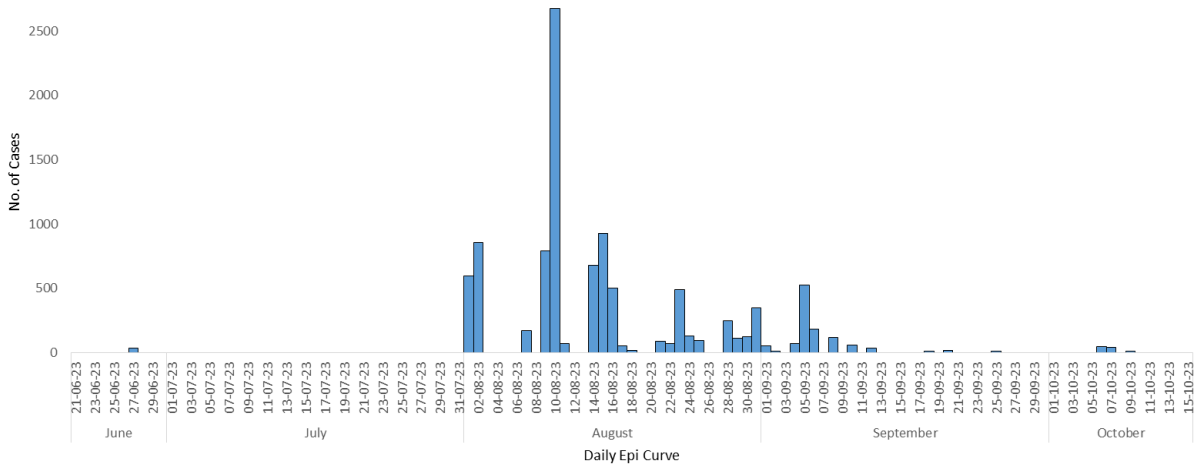

Figure 10: Epi curve showing the onset of conjunctivitis outbreak in Bhutan 2023

Most patients reported with symptoms of red eyes, itchiness, and discharge from the eyes. Few experienced fever with coryza, dry cough, and headache. None of the case have developed complications, none required hospital admission. The conjunctival swabs collected from patients were tested at RCDC to rule out the etiological agents. The samples was further shipped to AFRIMS in Thailand for further testing. The laboratory testing detected **Coxsackievirus A24 Variant** which

leads to acute hemorrhagic conjunctivitis. The cases were reported from almost all districts **(Figure 11)**

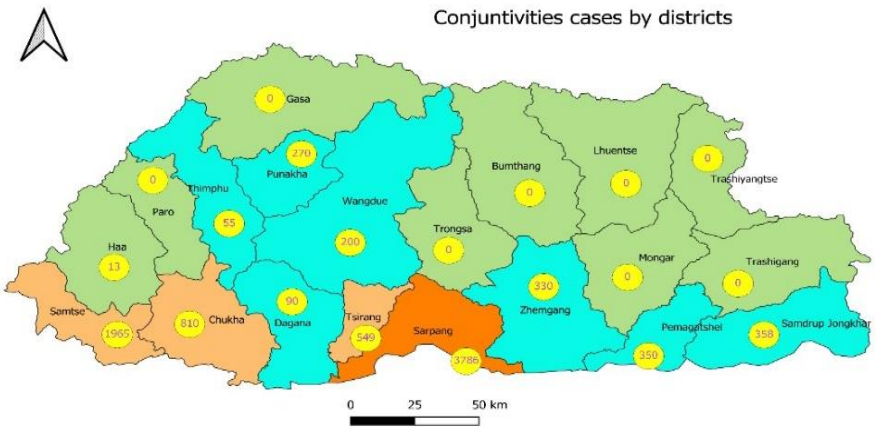

Figure 11: Distribution of Conjunctivitis outbreak by Dzongkhag

Public Health Intervention:

1. Students were advised to avoid close contact with others to prevent the spread of disease.
2. The health focal of the school has been debriefed on the outbreak and basic health information has been communicated.
3. Awareness and advocacy programs on basic hand hygiene practices and cough etiquette for prevention, and transmission of conjunctivitis were conducted in schools and local communities

Conclusion on conjunctivitis:

This was the first documented outbreak of conjunctivitis that affected the majority of School students across the country. The recent outbreak has left families and communities grappling with its rapid transmission. Laboratory testing of clinical samples confirmed the strain of adenovirus as the primary cause of "**Coxsackievirus A24 Variant"** with symptoms including swelling, inflammation,

itching, and tearing of the eyes, often accompanied by discharge and crusting. The infection was self-limiting and generally resolved within a week, the virus was highly contagious and the disease was transmitted throughout the country within a short period.

Rabies

On 25 June 2023, RCDC received notification of a possible rabies case from CRRH Hospital in NEWARS. On verification, it was learned that on May 31, 2023, a young man aged 29 years was bitten by an unknown dog while he was trying to prevent a cat from being bitten at Taraythang RBA outpost. Upon the recommendation from RCDC, DHRRT in coordination with District Livestock and Regional Veterinary Hospital & Epidemiology Center conducted the detailed field investigation and submitted the joint investigation report. The team from MoH, National Centre for Animal health (NCAH), RCDC and KJUMBS joined the district team for investigation.

Finding:

On May 31 the suspected dog also had bitten two cows, two cats, and an armed force personnel. Both the cats died on the same day following the bites. A cow died on June 22, it did not show classical rabid signs and syndrome and its samples were tested negative for rabies virus:, while the second cow which was cordoned off started showing signs and symptoms like rabies from June 29, 2023 and died on July 1, 2023 after suffering for three days. The clinical samples from the 2nd dead cow tested positive for rabies.

Human case:

The 29-year-old armed force personnel serving at Taraythang border outpost was the first case linked epidemiologically to that rabid suspected dog. He had 1st dose of ARV from Taraythang PHC on the same day he was bitten by a suspected dog, while he took 2nd and 3rd doses from Umling PHC. He was asymptomatic till June 21, his onset of symptoms backache, and muscular pain was from June 22, 2023. He was assessed by a Medical Specialist and managed in the ICU, survived for 23 days after the onset of symptoms, and passed away on July 17, 2023.

On 5th July 2023, there was a report of three children bitten by a rabid dog at RBA colony (RBA Wing 9), Lodaray, Gelephu. All the victims were given ARV and Immunoglobulin. A total of 76 primary contact and high-risk RBA families were also given ARV and suspected rabies dogs were neutralized by livestock staff at the outbreak site. None developed rabbis like symptoms following the second event.

Public Health Intervention

- All close contact with bitten animals, those who handled the carcasses of animals, consumed dairy products, and attendants of suspected cases were traced and given ARV, and PEP.
- The team has conducted advocacy/awareness programs for the general public through BBS, Radio, local TV channels, and multimedia.
- Community health workers, livestock staff, and local government officials had conducted house-to-house advocacy within their jurisdiction.

Conclusion on Rabies:

In conclusion, this was the first documented outbreak of rabies in the Taraythang Geog. Rabies outbreak in animals was confirmed by epidemiologically, clinical, and laboratory confirmation of the animal samples. An index human case fulfilled the case definition of rabies infection both clinically and epidemiologically. As a part of the global effort to eliminate human rabies with a goal of zero human death due to dog-mediated rabies by 2030: ***"Zero by 30".*** This rabies may pose a challenge to sustaining the rabies elimination goal as we are not able to establish the source of the outbreak. Rabies is expected in the bordering district but we need to establish the source of infection from outside the country.

Rabies outbreaks can cause significant morbidity and mortality, and adverse socio-economic impact, and require enormous resources to manage them effectively. Investigation of an outbreak is of urgent public health importance and requires immediate reporting to the concerned health authorities. The ability to manage disease outbreaks effectively is a key responsibility of public health services.

Recommendation:

- Since rabies suspected cases are reported continuously in rabies endemic districts, the surveillance system should be strengthened at the Dzongkhag for early response.
- The concept of One Health approach should be strengthened at all levels of the governance for coordination and timely response to a public health event of zoonotic diseases.
- Cross-border collaboration needs to be initiated to have a combined and collaborative response to cross border Public health issues and challenges.

4. Discussion and Conclusion

The overall timeliness of reporting of notifiable diseases (82.0% vs. 61.0%) and completeness (94.0% vs. 89.0%) have improved compared to 2022. The timeliness of reporting had significantly increased in 2023 compared with 2022 (82.0% vs. 61.0%) could be the impact of NEWARS training conducted in 2023 after a few years of gaps.

While comparing the number of cases reported by year, we observed that the total number of notifiable diseases reported by reporting centers in 2023 increased by about 10.0% compared with 2022. This suggests an increase in the utilization of health services by the population. The reporting of notifiable diseases may have also been influenced by the increase in number of NEWARS-trained health professionals. The rise in the number of cases could be attributed to many factors such as changes in behavior, an increase in number of people seeking healthcare services, and accessibility to services.

Overall, all the number of respiratory disease outbreaks had dropped in the past three years. There was a huge reduction of outbreaks observed in 2023, while the cases of respiratory diseases have increased in general over the past years. An unusually highest number of outbreaks reported in the system was of conjunctivitis. Since conjunctivitis is not a common illness in the community, as well as it is not in the list of weekly and immediately reportable diseases, the reporting center had reported even a single case as an event. An outbreak of Dengue fever was reported from Phuntsholing during the first week of November 2023.

Another finding was the variation in disease incidence by age group and district. The most commonly affected age group affected for the notifiable disease was pediatric cases, similarly, some

districts such as Samtse, Sarpang, and Thimphu reported higher numbers of respiratory and diarrheal diseases than others. This could indicate differences in local environmental or socio-economic factors that affect disease transmission and susceptibility.

In conclusion the overall notifiable diseases reporting status improved over the year, this could be because of strengthening communication with healthcare workers on the revival of diseases notification. Therefore, continuous monitoring and follow-up must be carried out with healthcare centers with low reporting rates.

Awareness especially ahead of peak seasons do help in reducing the disease morbidity mortality through timely response to any diseases outbreak. There is also a need to regularly train and update healthcare workers on the list of diseases that needs to be notified and the system updates. Integration of NEWARS with another laboratory-based surveillance system of RCDC will help in linking the epidemiological profile of the patient to their laboratory reports thereby further strengthening the surveillance system.

Further analysis to understand the implementation and impact of the surveillance system in the healthcare setup will be helpful in improving the relevancy and timeliness of the early warning system to reduce morbidity and mortality.

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II Sentinel Surveillance for Diarrheal Etiological Agents, 2023

Enteric and Invasive Disease Laboratory, Royal Center for Disease Control, Ministry of Health, Thimphu

1. Background

Diarrheal diseases represent a significant public health challenge worldwide, causing substantial morbidity and mortality, particularly in developing countries. Diarrheal disease is the second leading cause of death in children under five years old and is responsible for killing around 525,000 children every year. Globally, there are nearly 1.7 billion cases of childhood diarrheal disease every year. Diarrheal disease is a leading cause of child mortality and morbidity in the world and mostly results from contaminated food and water sources. Worldwide, 780 million individuals lack access to improved drinking water, and 2.5 billion lack improved sanitation. Diarrhoea due to infection is widespread throughout developing countries. Diarrhea can occur from a variety of causes. Still, the most common causes are the consumption of food or drinks that are contaminated with pathogens such as bacteria, viruses, and parasitic organisms or toxins.

Diarrheal disease remains a significant public health concern in Bhutan, despite efforts to improve sanitation and healthcare infrastructure. Diarrhea is the second-highest notified disease and a leading cause of morbidity in Bhutan particularly among children under five years of age. Diarrheal diseases account for a substantial proportion of outpatient visits and hospital admissions nationwide each year. A total of 11042 acute watery diarrhea (AWD) and 751 Acute Bloody Diarrhea (ABD) cases were reported in 2023 under the age of 9 years, double less than what was reported in 2022. (AHB).

The etiology of diarrheal disease in Bhutan is diverse, with bacterial, viral, and parasitic pathogens playing a significant role. Common causative agents include *Escherichia coli, Salmonella spp.*, rotavirus, norovirus, *Giardia lamblia*, and *Cryptosporidium* spp. These pathogens are often transmitted through contaminated food and water sources, inadequate hygiene practices, and poor sanitation conditions.

2. Methods

Case definition

Any patient with the passage of three or more loose or liquid stools in the past 24 hours.

Sampling Method

At each sentinel site, two aliquots of fecal specimens were prepared from every patient by trained laboratory staff or nurses. These aliquots, derived from a single sample, were intended for separate virological and bacteriological testing. For virological analysis, samples were divided and stored in cryovials without preservatives. These cryovials were maintained at temperatures between -20 to -40˚C to preserve the integrity of the specimens before shipping and subsequent processing.

For bacteriological testing, fresh whole stool specimens were collected in sterile containers. These containers were then placed in Cary-Blair Medium, a transport medium known for preserving bacterial viability, and stored at temperatures ranging from 2 to 4˚C to maintain sample integrity during transportation. To ensure the accuracy of surveillance data, patients who had likely consumed antibiotics before sample collection were excluded from enrollment.

Subsequently, all collected specimens underwent packaging following triple packaging protocols following IATA regulations and were shipped to RCDC for further processing and analysis.

Sampling sites:

There are 12 National Diarrheal Diseases Sentinel Sites (NDDS) **(Figure 1)**

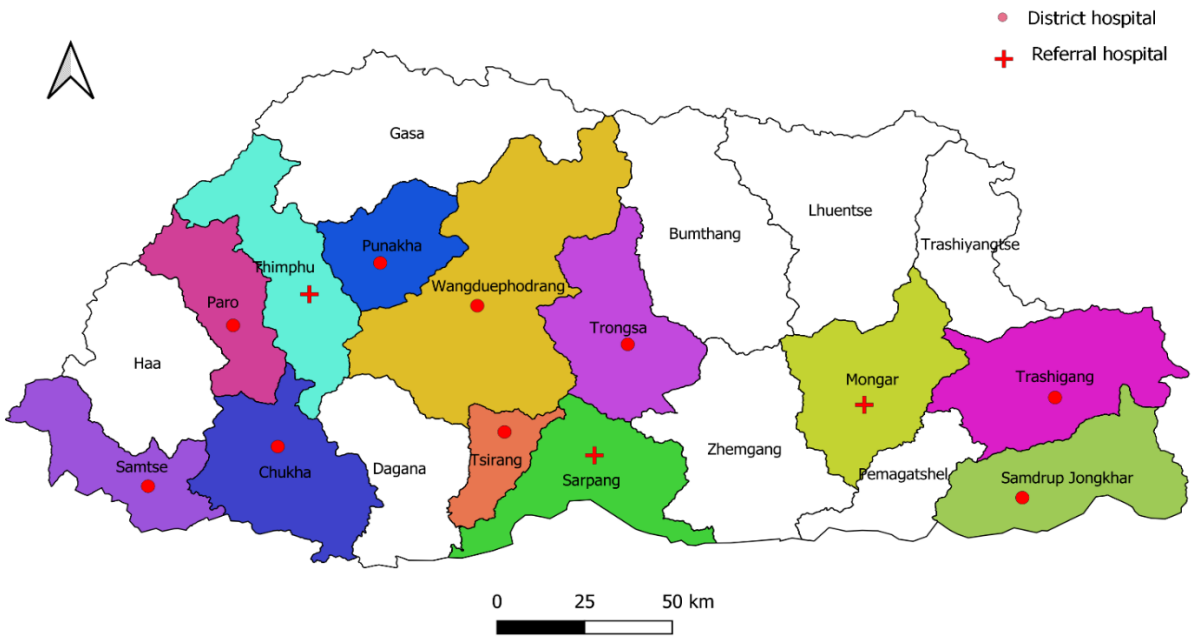

Figure 1. Sampling sites for NDDS

Enteropathogen testing

At sites equipped with microbiology facilities, a comprehensive diagnostic approach was employed to identify potential causative agents of diarrheal illness. This included the preparation of wet mount smears for microscopic examination and stool culture.

At Enteric, Zoonotic and Vector-borne Disease Laboratory, a thorough analysis was conducted on all samples to detect bacterial, viral, and parasitic pathogens using various techniques such as culture, serology, and polymerase chain reaction (PCR).

Bacterial pathogens including *Shigella, Salmonella, Vibrio, Aeromonas, Plesiomonas*, and *Campylobacter* were identified utilizing standard microbiological methods. Furthermore, isolates of *Escherichia coli* (*E. coli*) underwent multiplex PCR to determine their virulence and pathogenic potential. Common intestinal parasites such as *Giardia* and *Cryptosporidium*, as well as enteric viruses including rotavirus, astrovirus, and adenovirus, were detected using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits.

Antibiotic Susceptibility Testing

The antibiotic sensitivity testing of bacterial isolates was conducted using the modified Kirby-Bauer disc diffusion method, following the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI). This method is a standard procedure utilized for interpreting the susceptibility of bacterial isolates to various antibiotics based on the size of the inhibition zones surrounding antibiotic discs. CLSI recommendations ensure consistency and accuracy in interpreting susceptibility results, thereby facilitating informed decision-making regarding antibiotic therapy.

3. Results

In the period spanning from January 2023 to December 2023, stool specimens from a total of 347 patients presenting with diarrhea were collected and subjected to analysis. Notably, among the various sentinel sites involved in the surveillance, CRRH (Central Regional Referral Hospital) contributed the highest number of patients, as illustrated in **Figure 2 &3**.

Of all patients enrolled for surveillance, the majority (73.0%) sought treatment as outpatients (OPD), while the remaining 27.0% required inpatient care (IPD). Among the cases, 53.0% were male, and 47.0% were female, indicating a relatively balanced gender distribution among hospitalized patients. A significant proportion (48.0%) of patients belonged to the age group of < 10 years, highlighting the susceptibility of children to diarrheal illnesses.

The majority of specimens exhibited loose consistency (58.5%), followed by watery stools (38.5%). A smaller proportion (3.0%) of specimens presented with bloody stools, with mucus detected in 14.0% of samples. Additionally, 3.0% of samples tested positive for blood, as detailed in **Tables 1 and 2**.

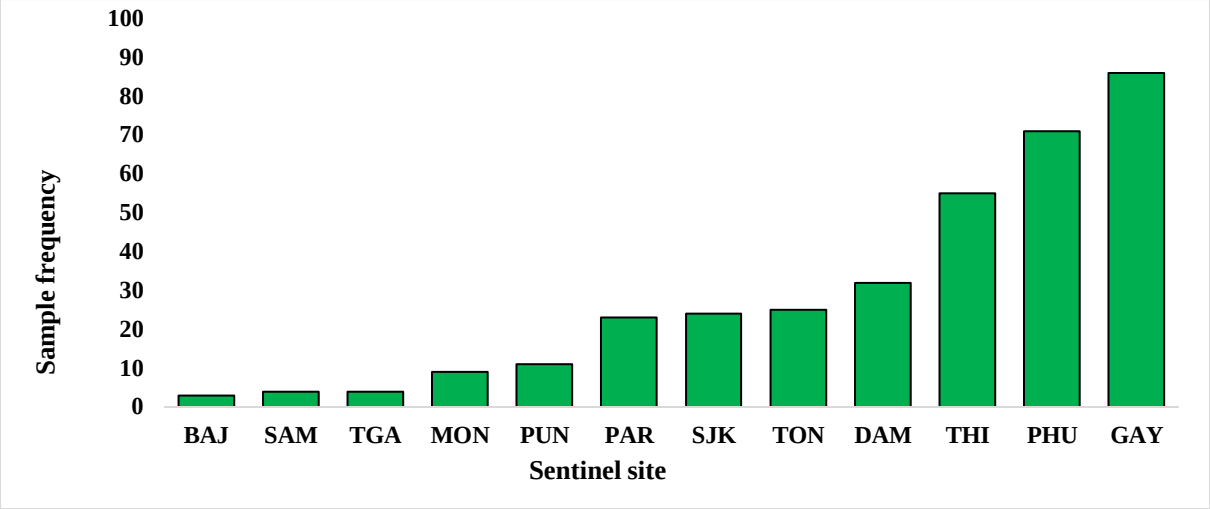

Figure 2: No. of samples received from Sentinel sites

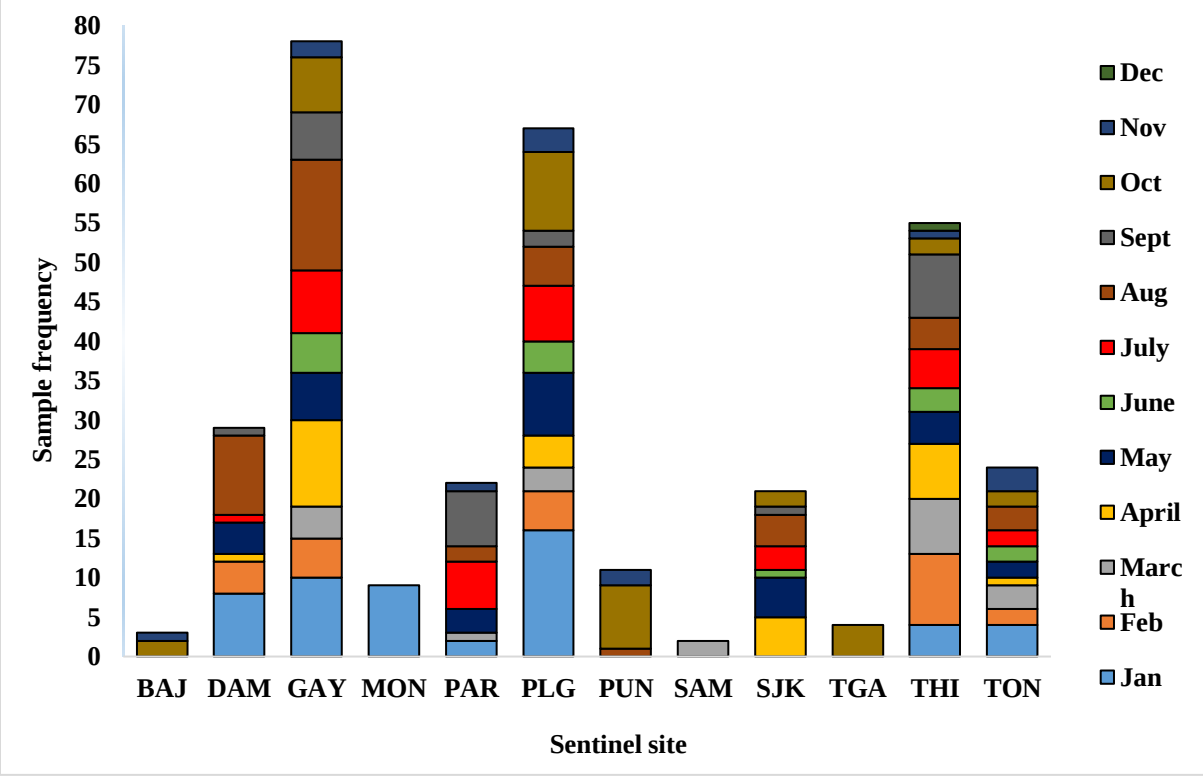

Figure 3: No. of samples received by site/month.

Table 1. Sociodemographic characteristics of the surveillance participants (n=347)

Characteristics		**n (%)**
Gender	Male	185 (53.0)
	Female	162 (47.0)
Age (Years)	0 – 9	168 (48.0)
	10 - 19	30 (9.0)
	20 - 29	30 (9.0)
	30 - 39	33 (10.0)
	40 – 49	31 (9.0)
	50 and above	55 (15.0)
Visit status	IPD	95 (27.0)
	OPD	252 (73.0)
Occupation	Civil servant	9 (2.6)
	Private	46 (13.3)
	Student	54 (15.6)
	Farmers	31 (8.9)
	Dependent	130 (37.5)
	Housewife	48 (13.8)
	Others	29 (8.4)

Table 2. Clinical characteristics of the sample (n=347)

Characteristics		**n (%)**
Consistency	Loose	203 (58.5)
	Watery	134 (38.5)
	Bloody	10 (3.0)
Color	Black	7 (2.0)
	Brown	83 (24.0)
	Clay	5 (5.0)

	Green	62 (18.0)
	Red	4 (1.0)
	Yellow	181 (52.0)
	Other	5 (1.0)
Mucus	Positive	49 (14.0)
	Negative	298 (86.0)
Blood	Yes	11 (3.0)
	No	336 (97.0)

The culture results

All (n=347) samples subjected to culture revealed the growth of various pathogens implicated in diarrheal diseases, with Diarrheagenic *Escherichia coli* (n=29), *Shigella sonnei* (n=7), enteric viruses (n=68), and *Giardia lamblia* (n=19) emerging as the predominant etiological agents. Notably, among the Diarrheagenic *E. coli*, Enteroaggregative *E. coli* (EAEC) and Enteropathogenic *E. coli* (EPEC) were identified as the most prevalent pathotypes, as depicted in **Figure 4**.

The analysis of age distribution highlighted those individuals under nine years of age constituted the most affected demographic group, irrespective of the identified causative agent. This finding underscores the vulnerability of children to diarrheal illnesses and emphasizes the need for targeted interventions and preventive measures tailored to this age group.

Figure 5 provides a detailed breakdown of the distribution of pathogens across different age groups, shedding light on the age-specific patterns of diarrheal disease incidence.

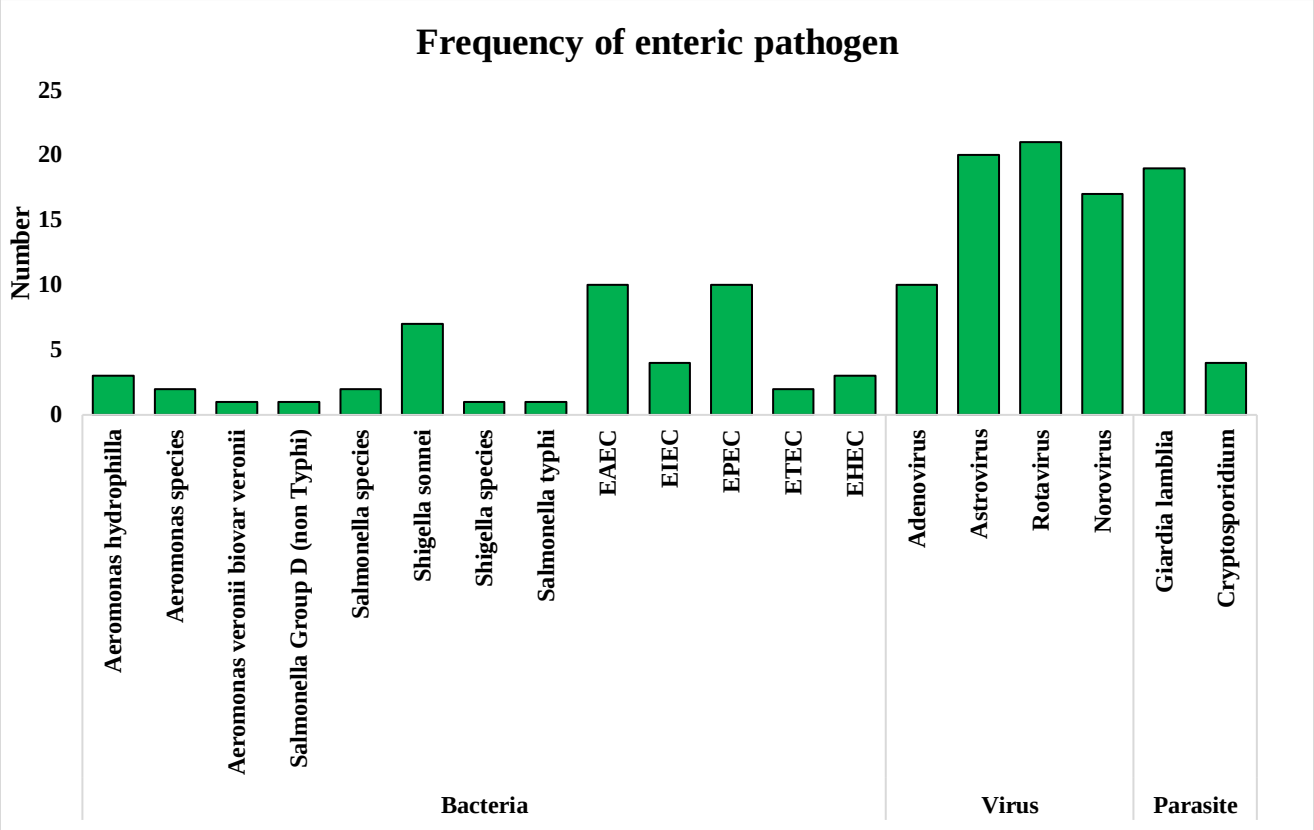

Figure 4. Etiology of enteric pathogens

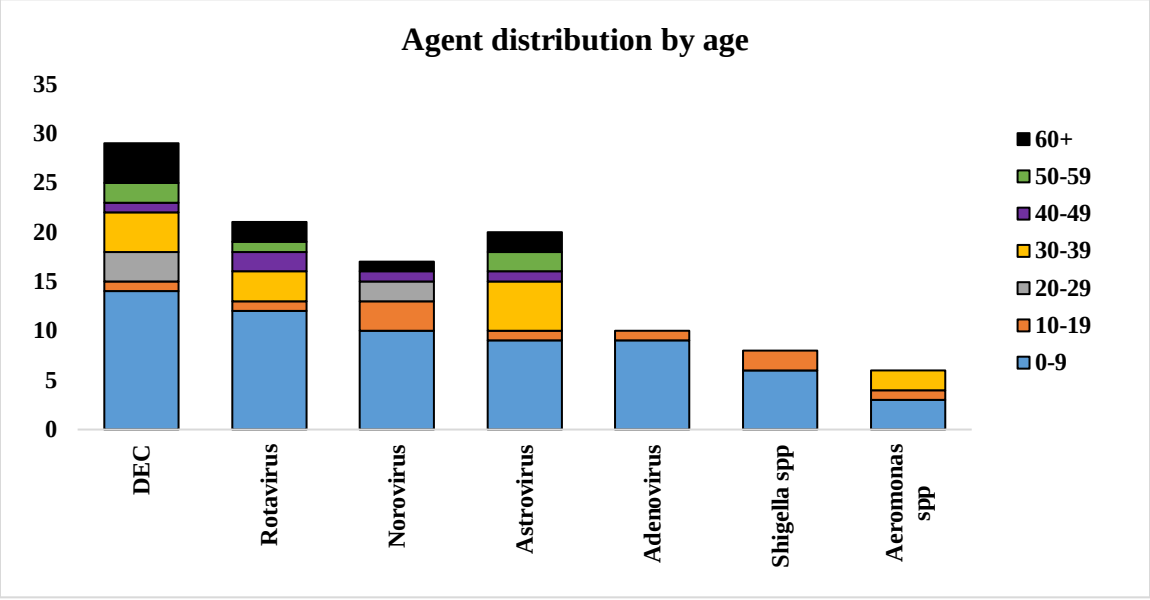

Figure 5: Agent distribution by age group

Seasonal distribution of agents

Enteric bacteria represent a prevalent cause of diarrhea year-round, with notable prominence observed during the summer and monsoon seasons, particularly among the most prevalent bacterial agents such as Diarrheagenic *Escherichia coli* (DEC) and *Shigella* species.

During the winter months, there was a notable upsurge in cases attributed to enteric viruses, specifically Astroviruses and Rotaviruses. Additionally, throughout the year, 19 cases of *Giardia lamblia* infection were identified, highlighting its significance as a causative agent of diarrheal illness.

These seasonal variations in the incidence of diarrheal pathogens underscore the dynamic nature of enteric infections and emphasize the importance of tailored surveillance and preventive measures to effectively manage diarrheal diseases across different seasons. By understanding these patterns, healthcare systems can implement targeted interventions to mitigate the burden of diarrheal illnesses and improve public health outcomes. (**Figure 6**).

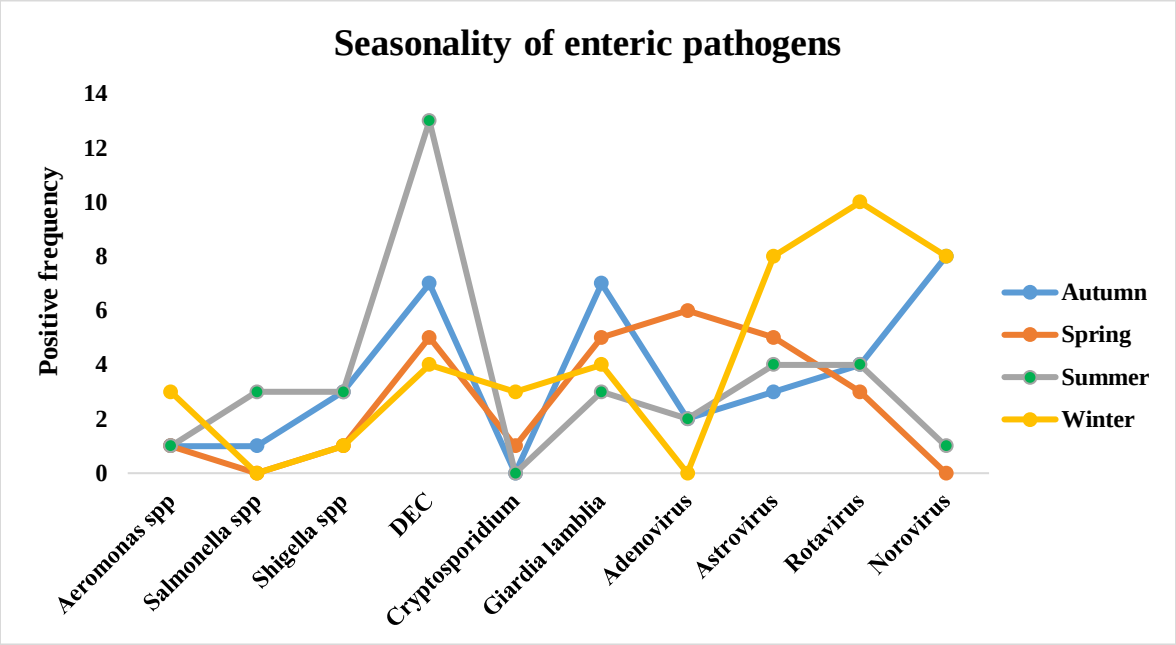

Figure 6. Seasonality of the enteric pathogens

Antibiotic susceptibility

The bacterial strains under study showed varying degrees of resistance to commonly used antibiotics. Among the *Aeromonas* spp strains, 6 out of the total number tested were found to be resistant to the antibiotics cephazolin and cephalexin. *Salmonella* spp, on the other hand, exhibited resistance to both ampicillin and cephazolin. The Shigella spp strains, which were tested in the study, showed resistance to a range of antibiotics including ampicillin, ciprofloxacin, nalidixic acid, and the sulfathemaxazole group of antibiotics. Finally, the DEC pathogens showed a high level of resistance to the antibiotic's ampicillin, cephazolin, cephalexin, and nalidixic acid, with more than 50.0% of the total number of strains tested demonstrating resistance.

The antimicrobial susceptibility tests revealed concerning findings regarding the resistance patterns of enteric bacteria. Overall, the results indicated a complete resistance rate of 100.0% to cephazolin, signifying a severe limitation in the efficacy of this antibiotic. Additionally, there were notable levels of resistance, exceeding 60.0%, observed against ampicillin, underscoring the urgent need for alternative treatment options. Furthermore, the tests highlighted significant intermediate resistance rates, with approximately 70.0% and 60.0% observed for ciprofloxacin and cephalexin, respectively. These findings suggest a concerning trend towards the development of resistance within the cephalosporins and fluoroquinolones groups of antibiotics (**Table 3 and Figure 7 &8)**.

Table 3. ABST pattern of the tested bacterial pathogen

Enteric pathogen	**Antibiotic**																													
	AMP			**CZO**			**CRO**			**LEX**			**CHL**			**CIP**			**GEN**			**NAL**			**TCY**			**SXT**		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Aeromonas spp	6	0	0	0	0	6	5	0	1	0	0	6	5	0	1	5	0	1	5	0	1	4	0	2	5	0	1	3	1	2
Salmonella spp	0	0	4	0	0	4	3	0	1	3	0	0	4	0	0	1	2	1	3	0	1	1	0	3	4	0	0	3	0	1
Shigella spp	0	0	8	0	0	4	8	0	0	5	3	0	8	0	0	0	1	7	8	0	0	0	1	7	7	0	1	2	0	6
DEC	12	2	29	0	0	39	26	1	14	6	10	25	38	3	0	7	23	11	37	0	4	14	5	22	31	0	10	28	1	12

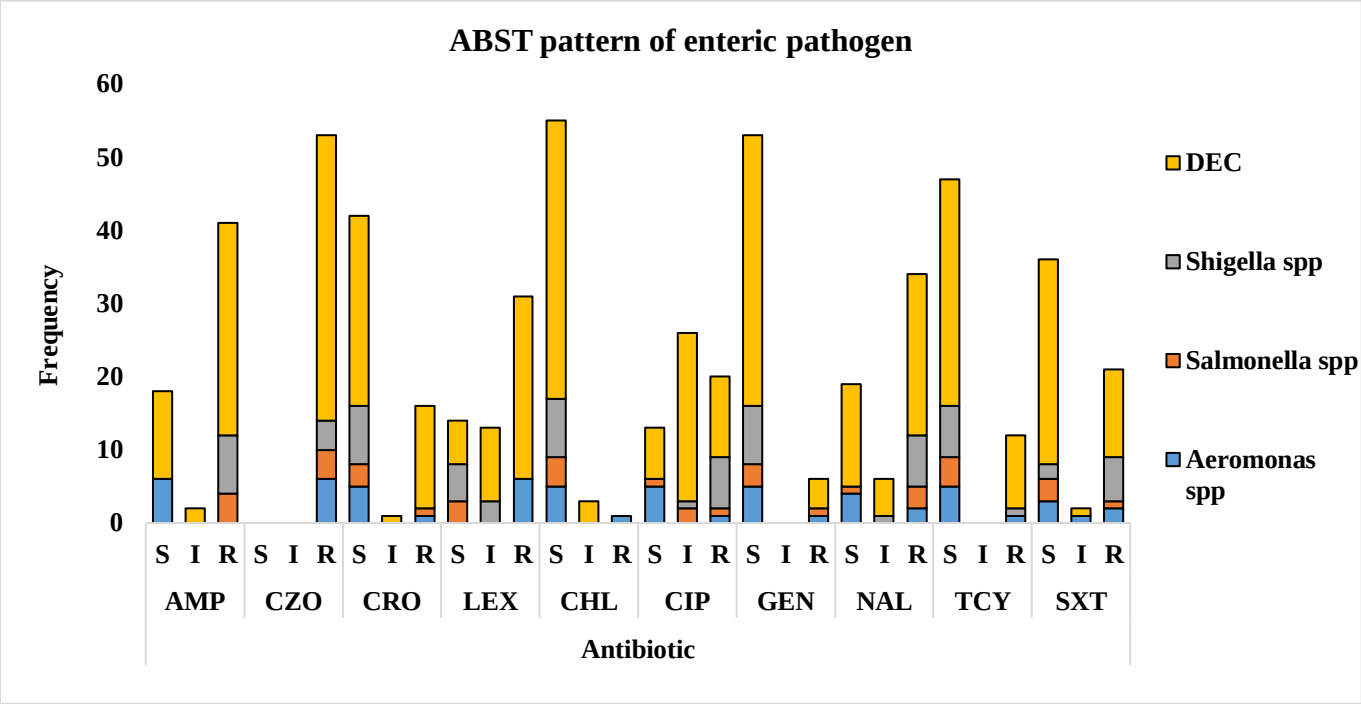

Figure 7. ABST pattern of the tested bacterial pathogen

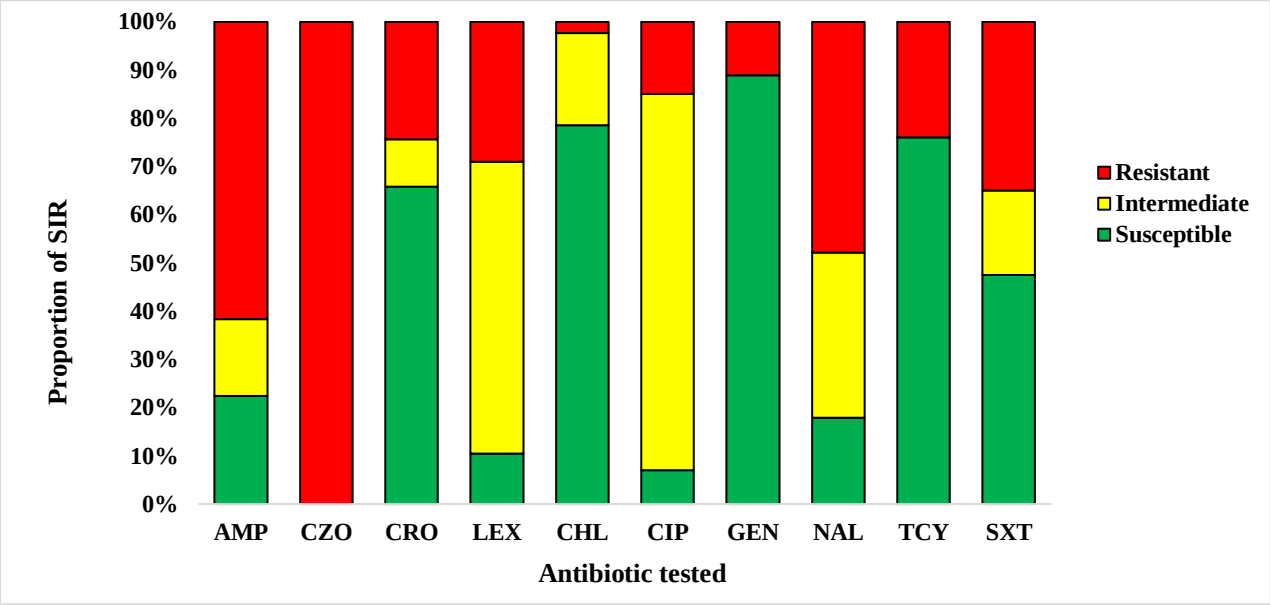

Figure 8. Antibiogram of enteric bacteria pathogens

AMX (Amoxicillin), AMP (Ampicillin), CZO (Cephazolin), CRO (Ceftriaxone), LEX (Cephalexin), CHL (Chloramphenicol), CIP (Ciprofloxacin), GEN (Gentamycin), NAL Nalidixic Acid, TCY (Tetracycline), SXT (Trimethoprim and sulfamethoxazole)

4. Discussion

A total of 347 samples were received from the 12 hospital-based sentinel surveillance. It has been observed that males (53%) and those under 9 years old (48.0%, n=168) were most affected. This surveillance study provided critical insights into the extensive burden of major bacterial, parasitic, and viral pathogens associated with diarrhea infections. The distribution of enteropathogens showed that DEC, *Shigella sonnei, Giardia lamblia,* Astrovirus and Rotavirus were the most potential enteric pathogen causing diarrhea than parasitic agents. The findings underscore the importance of considering strains such as Enteroaggregative *Escherichia coli (EAEC)* and *Enteropathogenic Escherichia coli (EPEC),* typically not included in routine screening protocols, as potential culprits in childhood diarrhea cases. However, the study also highlighted the need for further investigation into additional causative agents contributing to acute diarrhea, particularly emphasizing the significance of exploring rotavirus genotyping surveillance among children under 5 years of age.

The seasonal prevalence pattern varied among the detected pathogens indicating the dynamic nature of diarrheal diseases. Viruses were more prevalent in winter and autumn months (dry – colder temperatures), while bacteria were more prevalent in summer months and parasite infection remained constant throughout the year. The seasonal pattern is likely associated with environmental factors, such as ambient temperature, precipitation, and humidity, all of which may influence exposure frequency, host immunity, and pathogenicity.

Bacterial pathogens have demonstrated alarming levels of resistance, with a concerning 100% resistance observed against cephazolin antibiotics. Additionally, emerging resistance rates of more than 60.0% for ciprofloxacin and cephalexin antibiotics highlight the urgent need for reinforced antimicrobial resistance (AMR) surveillance and extensive sequencing of resistance isolates. Identifying the specific resistance genes and mutations driving this resistance is imperative for devising effective countermeasures.

The results of antimicrobial susceptibility tests underscore the escalating challenge posed by antimicrobial resistance among enteric bacteria. Consequently, there is an urgent need for cautious antibiotic selection and a pressing necessity to explore alternative treatment strategies to combat this growing threat effectively. Insufficient sample submissions for surveillance were noted, largely attributed to a lack of staff sensitization and the recent attrition rate within the workforce. This shortfall in sample collection poses a significant challenge to conducting comprehensive surveillance and hampers the accuracy of data analysis.

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III Surveillance of Measles and Rubella in fever with rash syndrome, 2023

Investigation of measles and rubella in fever with rash syndrome, 2023

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Vaccine Preventable and Venereal Disease Laboratory

1. Background

Measles and rubella (MR) are two highly contagious viral diseases with potentials to cause epidemics in unvaccinated or under-vaccinated population (1). Caused by *measles viruses* that belong to the genus *Morbillivirus* within the family *Paramyxoviridae* and *rubella viruses* belonging to the genus *rubivirus* of *Matonaviridae* family, these pathogens mostly affect children under the age of 5 (2,3). Severe measles is more likely among poorly nourished young children and immunocompromised individuals (3).

Global epidemics of measles were frequently reported before the introduction of the measles vaccine in 1963 (4). Even in the current situation, an estimated deaths of around 128,000 are reported from measles alone, most of whom were children under the age of 5 years. The widespread implementation of measles and rubella vaccination programs have led to significant reductions in the incidence of these diseases and their associated complications. Administered in the form of measles-mumps and rubella (MMR), the vaccination has been proven to be highly effective with two doses providing as high as 94% protection against measles (5).

Bhutan was certified as having eliminated endemic measles since 2017 and rubella in 2023 (6). This success story is largely attributed to a good immunization coverage which is more than 95% for both first and second dosage, as recorded in 2022; complimented by a robust surveillance system. Vaccination has played a pivotal role in achieving regional elimination goals for measles and rubella in many parts of the world, however, several countries have subsequently lost their status of elimination with waning vaccine derived immunity and importation of wild type viruses (7).

Laboratory based surveillance of measles and rubella has, therefore, become a cornerstone for generating evidence through active case finding and confirmation. MR surveillance in Bhutan is an active nation-wide surveillance that encompasses epidemiological and clinical data collection along

with laboratory sample testing (8). Measles and rubella are nationally notifiable diseases and reporting of fever with rash syndrome is captured by the National Early Warning and Alert Response System (NEWARSIS).

The Vaccine Preventable and Venereal Disease Laboratory (VPVDL) in Royal Centres for Disease Control (RCDC) is the national reference and the only laboratory in the country that performs detection of measles virus (MeV) and rubella virus (RuV) while the National Epidemiology and Disease Surveillance Unit in RCDC performs epidemiological investigations. This report presents the surveillance findings and associated laboratory results of MR for the year 2023.

2. Methodology

Case definition:

WHO case definition of measles and rubella is adopted, according to which, any person presenting with fever along with maculopapular rash (non-vesicular) in whom a clinician suspects measles infection irrespective of age, should be enrolled as a suspected measles/ rubella case.

Reporting:

Any suspected case of measles or rubella were mandatorily notified to RCDC through NEWARSIS and the specimen collected for testing.

Specimen Collection and shipment:

Blood specimens were collected from suspected cases for serological testing of measles/ rubella IgM antibody testing. Additionally, a throat or a nasopharyngeal specimen was collected in viral transport media which were to be used for molecular testing of MeV and RuV and for genotyping of virus if present. Serum was separated prior to shipment and packed in leak proof vials. Shipment to RCDC was made in a cold-chain box.

Laboratory testing:

Serological detection of IgM antibody was carried out using Anti IgM-Enzyme Linked Immunosorbent Assay (ELISA). Tests were performed in parallel for both measles and rubella for all the samples at VPVDL, RCDC using Euroimmun® kits. Tests were performed as per manufacturer's instructions.

As for molecular detection using quantitative real-time reverse transcriptase Polymerase Chain reaction, only throat swab samples qualifying the following criteria were selected:

- Throat swab accompanying serum that is positive for anti-IgM measles or rubella
- Throat swabs that were collected within 48 hours of onset of fever and rash, irrespective of anti-IgM test result

Protocol by United States- Centers for Disease Control (US-CDC) qRT-PCR for detection of measles and rubella viral RNA was used. Positive samples were subjected to end-point PCR testing for sequence analysis that was ultimately used for phylogenetic analysis and determining molecular epidemiology.

3. Results

A total of 437 samples were collected during the period, of which 304 samples were collected from the suspected cases and 133 samples collected through contact tracing. The cases were distributed all across the country (**Figure 1).**

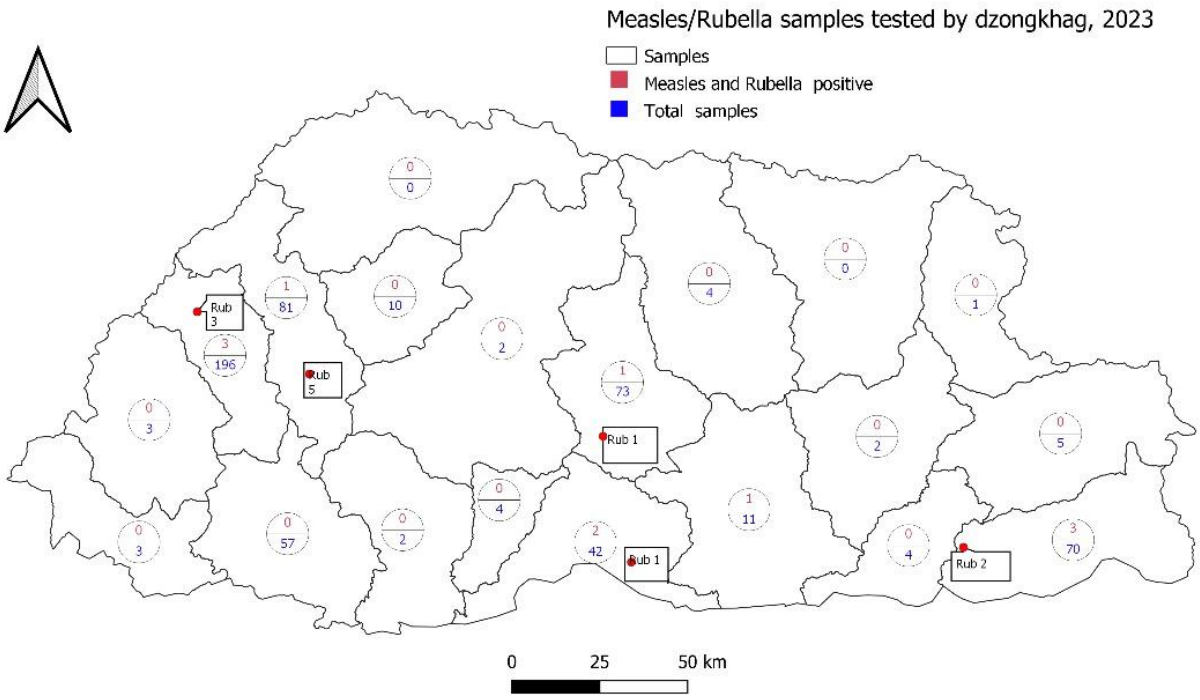

Figure 1: Distribution of sample collection along with sero-positives for MR, 2023

Monthly notification:

Cases were notified throughout the year from different parts of the country (**Figure.2**). Cases reported were high for the hotter months with cases from May to September comprising of 64.1% of the total. Cases reported were low during the colder months. During the months of January, February and December, only 8.9% of the total cases were reported.

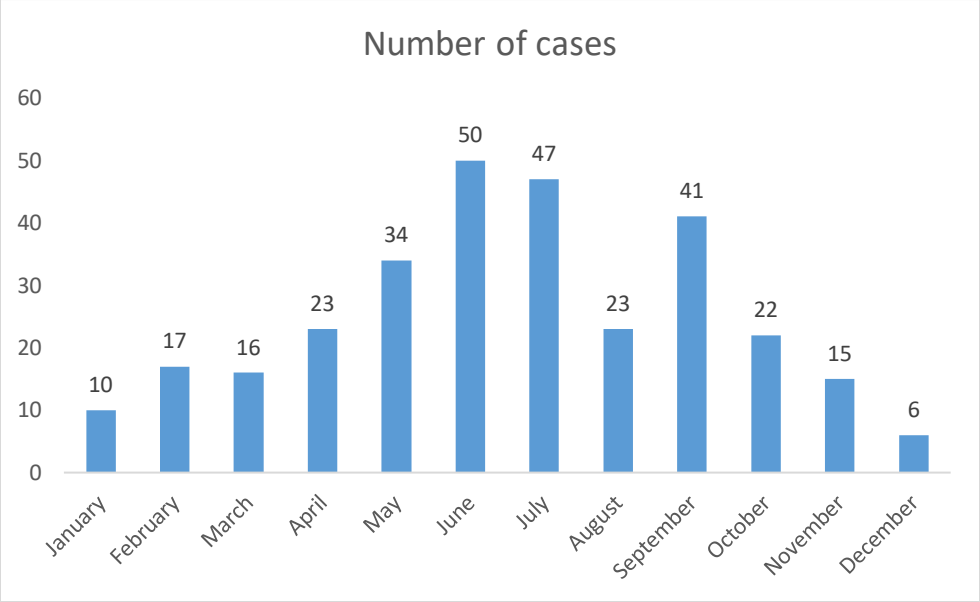

Figure 2: Monthly distribution of notified MR suspected cases

Age and Gender:

In 2023, the age group 1 to 9 years comprises of 62.2% of the total cases. Smaller number of cases were reported from older age group where only 0.7% of cases were reported from 50 years and above. The cases were mostly males (55.6%) than females (44.4%) with a rate ratio of 1.3:1(**Figure 3)**.

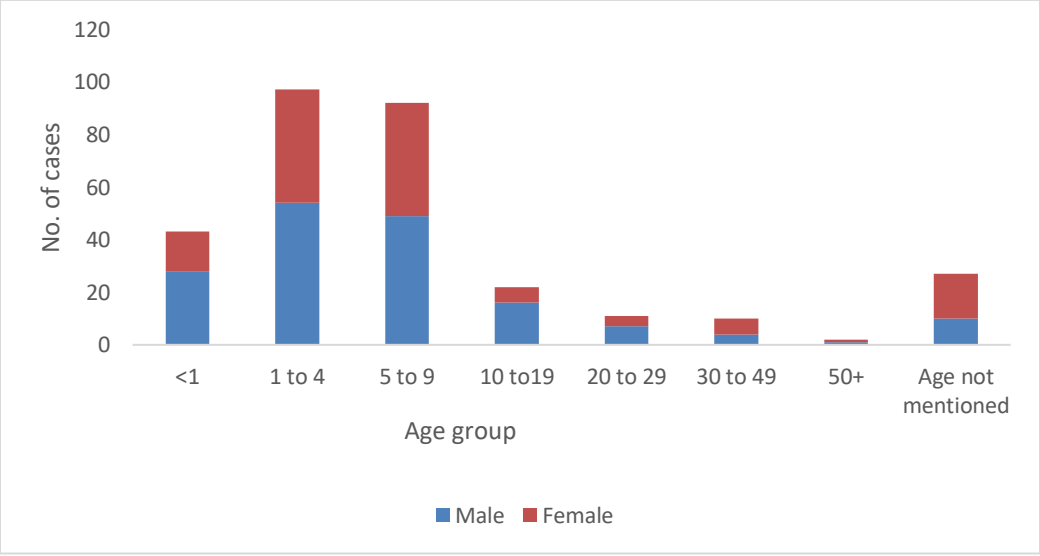

Figure 3: Age distribution of notified fever with rash cases

Positivity and Vaccination status

There were 9 samples positive for MeV IgM and 12 for RuV IgM. Of the 9 measles IgM positive, there was 1 sample positive for MeV ribonucleic acid (RNA) by qRT-PCR while an additional 1 sample negative for measles IgM was positive for MeV RNA.

The positive cases were assessed for their vaccination status. Except for the 32-year-old case of measles whose vaccination status could not be ascertained, the rest had all been vaccinated as per the national immunization schedule (Fig. 4). Further, 6 of the rubella positive cases who were of less than 1 year old were verified as " vaccine associated", while 1 was a case of congenital rubella syndrome (CRS) (**Figure 4**).

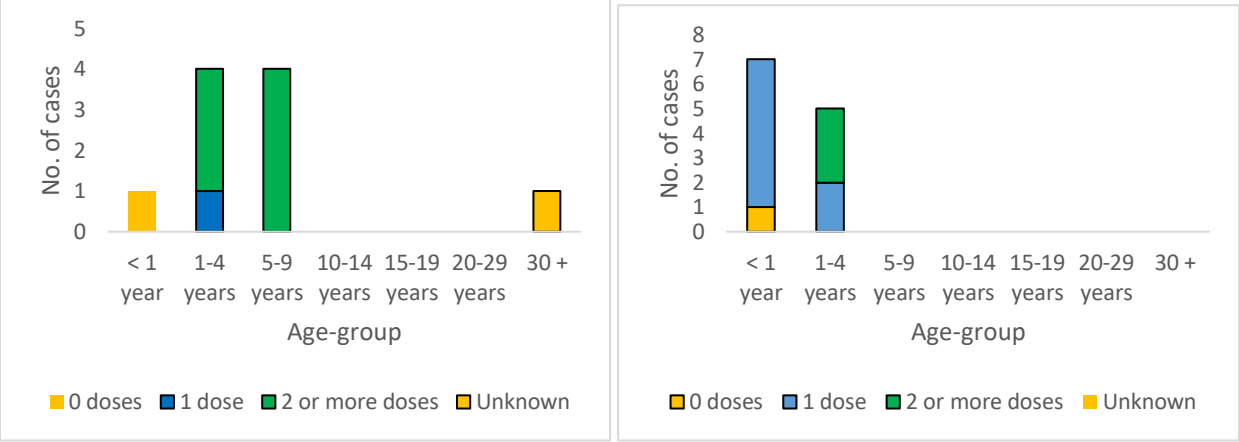

Figure 4. Age and vaccination details of serological positive cases for measles (left) and rubella(right)

Surveillance quality indicators

The laboratory surveillance quality indicators were assessed in two aspects, timeliness of specimen transport and timeliness of reporting lab results.

Number of days taken for samples to reach RCDC after collection range from 0 to 53 days, with an average of 3.1 and a median of 1.0. Of the 436 specimens that had complete information on date of collection, 283 (64.9%) fulfill the WHO criteria of reaching the laboratory within five days of collection **(Figure 5**).

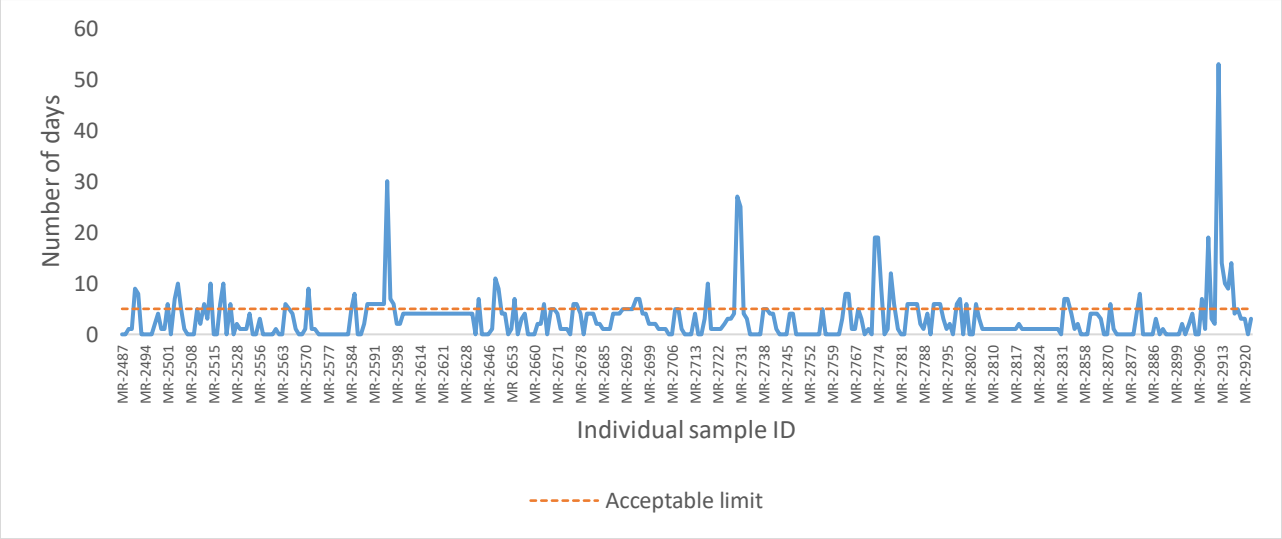

Figure 5. Number of days taken for specimens to reach RCDC from the day of collection

Similarly, number of days taken for completion of test after receiving samples range from 0 to 31 days. The mean and median for this indicator are 3.1and 3.0 respectively (**Figure 6**). Samples that were tested within 4 days of receiving in the laboratory amounts to 422 (96.6%).

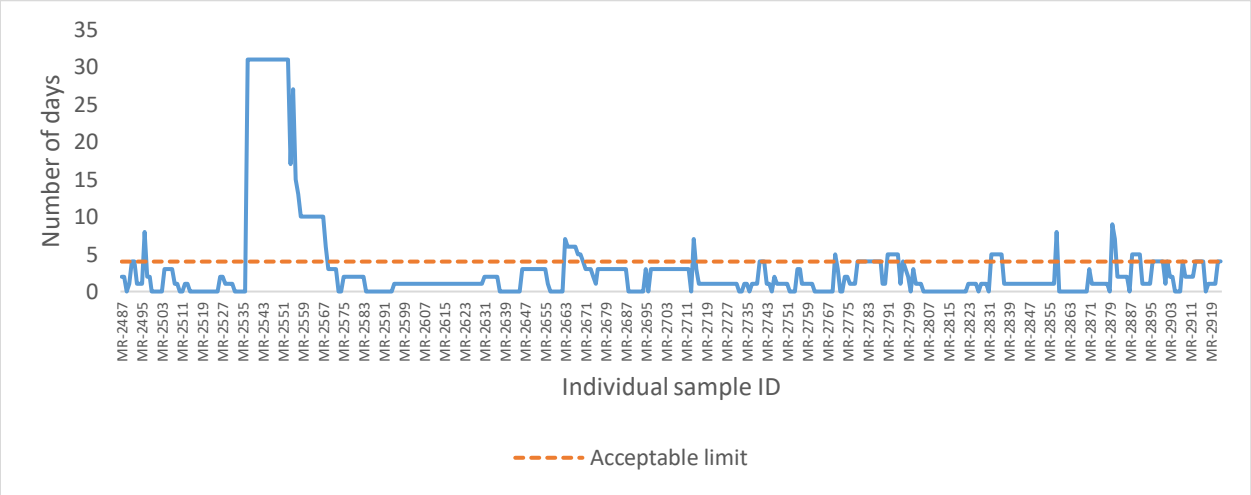

Figure 6. Number of days taken for specimens to be tested from the day of receipt

4. Discussion:

Since reporting of fever with rash cases is a part of NEWARS, there is active reporting from all over the country, leading to active case searching and detection. This continues even after Bhutan has achieved elimination of endemic measles and Rubella. In fact, number of samples received and tested by far surpasses that if the previous year where the total samples collected and tested was only 148 (9).

Our surveillance findings indicate that most of the suspected as well as laboratory positive cases of measles and rubella occurred in the pediatrics. Although measles and rubella are known to affect all age groups, countries worldwide have reported higher incidences in infants who are too young to be protected by vaccines (10). Similar picture can be found in Bhutan. We also encountered a number of vaccine-induced cases which were classified upon verification by external technical team from WHO-SEARO.

In 2023, we found 2 samples positive for MeV RNA detection by qRT-PCR. Both the cases were from different time and geographical areas but upon genetic analysis both turned out positive for D8 strain. Both the cases were verified as non-indigenous/ endemic as field investigation could not associate the cases to any endemic circulations. Moreover, D8 is a predominant strain in Europe, North America and Asia. Most importantly, it is commonly found in the neighboring country, India (11). As opposed to the previous practice, the laboratory has changed the algorithm for molecular testing of MeV and RuV. This has dramatically reduced the number of samples tested by molecular methods.

Our laboratory has achieved WHO recommended standard of testing at least 80% of samples within 4 days of receiving in the laboratory (12). Delay of testing for a group of samples in the months of March-April occurred due to shortage of reagents which in turn was attributable to testing asymptomatic contacts. With recommendations from WHO-REARO, this practice was discontinued.

Although RCDC recommends hospital laboratories to make weekly shipment of samples, we received only 64.9% of the specimens within 5 days of collection. While this is an improvement compared to the previous year (46%), we still need to find ways to enhance this turn-around-time.

Timely shipment and proper cold-chain maintenance are crucial as these factors are known to affect the test results (12).

Challenges of making timely shipment remains perennial along with other factors such as cold-chain maintenance of shipment boxes and sending complete clinical and epidemiological information. Huge turn-over staff in the recent years complimented with budgetary constraints have created barriers in regular training and sensitization of field staff with regard to case identification, specimen collection and shipment.

Conclusion

Year 2023 marked another achievement in our endeavor towards eliminating a disease of public health concern. As we declare ourselves free of endemic measles and rubella, it is important that we sustain the surveillance with robustness and quality. It is also imperative that the challenges mentioned be addressed through available means and the capacity of laboratory and data management be upgraded as a continuous process of quality improvement.

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IV National External Quality Assessment report of Serological Testing of Sexually Transmitted Infections, 2023

Sangay Zangmo, Sonam Jamtsho, Tenzin Dorji, Rinchen Wangdi

1. Introduction

Infections of Human Immunodeficiency Virus (HIV) and viral hepatitis due to Hepatitis B Virus (HBV) and Hepatitis C virus (HCV) are some of the most common communicable diseases and major causes of chronic disease worldwide (1). These infections, along with syphilis, a curable yet prevalent sexually transmitted infection (STI), share common transmission routes, epidemiological patterns, and public health interventions (2). The risk of STIs via blood transfusion remains a global concern. Since the 1970s, serological assays detecting virus-specific antibodies and antigens have effectively identified infected blood donors (3). While many countries have transitioned to Nucleic Acid Amplification Tests (NAATs) which shortens window period by several folds (3), health facilities in Bhutan continue to rely on serological methods for screening STI in blood transfusion services.

National External Quality Assessment Scheme (NEQAS) for STI, mainly pertaining to TTI was initiated in 2006 by the Royal Centre for Disease Control (RCDC), formerly known as Public Health Laboratory. Currently, the activity is spearheaded by the Vaccine Preventable and Venereal Disease Laboratory (VPVDL) in RCDC and is carried out as an annual activity. All state-owned health centers performing serological screening for STIs for either diagnostic or blood transfusion screening purposes are mandatory participants of the scheme. From 25 laboratories in 2006, the program has expanded to 58 health centers, including Health Information and Service Centers (HISC), military hospitals, primary health care centers, district hospitals, and the national referral hospital.

External quality assessment (EQA) is an important component of quality systems for diagnostic as well as blood transfusion services. It involves external evaluation of a laboratory's performance by testing unknown samples and benchmarking results against other laboratories. EQA helps the laboratories to raise standards of performance and provides an opportunity for continual quality

improvement through the identification of laboratory errors and the implementation of measures to prevent their recurrence (4).

Past assessments have been helpful in identifying errors in pre-analytical, analytical, and post-analytical phases, leading to targeted on-site supervision and training by RCDC officials. As in most disease surveillance activities across the globe, COVID-19 has immensely impacted the activity and the program still struggles to recover from the consequences (5). In 2021 and 2022, panel could not be dispatched due to lockdown measures imposed that would directly affect the shipment arrival in various places across the country. The health workers were mobilized to various places for COVID-19 activities because of which the panel would not have received due importance. The panels were successfully dispatched in 2023, and here we present the findings, underscoring both strengths and areas for improvement in STI serology testing across Bhutan.

2. Methodology:

There are currently 58 participating laboratories that include clinical laboratories of 32 hospitals and 6 HISCs that provide voluntary counselling and testing services. These participants were required to test the panel samples for anti-HIV, Hepatitis B surface antigen (HBsAg), anti-HCV and anti-*Treponema pallidum* (anti-TP). For anti-TP, participants were given a provision for reporting rapid plasma reagin (RPR) as well as anti-TP test results, but the final result of anti-TP was considered for analysis.

Panel preparation and distribution

The panel samples were prepared following the methods outlined in the guidelines (6). Whole blood samples obtained from the blood bank at JDWNRH was used for preparation of negative stock sample, and tested for anti-HIV, anti-HBV, HBsAg, RPR and TPHA to ensure that the sample is true negative. Re-calcification was carried out to extract serum and plasma. For that, a 2 moL/L solution of $CaCl_2.2H_2O$ was prepared by adding 3g of $CaCl_2.2H_2O$ to10 mL ofdistilled water and then further adding 0.5ml of the freshly prepared $CaCl_2$ solution to100 mL of volume of plasma.The final concentration of 0 .01 M $CaCl_2$ was made. This solution of plasme was mixed well and

incubated in water bath at 37ºC for 1hour or more until the plasma has clotted. The mixture was removed from the water bath and transfered to a bottle and placed in a freezer at –20ºCover night. This mixture was removed from freezer and thawed the next day for preparation of panel samples.

For the preparation of positive controls, known strong positive samples for HIV, HBV, HCV and syphilis were used. The samples were heat inactivated at 56°C for 60 minutes and then diluted using a serial 2-fold dilution with the negative stock. The heat inactivated positive and negative samples were then centrifuged at 10,000 RPM at 4°C for 10 minutes and filtered through a membrane filter of 0.45µ pore size. 50 µl of 0.05% proclin300 and 0.05% Bronidox L (5bromo-5-nitro-1,3-dioxane in propylene glycol) to every 100 ml of serum as preservative. Prior to distribution, the panel samples were validated with the available test kits to confirm the expected results for the four parameters of interest. Finally, 500 µl of panel samples were dispensed in leak proof, screw capped plastic microvials, which were then labelled and packed in bubble envelopes.

Reporting, data analysis and feedback

The sites were instructed to mandatorily report the results within one month of mail dispatch from RCDC. Reporting using e-mail, post and any form of social media platform was accepted. Submissions beyond this period were recorded as "delayed reporting" and, while recorded, were excluded from the primary analysis. Results received from the sites were recorded in an excel sheet and analyzed in two parts. Part A comprised of 80% of the total score and consisted of test conformity evaluation by comparing submitted test results with actual results. Part B consisted of evaluation of information provided by the laboratory personnel on test kit and other aspects of testing. This comprised of 20% of the total score. The participants were provided an overall score for their performance by adding 80% of the total scores obtained in the part A and 20% of the total scores obtained in part B. Accordingly the scores were interpreted as excellent (100%), very good (95-99%), good (90-94%), satisfactory (80-89%) and need improvement (<79%). VPVDL ensured the dissemination of a preliminary report to individual sites within one month of the closing date. The final summary report was provided later after compiling all the reports. The overall process of NEQAS mechanism is given in **Figure 1.**

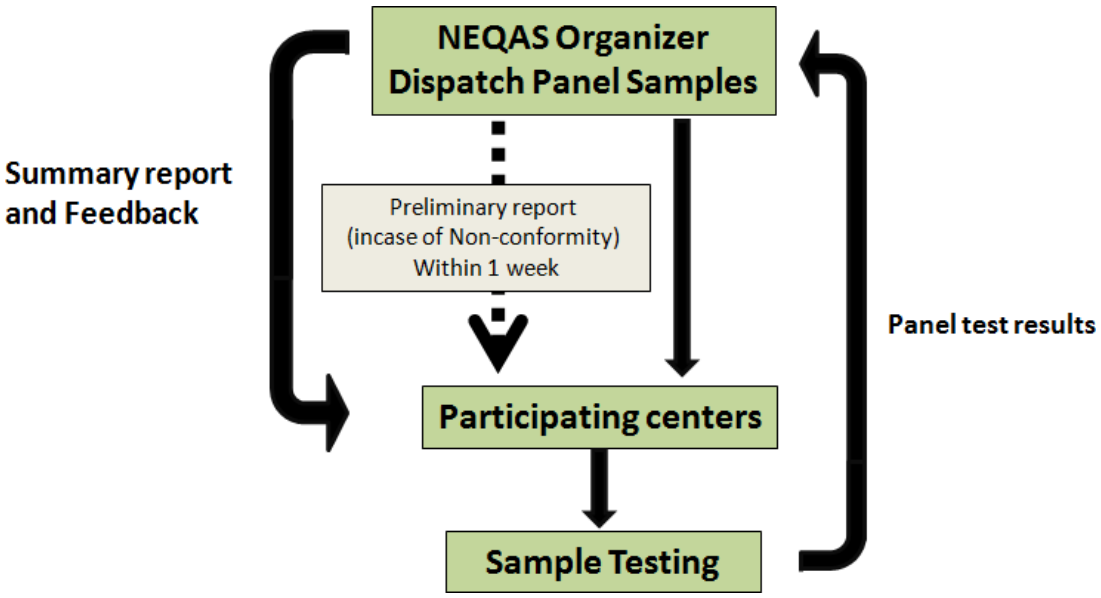

Figure 1. Flow process of STI NEQAS (figure adopted from Guideline NEQAS STI/TTI Serology, RCDC)

3. Results

Forty-three (74.1%) participants responded within the given deadline with the test results as shown in Figure 27. A prompt 9 (20.1%) of participants responded within the initial week following panel dispatch, while 6 (13.6%) took up to three weeks. Unfortunately, 15 (34.9%) participants did not respond, and 1 participant failed to record the test date. There were no instances of late responses.

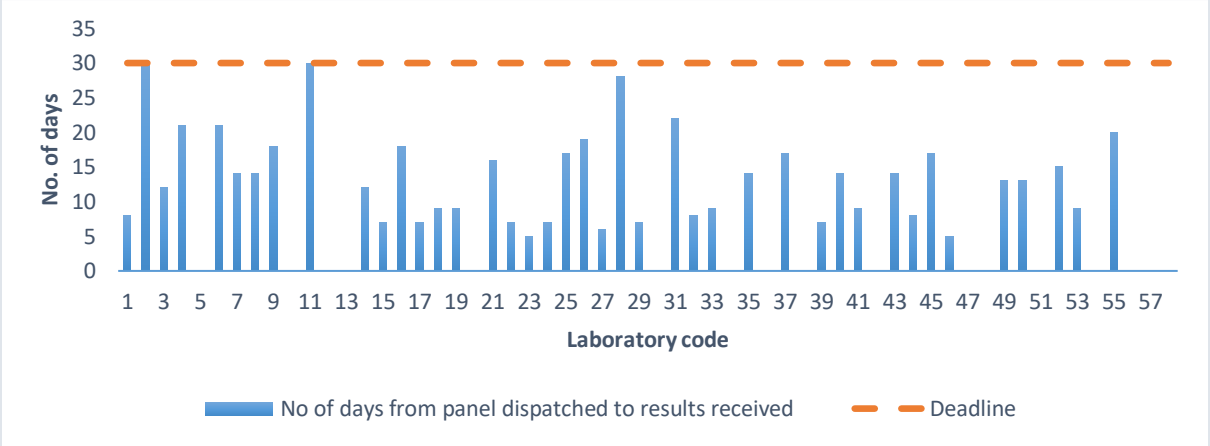

Figure 1: Number of days taken from dispatch of panel till the results received.

Part A: Test result conformity

In the analytical part, laboratories performed better in anti-HIV testing with 38 (88.4%) of participants scoring in excellent category, followed by testing of HBsAg, anti-HCV and anti-TP with 37 (86%), 23 (53.5%) and 17 (39.5%) respectively (**Figure 28**). Laboratories did not perform well in testing anti-TP with 4 (9.3%) of them needing improvement. Subsequently, 3 (7%), 2 (4.3%) and 1 (2.3%) laboratories also needed to improve in the testing of HBsAg, anti-HCV and anti-HIV respectively. Notably, 2 did not conduct the anti-HCV test; one cited the absence of a test kit, while the other provided no explanation. The latter also omitted the anti-TP test.

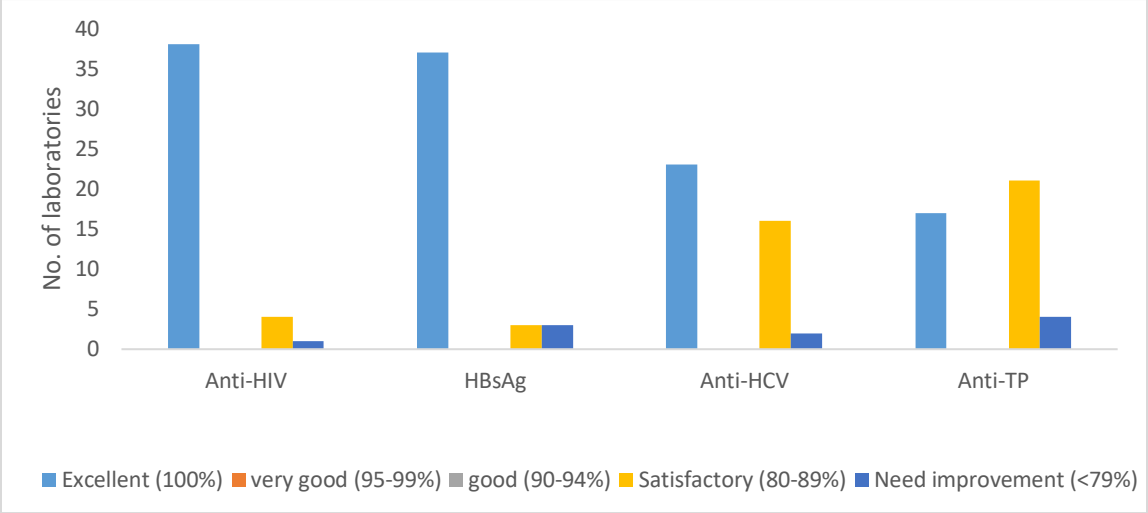

Figure 1: Performance of participating laboratories based on the conformity of their test results

Part B: Testing information

It was found that the laboratories largely used test kits from two different manufacturers. Bioline™ HIV 1/2 3.0 from Abbot, Korea and SD Bioline HIV ½ 3.0 from Standard Diagnostics, Korea were used almost equally. In this round of NEQAS, no laboratories used expired test kits, however, one laboratory had no test kit in stock for ant-HCV and anti TP. Overall, the majority of laboratories demonstrated proficiency in providing both test kit and testing details, as illustrated in **Figure 2**. Approximately 78.0% of them scored in very good and excellent category. However, there was also around 8.0% of the laboratories that failed to submit the required information in a correct format along with the test results.

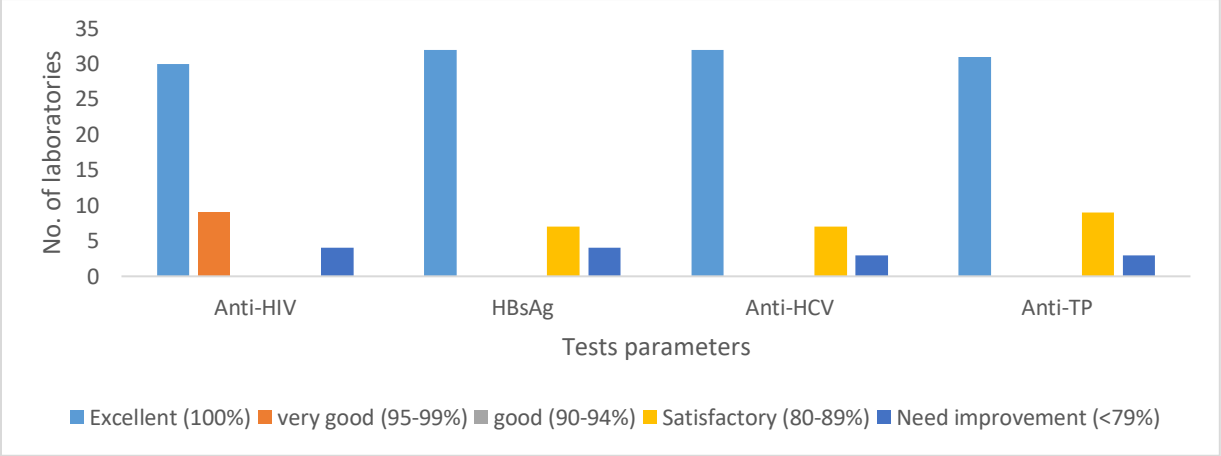

Figure 2: Performance of participating laboratories based on the testing information provided

Overall scoring

Laboratories performed better in HBsAg testing with 69.8% in the excellent category, followed by anti-HIV, anti-HCV and anti-TP, securing 62.8%, 41.9% and 27.9% respectively (**Figure 3**). Performance for the anti-TP test saw the worst results with 11.6% of the participants falling in the need improvement category. Accordingly, participants falling in need improvement for anti-HCV, HBsAg and anti-HIV testing were 7.0%, 4.7% and 2.3% respectively.

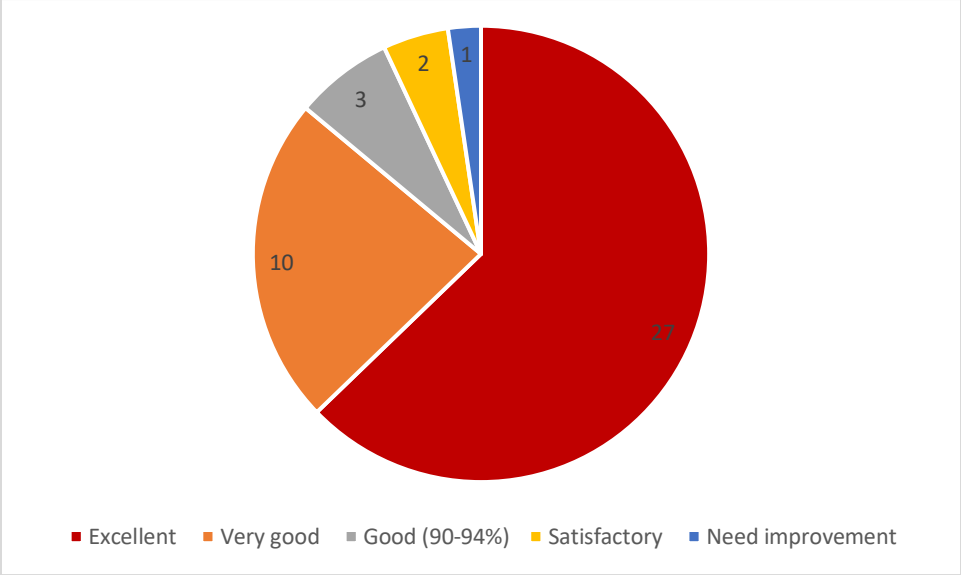

Figure 3: Number of laboratories falling in different criteria as per their overall performance in NEQAS STI/TTI panel 20, 2023

4. Discussion

Continuous quality improvement requires ongoing monitoring and review of all elements of the quality system, using both internal and external mechanisms, to ensure that the defined quality standards are being met consistently. EQA serves as a critical tool among various mechanisms to identify areas for enhancement and to benchmark a laboratory's performance against peers. (4).

The 20th round of NEQAS panel for STI/TTI was conducted after a gap of 2 years owing to COVID-19 pandemic restrictions in the previous years. Response from the participating laboratories was not satisfactory as there were 15 non-responders. While the absence of a laboratory technician was confirmed in one case, the reasons for non-participation in other instances remain speculative. It is plausible that the pandemic's impact, whereby by high staff turnover, left many positions filled by inadequately trained personnel. (7).

A similar reason could be associated to the unexpected high number of non-conforming test results. The test employed is a rapid immunochromatographic test, which is technically easier to perform

compared to most other tests. However, many failing to provide the test results correctly is a concern. There has been no sensitization or refresher workshop, or training given in any form as it should have been ideally done (2).

Laboratories have performed better in testing of anti-HIV and HBsAg while for anti-HCV and anti-TP, 8 laboratories fell in need improvement category. While it is difficult to exactly point out the reasons for this discrepancy, it is important that the laboratories strictly adhere to their standard operating procedures (SOP) and monitor the storage of their test kits as the temperature and humidity under which the test kits have been stored can affect its shelf life (8). A study has revealed that 62% of the laboratory errors were the results from pre-analytical phase, and a further 23% from the postanalytical phase (9).

We have also noted transcription errors in the results reported. Such errors can have a huge impact on the patient's test result and treatment decisions. It is important that the results generated from the tests are immediately recorded on the result sheet by the same person performing the test. Many laboratory workers have the tendency to perform the EQAS panel separately from routine samples. Since the results of the EQAS panel are meant to reflect the laboratory's day to day performance, the panel samples should be treated the same way as the routine and tested together (4).

Although VPVDL has communicated with few of the laboratories with gross discrepancies, we were limited in resources that did not allow us to get back to individual laboratory and find out the source of discrepancy or non-conformity. The absence of supervisory visits, on-site monitoring, and training in recent years has diminished the emphasis on NEQAS STI/TTI at the operational level, suggesting a need for renewed focus and support. .

Conclusion

Analysis of the NEQAS STI/TTI round 20 shows that laboratory personnels in general need to improve their performances in all pre-analytical, analytical and post-analytical phases of the rapid STI/TTI immunochromatographic assays. The report also highlights the importance of conducting NEQAS activities on a regular basis and follow up activities such as on-site assessment and trainings

for continuous quality improvement. Such systematic and consistent efforts are vital for elevating laboratory standards and ensuring reliable patient outcomes.

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V COVID-19 Integrated Influenza Surveillance for 2023

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1. Introduction

Influenza viruses are enveloped viruses under the family Orthomyxoviridae that contain a segmented RNA genome. Of the four types of influenza viruses, A, B, C, and D the first two are associated with significant seasonal morbidity and mortality. [1] Influenza or seasonal Influenza has caused major public health concerns infecting 5 – 15% of the global population [2]. Influenza virus infections contribute considerably to morbidity and mortality both in the temperate and tropical regions, causing 3 – 5 million cases of severe illness annually and 250,000 – 650,000 deaths annually. [2, 3]

In Bhutan, acute respiratory infection (ARI) is one of the top 10 diseases reported annually for the last ten years through the National Early Warning Notifiable Disease System (NEWARS).[4]A study showed that the incidence of influenza-associated hospitalizations among all age groups was 50/100,000 persons in 2015 and 118/100,000 persons in 2016. The highest rates were among children <5 years: 182/100 000 in 2015 and 532/100 000 in 2016.[5]

Influenza-associated infection has contributed to a substantial burden of severe illness requiring hospitalization, especially among children, older adults and those with underlying conditions. This annual report of COVID-19 integrated Influenza surveillance describes the Influenza activity and COVID situation in the country with epidemiological and virological analysis.

2. Method

We have adopted a prospective sentinel-based surveillance protocol design for influenza and other viral respiratory pathogens including ARI outbreaks from non-sentinel sites as per the COVID-19 Integrated Influenza surveillance guideline.[8] Influenza sentinel hospitals are: Jigme DorjiWanghck National Referral Hospital, Thimphu, Punakha Hospital, Paro Hospital, Monggar Regional Referral Hospital, Phuntsholing Hospital, Gelephu Regional Referral Hospital, Trongse Hospital, Tsirang

Hospital and Trashigang Hospital. The sentinel sites were selected based on strategic geographical locations and demographic representation (**Figure 1**).

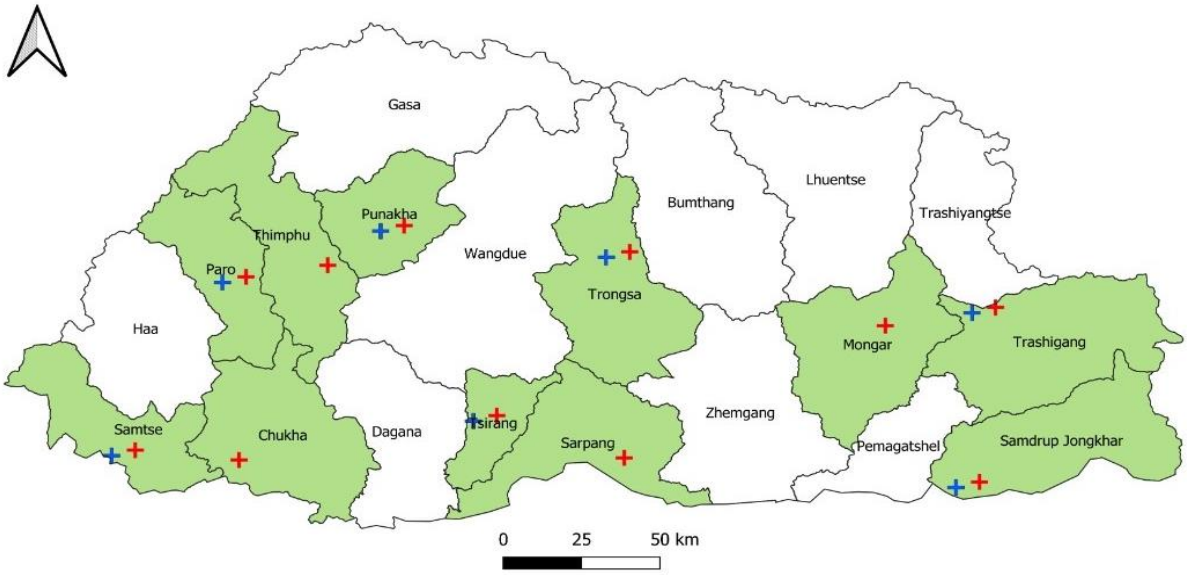

Figure 1: ILI Surveillance sites & SARI Surveillance Sites

Participants and Sample Size

Clinical and epidemiological information was collected from patients as per the case definition for influenza-like illness (ILI) and severe acute respiratory infection (SARI). A respiratory swab specimen was collected after getting the informed consent. Specimens were collected as part of routine patient care through a surveillance network of outpatient clinics, inpatient clinics, or from the Flu clinic.

As per the guideline, each identified sentinel site for ILI enrolled 10-15 cases per week (20-40 cases per month) for specimen collection, representing all age groups. Meanwhile, all the SARI cases were recommended for specimen collection.

Case Definition

Severe acute respiratory infections (SARI): An acute respiratory infection with a history of fever or measured fever of ≥ 38° C and cough with onset within the last 10 days and requires hospitalization.[9]

Influenza-like Illness (ILI): An acute respiratory infection with a measured fever of ≥ 38° C and cough with onset within the last 10 days.[9]

Inclusion and Exclusion criteria

Inclusion Criteria:

Subjects meeting all the following criteria were considered eligible for enrolment in the study:

- Male or female (including pregnant women) patients ≥ 6 months of age
- Fever (oral temperature ≥ 100.5 F or 38˚ C; axillary temperature≥ 99.5˚ F or 37.4˚ C; rectal temperature ≥ 101˚ F or 38.6˚ C) AND cough or sore throat
- Presentation to health care within 10 days of reported fever onset
- Signed informed consent by patient and parent or legal guardian. For minor's ages between 7-17 years, a signed assent is required.

Exclusion Criteria: Subjects meeting any of the following criteria were excluded from the study:

- Immunocompromised host such as those with Acquired Immune Deficiency Syndrome, Lymphoma or Leukemia
- Suspected case of TB

Laboratory Detection

RT-PCR

Viral RNA was extracted from 200µl of Throat/Nasal swabs collected in Viral Transport media using Spin-X Viral RNA Extraction Kit (SD Biosensor, South Korea) and KingFisherTM Flex Purification system (Thermo Fisher, India) following the manufacturer's instructions. The extracted viral RNA was subjected to RT-PCR using Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay (US-CDC). 5µl of viral RNA was added in the tube containing the mixture of 7.75µl of nuclease-free

water, 3µl of Flu SC2 combined primer mix, 3µl of Flu SC2 combined probe mix and 6.25µl TaqPathTM 1-Step Multiplex Master Mix (No ROX).

The cycling condition was set at 1 cycle of reverse-transcription step at 20ºC for 2 minutes and 50ºC for 15 minutes followed by 1 cycle of pre-denaturation at 95ºC for 3 minutes followed by 45 cycles of 95ºC at 15 seconds, 55 ºC for 33 seconds. Ct value <40 for InfA, InfB, SC2 and <35.00 Ct for RP was considered positive whereas, ct value ≥40.00 for InfA, InfB, SC2 and ≥35.00 Ct for RP were considered negative. For each protocol positive and negative controls were run to validate the test result. Furthermore, samples that tested positive for Influenza A and B virus were subtyped and lineage determined respectively using Human Influenza Virus Real-Time RT-PCR Diagnostic Influenza A/B Typing Kit (US-CDC) based on manufacturer's instruction.

Data Collection

All the demographic details including clinical and laboratory information were collected through a structured surveillance questionnaire, which were verified by RCDC before entering into the COVID-19 Integrated Influenza surveillance system. All the laboratory results were entered into the system and the same were shared in PDF to relevant stakeholders and sentinel sites.

Data Analysis

Surveillance data were extracted from the COVID-19 Influenza Integrated Surveillance System and NEWARS. Descriptive statistics were used to analyses the results including patient demographics to see the proportion of Influenza-associated ILI and SARI. All data were analyzed using Epi Info7. QGIS 3.16 was used to generate a mapping distribution of the Influenza positivity in the country.

3. Result

Weekly ILI and SARI cases were reported from 7 ILI sentinel sites and 11 SARI sentinel sites respectively to RCDC. A total of 8,379 ILI cases were reported, 48.8% more compared to the previous year (4,287). Similarly, a total of 1,250 SARI cases were reported, which is 47.9% more compared to the previous year (651). Weekly, on average 19.2 ILI cases per 1000 outpatient visits and 78.7 SARI cases per 1000 admitted patients were reported. Though ILI were reported throughout the year, more cases were observed in weeks 31 – 40 (Aug – Oct) (**Figure 2**).

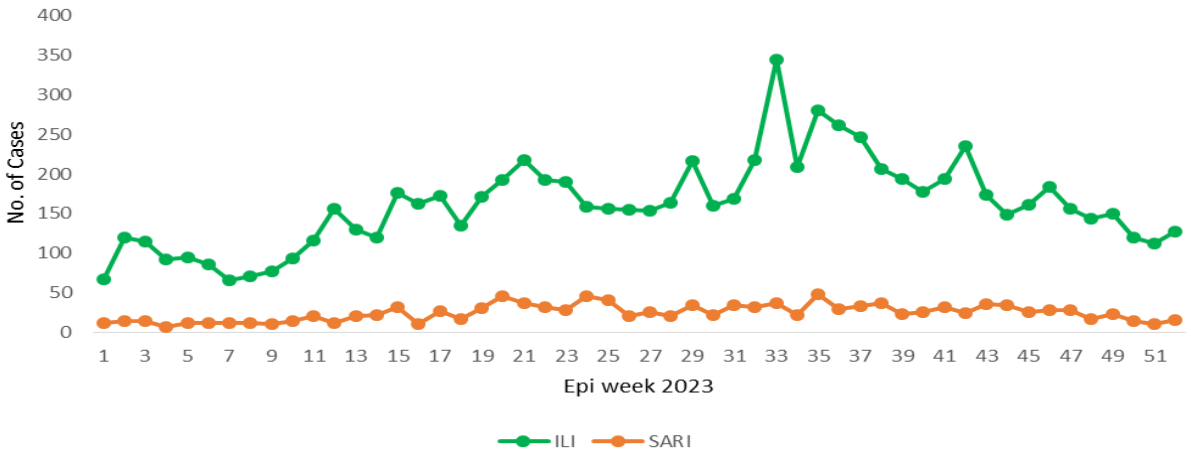

Figure 2: Weekly ILI and SARI cases reported by sentinel hospitals in 2023

The most affected age group for ILI was 5 – 14 years [27.4% (2319)], followed by 15 – 29 years [22.0% (1842)] and 30 – 64 years [21.5% (1803)]. While for SARI, the most affected age group was 0 – 1 years [(28.4% (355)], followed by above 65 years (22.2%) and 2 – 4 years (19.8%) (**Figure 3**).

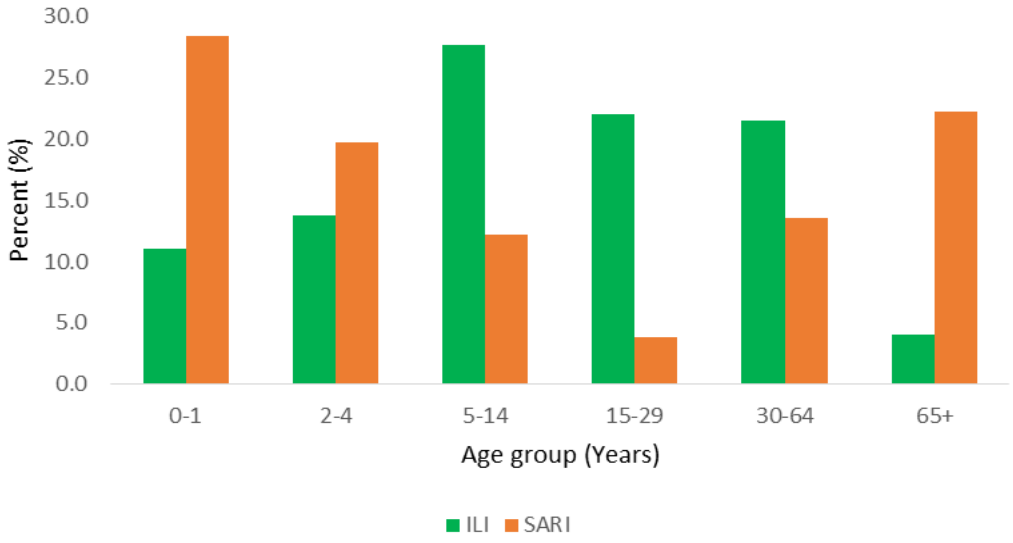

Figure 3: ILI and SARI cases by age group in 2023

Paro Hospital (2,440) has reported the maximum number of ILI cases, followed by Samdrup Jongkhar Hospital (2,178). Similarly, Gelephu CRRH (290) has reported the majority of SARI cases compared to other sentinel hospitals (**Figure 4**).

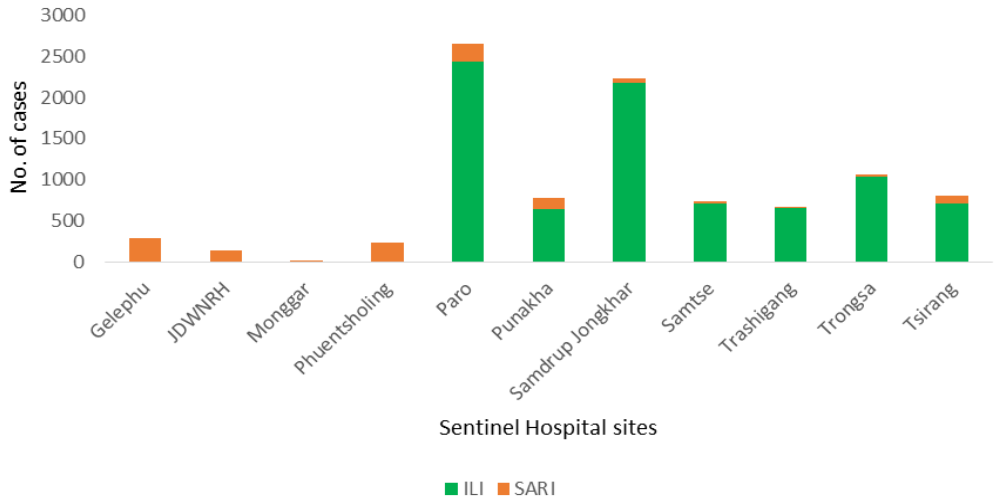

Figure 4: Number of ILI and SARI cases reported by Sentinel Hospitals in 2023

Virological surveillance

A total of 4178 respiratory swab specimens were received, of which 71.3% (2978) were from ILI, 23.4% (978) were SARI, and 5.3% (222) were from ARI outbreaks. The weekly influenza positivity rate was monitored throughout the year. On average, the weekly influenza positivity rate was 24.2% (range: 1.8 – 47.1%) and SARS-CoV-2 was 3.8% (range: 0 – 21%). The overall influenza positivity rate among ILI was 30.3% (901), SARI was 10.4% (102) and ARI outbreak was 58.1% (129). The influenza A/H3 subtype (30%) was the most predominant circulating strain, followed by influenza B/Victoria (27.9%), and influenza A/H1pdm09 subtype (15.3%) (**Figure 5**).

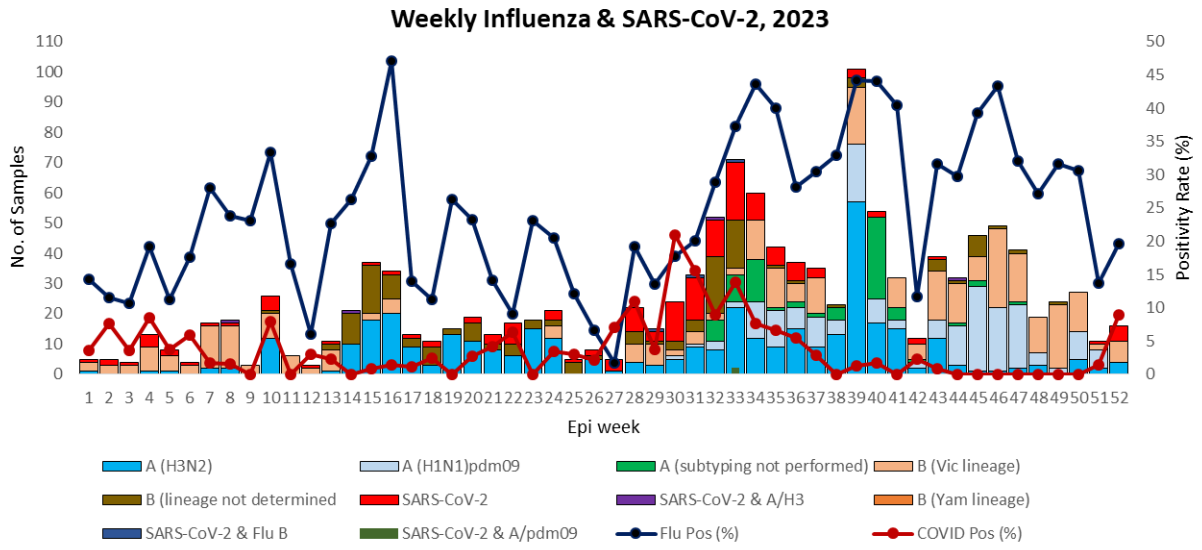

Figure 5: Weekly trend of Influenza subtypes and SARS-CoV-2 (source: RCDC)

The mean age for Influenza among ILI was 21.7 (0.5 – 79) years and the median was 18 (9 – 79), while the mean age for Influenza among SARI was 22.9 (0.08 – 90) years and median age was 6 (1.4 – 48) years. The most affected age group for Influenza among ILI was 5 – 14 years (34.3%) followed by 30 – 64 years (29.7%) and 15 – 29 years (26.6%). While, the affected age group for Influenza among SARI was 0 – 1 years (30.4%) followed by 5 – 14 years (17.6%). Clinically the most common symptoms presented for Influenza were cough (95.2%), fever (92.7%) and sore throat (72.1%) as shown in the table 1 (**Table 1).**

Table 1: Patient demographic and clinical characteristics of ILI and SARI

Variables	All ILI n	Influenza n(%)	SARS-CoV-2 n(%)	Coinfection n(%)	All SARI n(%)	Influenza n(%)	SARS-CoV-2 n(%)
Total	2987 (71.3)	901 (30.3)	131 (4.4)	8 (0.3)	978 (23.4)	102 (10.4)	20 (2.0)
Age							
Mean age (range), years	22 (0.08 – 85)	21.7 (0.5 – 79)	32.8 (0.4 – 76)	30.8 (7 – 75)	21.9 (0.08 – 99)	22.9 (0.08 – 90)	34.1 (0.1 – 96)
Median age (IQR), years	18 (8 – 33)	18 (9 – 79)	34 (17 – 45)	25 (12 – 45)	3.9 (1 – 45)	6 (1.4 – 48)	19 (0.5 – 72.5)
Age group							
0 – 1 years	129 (4.3)	18 (2.0)	3 (2.3)	0	357 (36.5)	31 (30.4)	9 (45.0)
2 – 4 years	266 (8.9)	56 (6.2)	5 (3.8)	0	160 (16.4)	15 (14.7)	0
5 – 14 years	889 (29.8)	309 (34.3)	18 (13.7)	3 (37.5)	127 (12.9)	18 (17.6)	1 (5.0)
15 – 29 years	795 (26.7)	240 (26.6)	28 (21.4)	1 (12.5)	38 (3.9)	5 (4.9)	1 (5.0)
30 – 64 years	823 (27.6)	268 (29.7)	70 (53.4)	3 (37.5)	127 (12.9)	18 (17.6)	3 (15.0)
>65 years	76 (2.6)	10 (1.1)	7 (5.3)	1 (12.5)	169 (17.3)	15 (14.7)	6 (30)
Sex							
Female	1353 (45.4)	414 (45.9)	66 (50.4)	3 (37.5)	463 (47.3)	52 (51.0)	11 (55.0)
Male	1625 (54.6)	487 (54.1)	65 (49.6)	5 (62.5)	515 (52.7)	50 (49.0)	9 (45.0)
Clinical symptoms							
Fever	2661 (89.4)	835 (92.7)	120 (91.6)	6 (75)	833 (85.2)	98 (96.1)	18 (90)
Cough	2811 (94.4)	858 (95.2)	128 (97.7)	7 (87.5)	850 (86.9)	92 (90.2)	20 (100)
Sore throat	2016 (67.7)	650 (72.1)	98 (74.8)	3 (37.5)	406 (41.5)	41 (40.2)	8 (40)
Breathing problem	321 (10.7)	103 (11.4)	14 (10.7)	1 (12.5)	565 (57.8)	56 (54.9)	15 (75)
Headache	1543 (51.8)	559 (62)	78 (59.5)	5 (62.5)	99 (10.1)	12 (11.8)	3 (15)

We have received a total of 4108 samples (ILI- 2978, SARI- 976). Samtse Hospital (661) has enrolled more ILI cases followed by Trongsa Hospital (553). Influenza and COVID-19 positives are detected from all the sentinel hospitals with flu-positive percent ranging from 14.8 – 44.4% (**Figure 6**). The average time taken for receiving samples from sentinel sites to nearest COVID-19 testing lab was 6.7 days (range: 1.8 – 14.7 days), while the average time taken for testing was 3.3 days (range: 1.2 – 9.8 days) (**Figure 7**). More ILI and SARI samples received than the previous year as shown below (**Table 2a & 2b**).

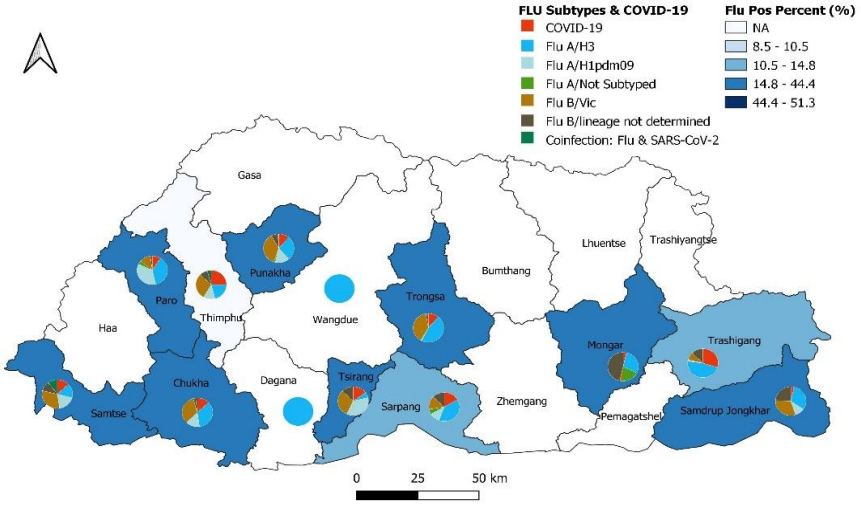

Figure 6: Proportion of Influenza subtypes and SARS-CoV-2 by districts

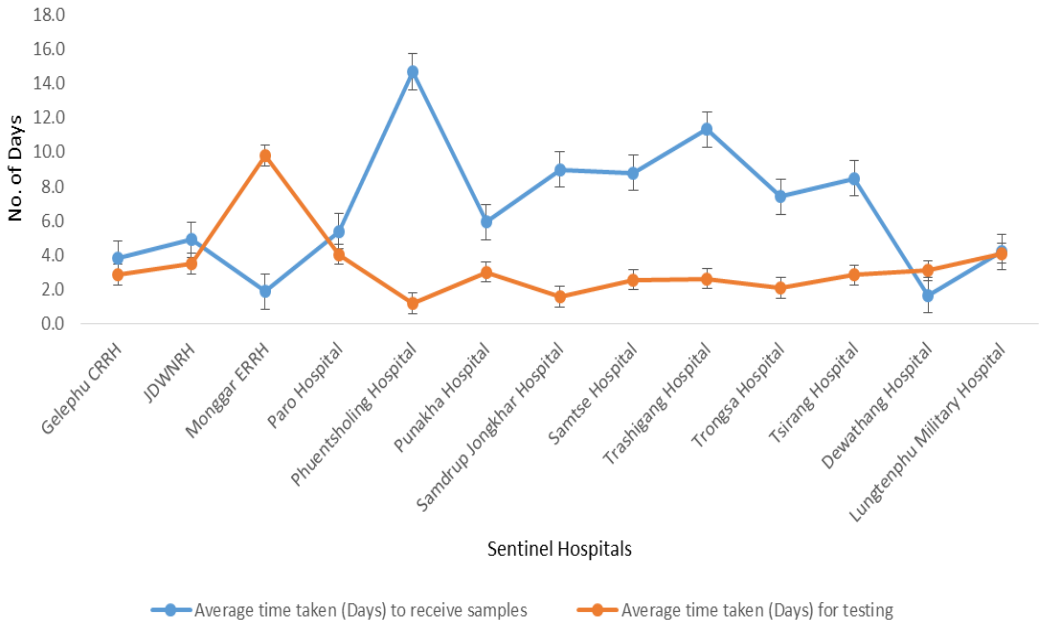

Figure 7: Average turn-around time for receiving and testing ILI and SARI samples

Table 2a: Overall ILI and SARI surveillance status in 2023

	ILI Surveillance				SARI Surveillance		
Sentinel Hospital sites	**ILI cases Reported**	**No. of ILI Samples collected**	**Percent (%) enrolled**		**SARI cases Reported**	**No. of SARI Samples**	**Percent (%) enrolled**
Gelephu	NA	89			290	242	83.4
JDWNRH	NA	5			141	178	126.2
Monggar	NA	68			15	5	33.3
Phuentsholing	NA	283			233	209	89.7
Paro	2440	160	6.6		219	143	65.3
Punakha	640	295	46.1		140	33	23.6
Samdrup Jongkhar	2178	168	7.7		49	16	32.7
Samtse	709	661	93.2		29	28	96.6
Trashigang	661	204	30.9		8	8	100.0
Trongsa	1040	553	53.2		25	25	100.0
Tsirang	711	306	43.0		101	89	88.1
Grand Total	**8379**	**2792**			**1250**	**976**	

Table 2b: Number of Influenza subtypes and SARS-CoV-2 received and tested in 2023

	ILI												SARI								
Seninel Sites/ Hospitals	A/H3 & SARS-CoV-2	A/Pdm09 & SARS-CoV-2	B/Vic & SARS-CoV-2	A/ Pdm09 & B/Vic	A/H3	A/Not Subtyped	A/ Pdm09	B/Lineage Not Determined	B/Vic	SARS-CoV-2	Neg	ILI Total	A/H3	A/Not Subtyped	A/ Pdm09	B/Lineage Not Determined	B/Vic	SARS-CoV-2	Neg	SARI Total	Grand Total
Gelephu CRRH					13	2	1	3	7	6	57	89	7	1	5	4	1	3	221	242	346
JDWNRH											5	5	1		3	1	3	3	167	178	183
Monggar ERRH					8	4		12	1	1	42	68		1		1			3	5	86
Paro					29	2	27	2	11	3	86	160	9	2	9		1	6	116	143	308
Phuentsholing	1			1	29		10	3	35	11	193	283	11		7		3	4	184	209	492
Punakha		1			35	2	20	7	47	14	169	295	2		1	3	3	1	23	33	362
Samdrup Jongkhar					4		6	3	17	2	136	168				1			15	16	184
Samtse	1	1	2		38		42	25	71	30	451	661			1		2		25	28	689
Trashigang					27		1	7	5	17	147	204							8	8	276
Trongsa			1		99		1	5	84	23	340	553	2		1		1	1	20	25	578
Tsirang					7		43	18	45	21	172	306	2		11	1	1	2	72	89	395
Wangduephodrang					1							1									1
Chhukha BHU I											1	1									1
Dagana BHU I					5						2	7									7
Dewathang					14			10	1		73	98							1	1	110
Haa											5	5									15
Lungtenphu	1				4			1	4	3	56	69							1	1	70
RCDC							1				1	2									2
Sarpang					1						2	3									3
Grand Total	**3**	**2**	**3**	**1**	**314**	**10**	**152**	**96**	**328**	**131**	**1938**	**2978**	**34**	**4**	**38**	**11**	**15**	**20**	**856**	**978**	**4108**

4. Discussion

In 2023, Bhutan recorded more Influenza activity, despite the disruption caused to the surveillance by the COVID-19 pandemic for the past two years. [10] Influenza positivity rate was monitored weekly along with the SARS-CoV-2. Influenza activity has increased in Bhutan, especially towards the end of the year from October – December 2023. Influenza positivity was substantially high (15.2%) with Influenza A(H3N2) (70.2%) being the most predominating strain during the year. Globally, influenza activity remained elevated in the northern hemisphere where subtypes, influenza A viruses predominated with a slightly larger proportion of A(H3N2) viruses.[11]

The sudden surge in cases could be due to many ARI outbreaks occurring in schools. This is the time of Flu season particularly in the Northern Hemisphere. In temperate regions, influenza activity is known to peak during the winter months. In the Northern Hemisphere, influenza outbreaks and epidemics typically occur between October and May, whereas in the Southern Hemisphere, influenza activity occurs between April and September. In tropical regions, influenza can occur throughout the year. [1, 2, 4]

SARS-CoV-2 (20.6%) positivity among ILI and SARI cases was found proportionately high and significantly contributing to the disease burden. It was observed globally that SARS-CoV-2 positivity from sentinel surveillance continued to increase in the WHO Regions of the Americas and Western Pacific too. [11]

This study found the median age of Influenza among ILI was higher than the SARI. The findings were similar to the previous studies that showed seasonal Influenza morbidity is more in younger ages particularly below 30 years, though the mortality age group differs significantly. [12]

Challenges and Limitations

Sustenance of equipment, reagents, and consumables, lack of dedicated fund allocation, and less priority, etc., are some of the challenges faced in sustaining the surveillance system. We are unable to initiate routine testing of other respiratory viruses like RSV, Adenovirus, Para influenza virus, and hPMV, due to the unavailability of RT-PCR reagents.

Recommendations/Way forward

Surveillance data is evidence-based, critical for policy decisions, helps in the prevention and control of the disease, and needs continuous monitoring by surveillance. Therefore, the following are some of the recommendations we put forward for strengthening and sustaining the surveillance:

- Decentralize the public health surveillance to district hospitals as a responsibility, not an opportunity.
- Provide regular/periodic training for healthcare providers to ensure continuity of surveillance.
- Conduct periodic monitoring and supervisory visits to surveillance sites.
- Encourage surveillance sites to initiate regular sensitization program.
- Establish a shipping mechanism for non-surveillance sites.
- Develop a national laboratory policy for and implement laboratory networking.
- Provide expertise in relevant fields and transfer technology to sub-national PCR laboratories.
- Streamline the procurement procedure for equipment, reagents, and consumables for high-end equipment.

- Establish direct service contracts for high-end equipment maintenance with the manufacturer or principal dealer.

Conclusion

Influenza activity increased in 2023 compared to the previous years. Influenza was detected with 24.4% positive and 3.7% SARS-CoV-2 positive from the surveillance. The ARI outbreaks were reported occasionally from the schools and community, the outbreaks were caused mostly by Influenza.

Influenza and SARS-CoV-2 positivity rates were detected substantially high particularly during Flu seasons in 2023. Influenza A(H3N2) subtype was found to be the most predominant circulating strain in the country and globally as well. Continuous monitoring and laboratory surveillance are recommended to strengthen and enhance sustainable robust COVID-19-integrated Influenza surveillance in the country.

Influenza and other respiratory pathogens have the potential to cause public health threats for future pandemics, therefore, strengthening and sustenance of the surveillance is imperative. However, the sustenance of the surveillance is challenging particularly due to the lack of dedicated fund allocation, policy, and low priority.

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VI National Drinking Water Quality Surveillance, report 2023

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1. Introduction

Access to clean and safe drinking water is a fundamental human right and plays a crucial role in improving global well-being and sustainable development. Ensuring access to safe drinking water serves as a crucial factor to reduce waterborne diseases and significantly reducing the burden of illnesses like diarrhea and preventing untimely deaths (1) (2). The World Health Organization and the United Nations Children's Fund have been at the forefront of promoting safe drinking water and sanitation, and has been working with countries to improve access to safe drinking water. The Sustainable Development Goal 6 (SDG 6) - "Clean Water and Sanitation," emphasizes the significance of having universal access to water and sanitation by 2030 (3). Within this wider context, the emphasis on drinking water quality surveillance stands as a key element in achieving SDG 6.

Bhutan, in line with these global objectives, is determined in its efforts to provide clean and safe drinking water for its population. Despite the challenges posed by climate change, industrialization, and increased developmental activities, Bhutan is striving to improve the drinking water quality surveillance which plays crucial role to help maintain the integrity and safety of water sources. The continuous monitoring and assessment of water quality not only serve to identify and mitigate potential health risks but also contribute in building a resilient community in the face of environmental changes. The Ministry of Health (MoH) as mandated by Water Act of Bhutan (2011), is responsible for monitoring drinking water quality nationwide (4). The Royal Center for Disease Control (RCDC), in collaboration with health centers nationwide, has been at the forefront of conducting drinking water quality surveillance activities throughout the country. This report attempts to present the state of drinking water quality in Bhutan for the year 2023. By looking at the details of drinking water quality, we can gain valuable insights into the existing water quality, efficacy of the treatment process and identify areas that requires specific interventions.

2. Methodology

As per Bhutan Drinking Water Quality Standard (BDWQS) 2016, there are two types of drinking water surveillance systems in Bhutan, one for urban areas and another for rural area. There are a total of 39 health centers catering to urban drinking water quality surveillance, conducting water quality tests every month. On the other hand, rural water quality surveillance in conducted by 257 health centers on bi-annual basis (representing dry and wet season). The water quality monitoring reporting is done through the web-based water quality monitoring system (WaQMIS) which is developed and hosted by RCDC. The overall dataflow for the surveillance is as shown in **Figure 1**.

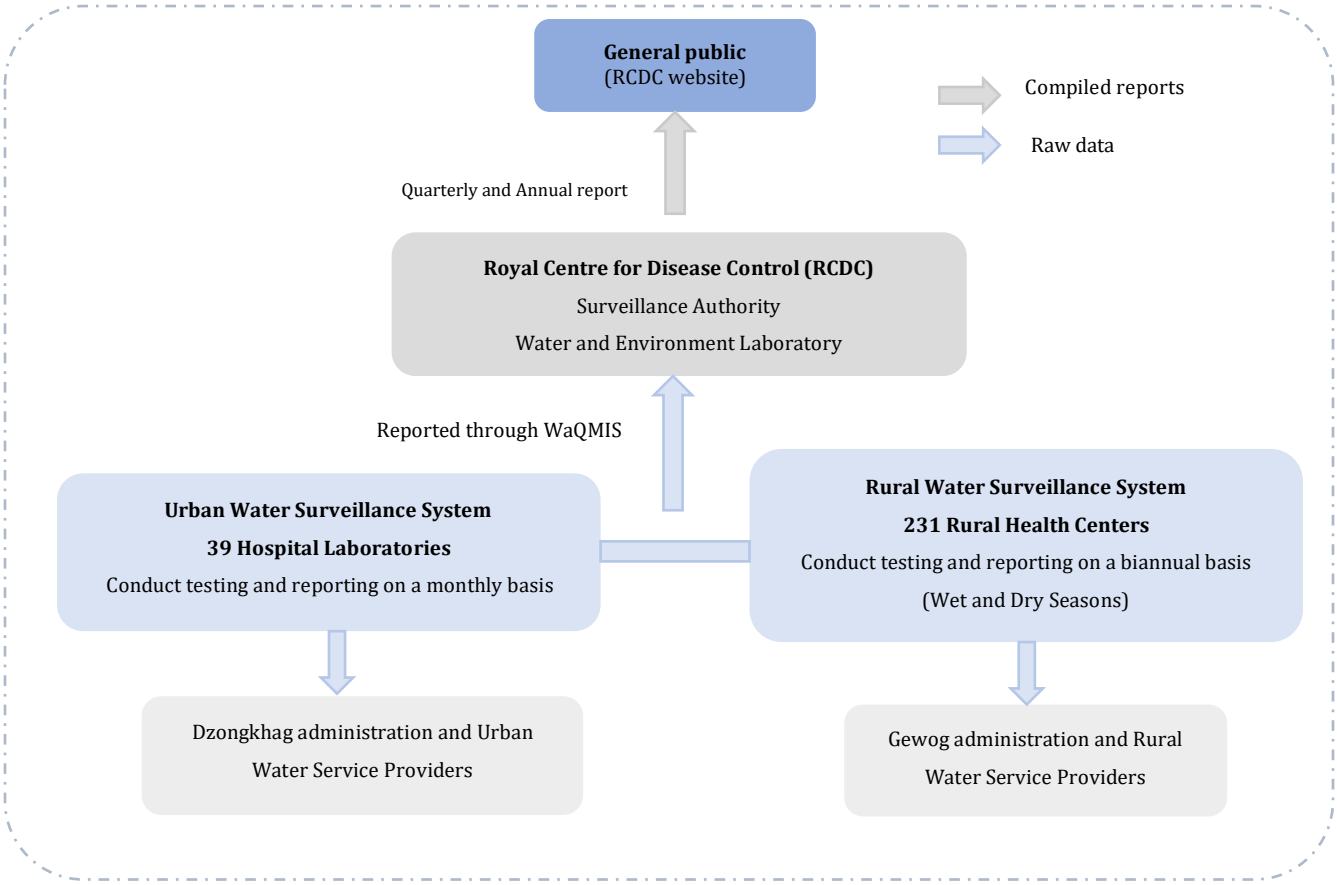

Figure 1 Existing data flow mechanism

In accordance with the National Guideline for Drinking Water Quality Surveillance (NGDWQS) 2019 (5) or the laboratory standard operating procedures, water samples are collected in sterile

containers from preidentified sampling points within the respective surveillance centers. The parameters that are currently analyzed by the urban and the rural surveillance centers are shown in **Table 1**. The laboratory analyses of the samples are performed in accordance with the standard operating procedure for the specific test parameters. All the data from the surveillance sites are reported through WaQMIS as per the requirement given in the BDWQS and NGDWQS. All the data in this report is presented as the mean ± SEM.

Table 1: List of test parameters in surveillance health centers for monitoring drinking water quality in urban and rural health areas.

Test parameters (Urban)	**Test parameters (Rural)**
E. coli	*E. coli*
Turbidity	Turbidity
pH	pH
Conductivity	Conductivity
Free residual chlorine	Color
Color	Odor
Odor	

3. Results

Urban Drinking Water Quality surveillance

In 2023, a total of 2341 samples were collected and tested from 38 health centers. As shown in **figure** 2, the majority of these samples, about 2140, were sourced from stream, followed by around 50 from spring and 40 from rivers. The remaining samples were obtained from groundwater or other sources. Out of the tested samples, approximately 38.0% were treated and supplied by Thromde or municipalities, while the rest were untreated water supplies.

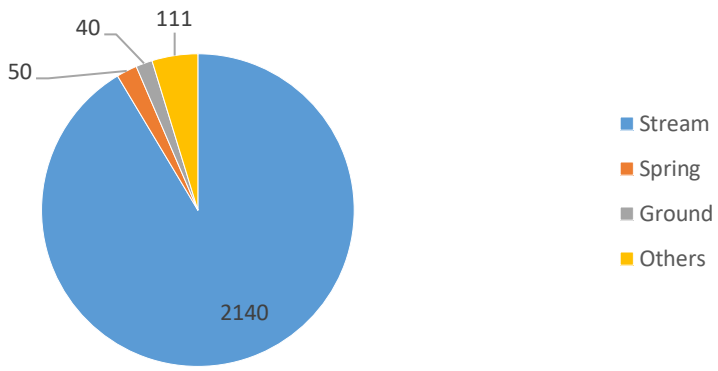

Figure 2. Different types of water sources included in the report

***E. coli* (Urban)**

From the total sample, 2214 samples were tested for *E. coli*. The test result showed that 58.7% samples were in compliance with the Bhutan drinking water quality standard for *E. coli* (0 CFU/100mL) and the rest were non-compliant and unsafe for consumption. Additionally, 6.5% of the total non-compliant samples were found to be grossly contaminated (>50 CFU/100mL). **Figure** 3 entails the overview of the compliance proportions across 19 dzongkhags (data from Gasa dzongkhag were not available through WaQMIS). **Figure 4** shows the details of compliance status for respective health centers. The number of samples tested were higher during rainy seasons with highest number of sample (352) tested during July (**Figure 5**).

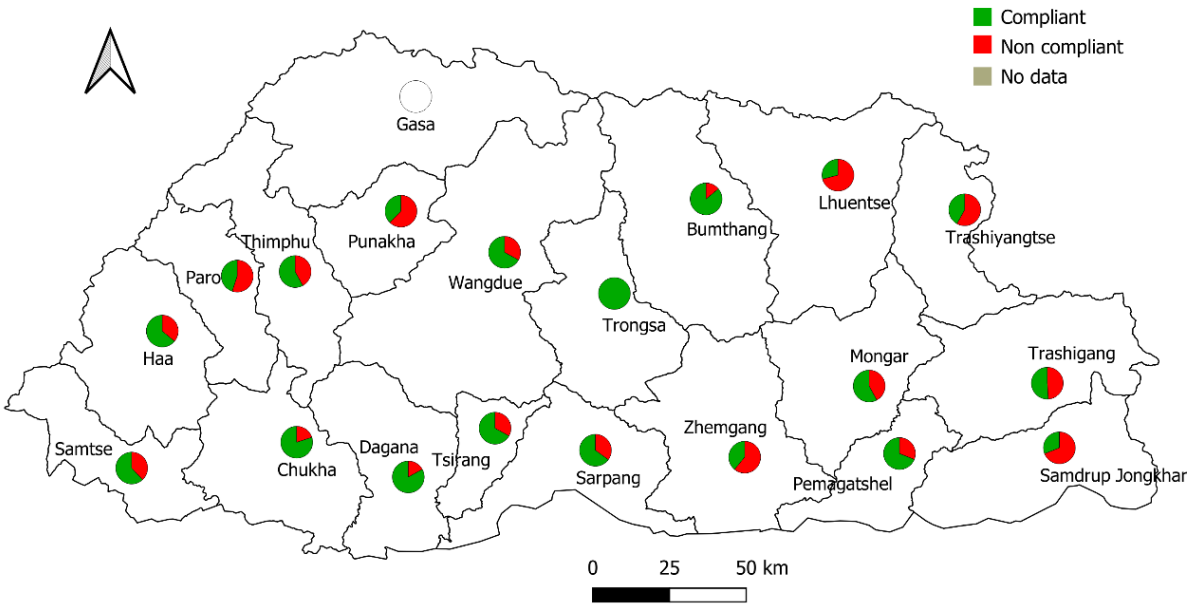

Figure 3. Overview of *E. coli* compliance in urban areas.

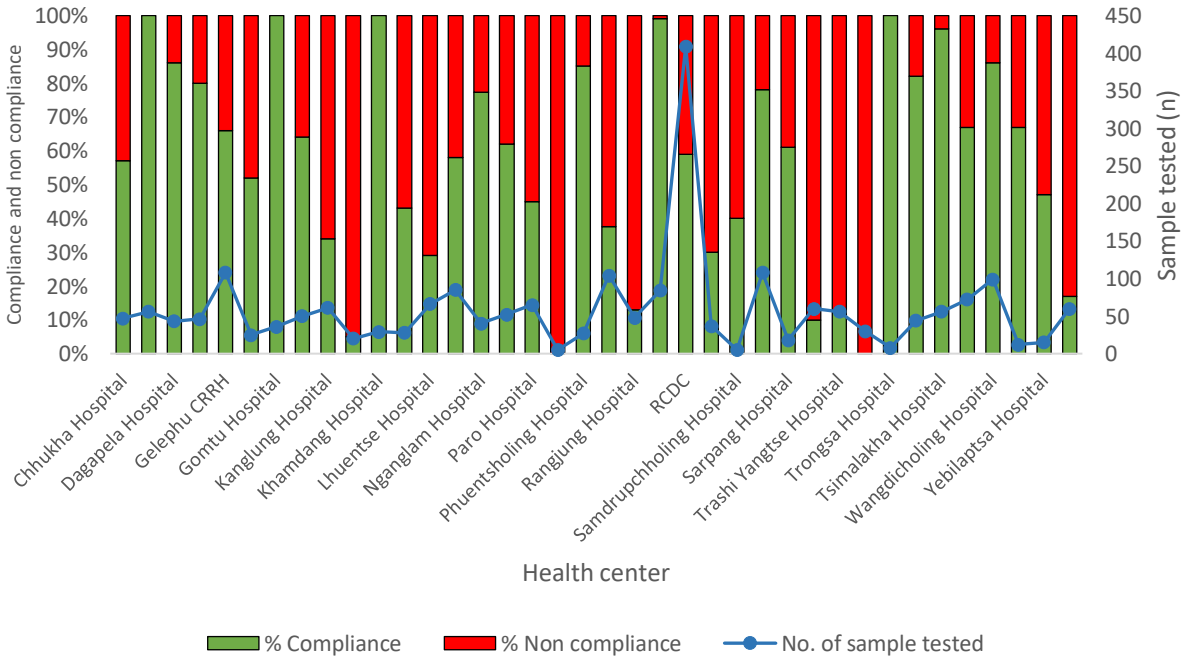

Figure 4. Individual Health Center Compliance status from individual health centers

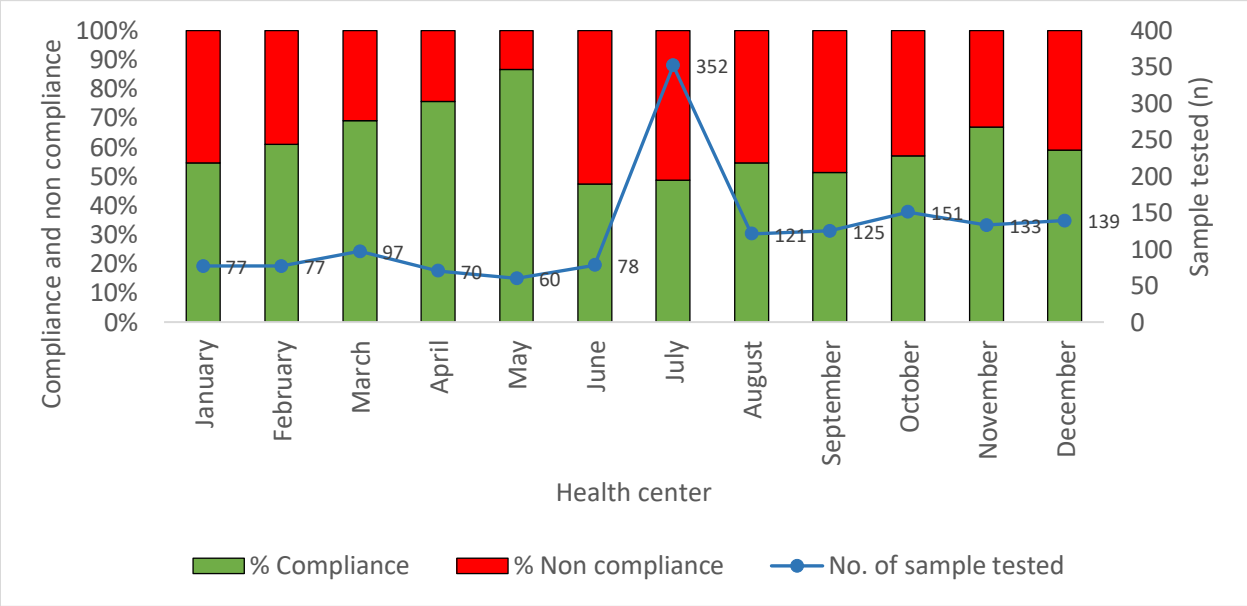

Figure 5. Number of samples tested by month

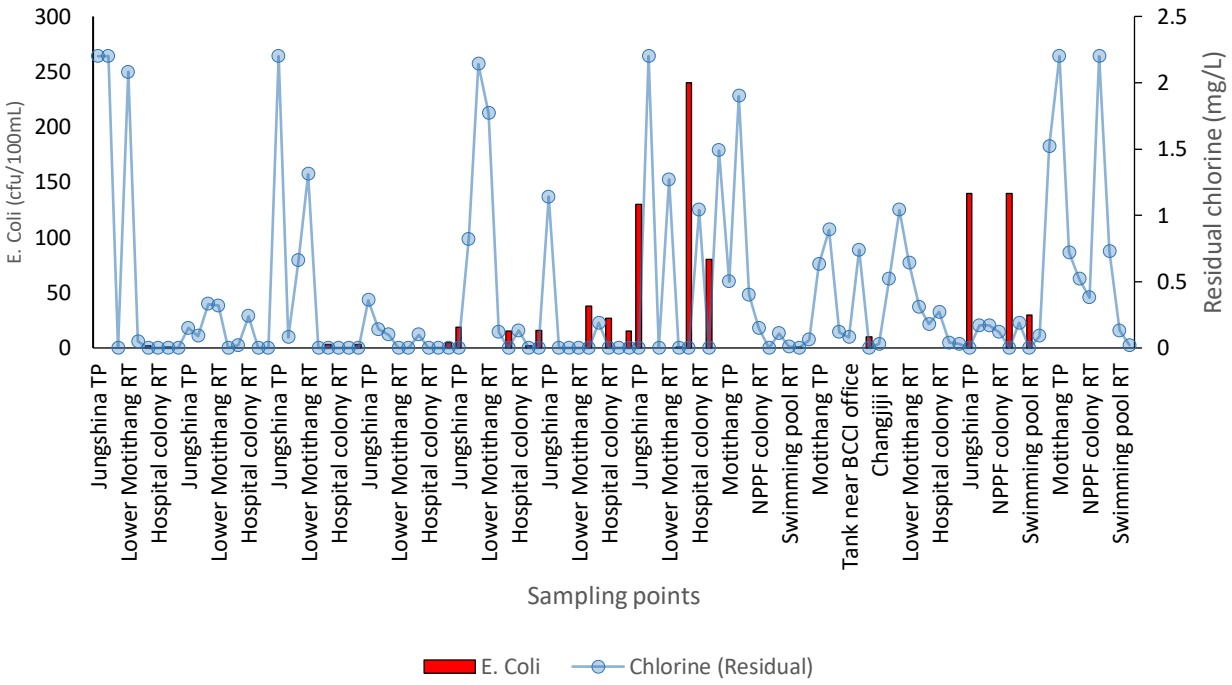

Figure 6. *E. coli* and residual chlorine level in treated water (before water meter)

A total of 108 water sample were collected from sampling points before water meter for Thimphu Thromde (As per the Water regulation of Bhutan 2014, it is responsible for municipal or Thormde to ensure safe water till water meter, after the water meter, individual household owner should take responsibility and clean their reservoir regularly) (6). It was found that out of the total samples before water meter, 40 (37. 0%) samples did not have residual chlorine (0 mg/L) subsequently 21 (52.5%) of these samples had *E. coli* in them. On the other hand, the remaining samples had residual chlorine ranging from 0.01 to 2.2 mg/L of residual chlorine and did not contain *E. coli* in these samples (**Figure 6Figure**).

Physio-Chemical Report (Urban)

Except for residual chlorine, most of other physical parameters including conductivity, TDS, pH, color, odor, turbidity were within acceptable limit required as per BDWQS. This report highlights only pH and turbidity and residual chlorine for 2023.

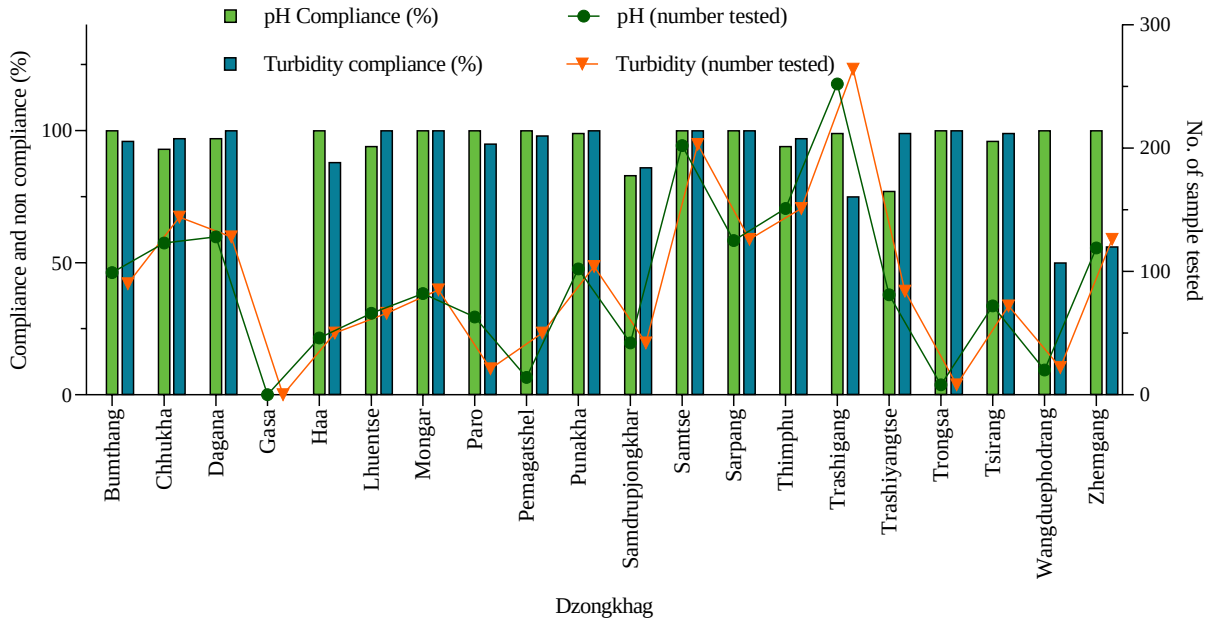

Figure 7. pH and turbidity compliance at dzongkhag level

pH (Urban)

A total of 1795 samples were tested for pH and 96.9% were found to have pH within acceptable limit as per BDWQS (6.5-8.5). **Figure** 7 shows overall compliance of pH and number of samples tested across 20 dzongkhags. The mean pH value was found to be 7.1 ± 0.01. The highest pH tested was found to be 9.0 and the lowest value was tested as 5.0.

Turbidity (Urban)

A total of 1836 samples were tested for turbidity across the country and 91.5% were found in compliance with BDWQS (<5 NTU) **(Figure 8).** The mean turbidity value was found to be 2.1 ± 0.18 NTU. It was observed that the mean turbidity values varied with seasonal change which gradually increases from May with peak reached during July and gradually declined from August to September.

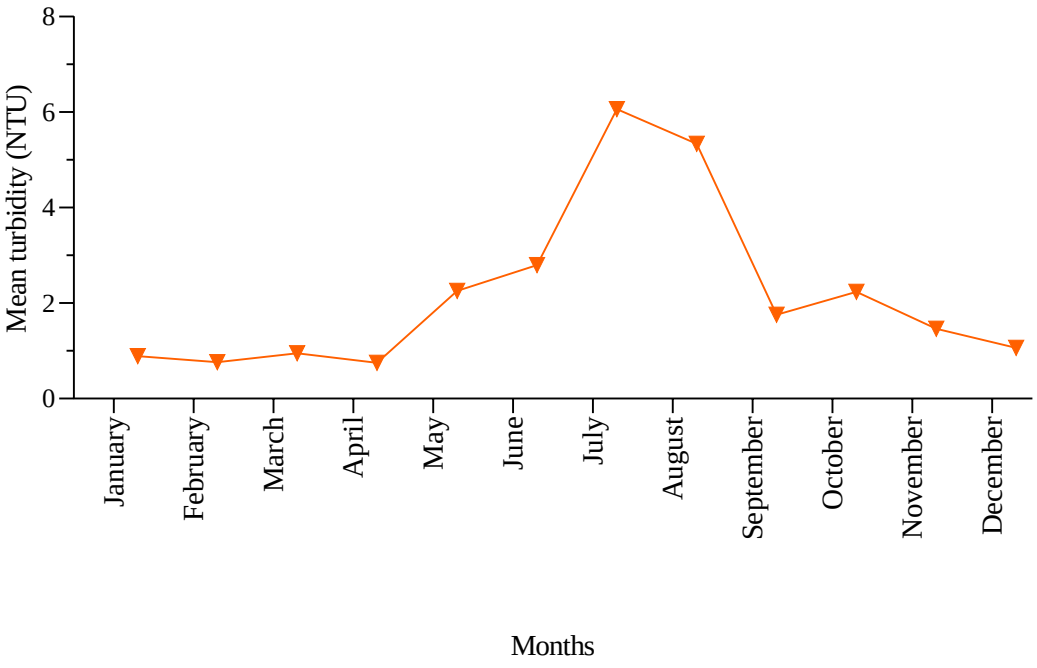

Figure 8 Seasonal variation of turbidity in urban area

Free Residual Chlorine (Urban)

Out of 39 urban health centers, 13 health centers reported for free residual chlorine as shown in Figure 9. A total of 568 samples were tested for free residual chlorine. A predominant proportion (84.1%) of the treated water had residual chlorine level below the minimum acceptable limit (0.2-0.5 ppm). Only 11.6% of the total samples tested had residual chlorine level within acceptable limit and the remaining samples had residual chlorine value above the maximum recommended level.

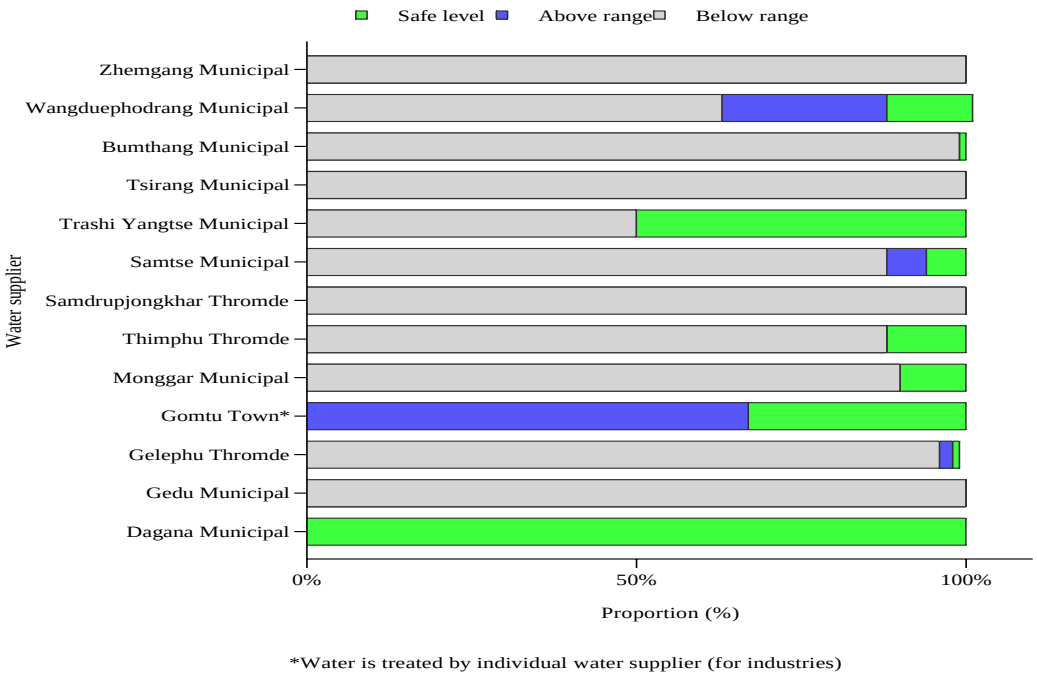

Figure 9. Residual chlorine level in treated water

Chemical parameters (Urban)

Table 2: Details of chemical parameters in drinking water

Elements (mg/l)	**Cd**	**Cr**	**Pb**	**Mercury**	**As**	**Se**	**Mn**	**Zn**
Number of samples	20	11	17	1	6	7	39	44
Mean	0.0017	0.0012	0.0024	0.0002	0.0020	0.0030	0.0110	0.4100
SEM	0.0007	0.0004	0.0005	0.0000	0.0004	0.0008	0.0074	0.2100
Minimum	0.0001	0.0001	0.0003	0.0002	0.0010	0.0010	0.0001	0.0002
Maximum	0.009	0.0044	0.0070	0.0002	0.0030	0.0070	0.2900	6.3000
MPL (mg/l)	0.003	0.05	0.01	0.006	0.01	0.0400	0.4	5
LOD	0.0002	0.0003	0.0020	NA	0.0150	0.0102	0.0001	0.0022

As shown in the Table 2, most of the chemical parameters were within acceptable limit required for drinking water. One sample from Dechenchholing, Thimphu had Cadmium level 0.009 ± 0.0001 mg/l, which was higher than recommended level for drinking water 0.003 mg/l. Likewise, zinc in one of sample from Gelephu was found to have Zinc level 6.245 ± 0.042 mg/l, which is higher than recommended level of Zinc in drinking water 5.00 mg/l.

Rural Drinking Water Quality surveillance

In 2023, a total of 1912 samples were collected and tested from 146 rural health centers. The majority of these samples, 93.5%, were sourced from stream, followed by around 5.4% from spring and rest from other sources. There are no water treatment facilities for rural water system, none of the water samples tested were treated.

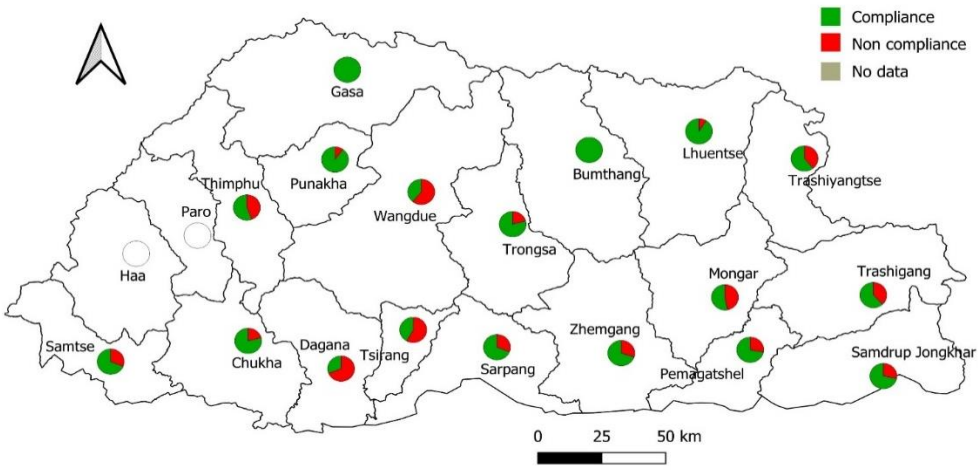

Figure 10. Overview of *E. coli* compliance in rural areas

***E. Coli* (Rural)**

From the total sample, 947 were tested for *E. coli*. Among these, 73.8% were found to comply with the Bhutan drinking water quality standard for *E. coli* (0 CFU/100mL) and rest were non-compliant. **Figure 10** gives the overview of the *E. coli* compliance proportions in rural areas across 18 dzongkhags (no data were available in WaQMIS for Haa and Paro dzongkhags).

Physio-Chemical Report (Rural)

In rural areas, the compliance rates for both pH and turbidity were lower in comparison to the samples from urban areas. The details of the compliance rate for both pH and turbidity in rural aera for individual dzongkhag is given in the **Figure 11**.

pH (Rural)

A total of 671 samples were tested for pH of which 61.3% were found to have pH within acceptable limit as per BDWQS (6.5-8.5). The mean pH value was found to be 7.1 ± 0.02. The highest pH tested was found to be 9.0 and the lowest value was tested at 5.0.

Turbidity (Rural)

A total of 972 samples were tested for turbidity of which 68.9% were found to comply with BDWQS (<5 NTU). The mean turbidity value was found to be 2.7 ± 0.19 NTU. It was observed that mean turbidity values were higher during rainy seasons (June – September) compared to dry season (November – March) as shown in **Figure 12**.

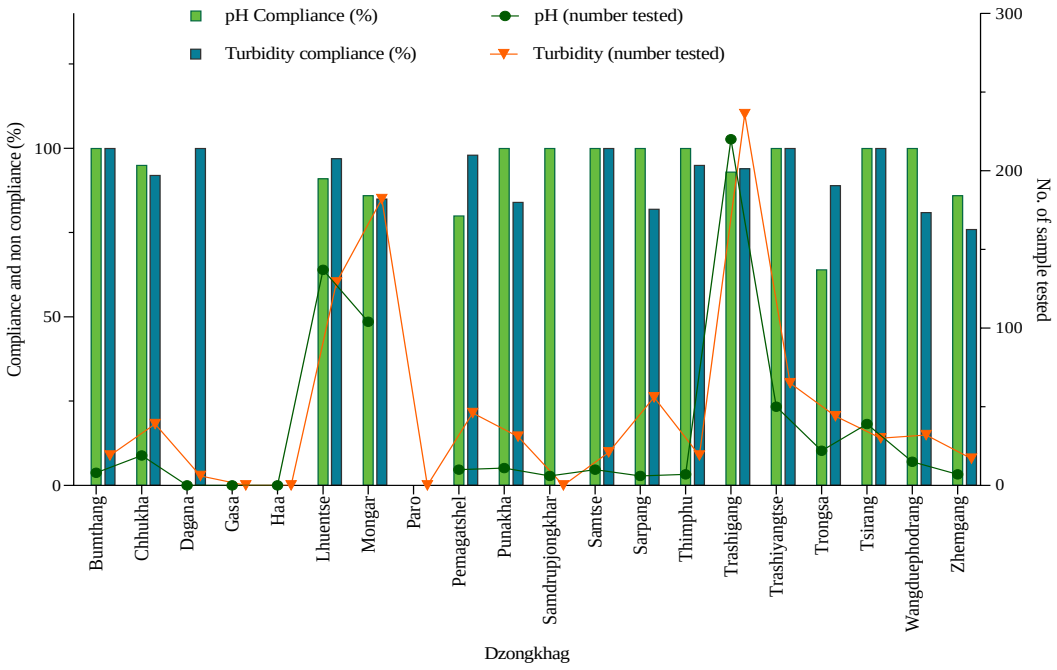

Figure 11. pH and turbidity compliance at dzongkhag level for rural area

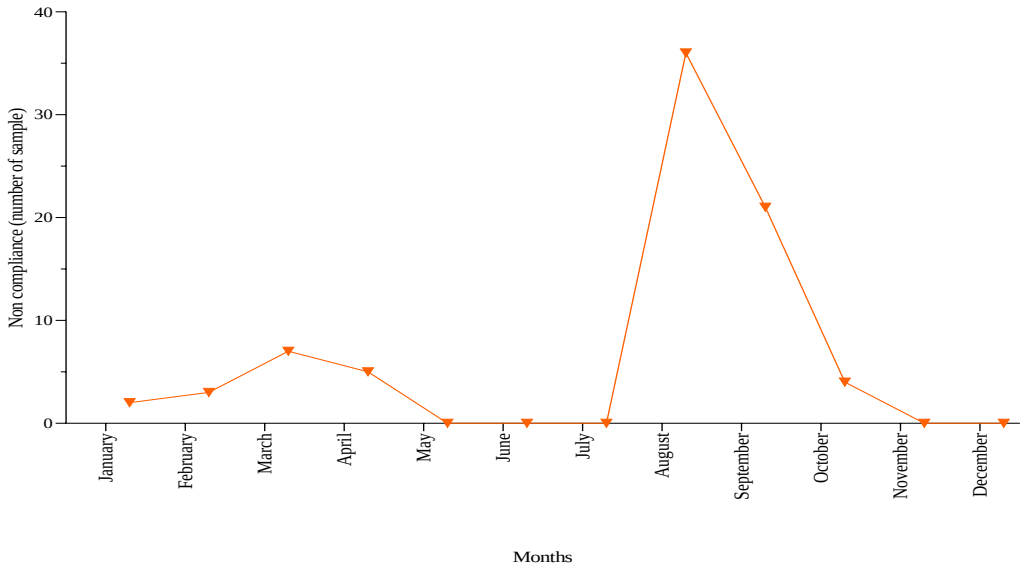

Figure 12. Seasonal variation of turbidity in rural area

4. Discussion

The findings in this report reveal a notable proportion of samples contaminated with *E. coli*, with 41.3% of total samples in urban. Similar results were observed in the preceding year's report for urban, having a non-compliance rate of 44.2%. However there is remarkable improvement in rural water quality in 2023 having only 26.2% non-compliant in 2023, compared to 41.8% in 2022 (7). An improvement in water quality for 2023 compared to the 2022 report, possibly due to improved awareness and corrective measures.

The presence of *E. coli* in drinking water signifies contamination by feces, suggesting the potential presence of harmful pathogens in the water (8). Moreover, the existence of grossly contaminated samples underscores the urgent need for enhanced sanitation and water treatment practices to mitigate health risks associated with fecal contamination.

The report from drinking water samples before water meter in Thimphu Thromde provides overview of the water quality supplied by Thimphu Thromde. The presence of *E. coli* in almost half the samples along with inadequate disinfection is matter of concern and pose a potential health risk to the consumers. Similarly, most of other dzongkhags also had residual chlorine level below acceptable limit. Notably, the presence of residual chlorine in Thimphu thromde water supply did not show the presence of *E. coli*, indicating that even minimal concentration of disinfectant can ensure microbial safety of water supply highlighting the importance of disinfection. The Thromdes and municipalities should investigate the underlying reasons for inadequate residual chlorine and revisit chlorination practices, monitor equipment functionality and quality of disinfectant.

Although most of the physical parameters, including pH and turbidity were within acceptable limit, notable variation in pH, ranging from 5-9 in some samples, and seasonal fluctuation of turbidity particularly observed during wet seasons, emphasize importance of continuous monitoring of these parameters. Variation in pH in water are associated with increased risk of chemical contamination and interfere with disinfection (8) (9). Similar results are observed in the neighboring country, where turbidity fluctuates especially during rainy season (10). Increased turbidity are associated with increased microbial contamination and can also interfere with disinfection by providing protection for microorganisms (8). These results emphasized need for regular operational monitoring and adjustment of treatment processes.

When comparing water quality between urban and rural water supply systems, rural water was had a better microbial quality, this could be attributed to fewer settlements and lower anthropogenic activities and also many of the sources are from natural spring. On the other hand, physio-chemical parameters were better in urban areas possibly due to the availability of water treatment facilities. Similar seasonal trend was observed between both urban and rural water in terms of physical parameters, with increased turbidity during rainy season, highlighting the need for necessary public advocacy, such as improving point of use water treatment practices, particularly during rainy season.

In regard to chemical properties, most of the analyzed samples showed overall compliance with the national and international requirements as shown in the previous study conducted on chemical parameters in drinking water (11). However, higher concentration of Cadmium was identified in one of the samples Dechenchholing, Thimphu, having cadmium level of 0.009 which is three times higher than recommended level of cadmium in drinking water. Cadmium is one of the heavy metals that can enter into drinking water though natural and anthropogenic activities (12). Chronic exposure to even low concentration of Cd can pose significant health risks including toxicity of lungs, kidneys, liver, bones and blood (13).

Zinc is another metal that was observed to have exceeded the recommended level in drinking water from one of the samples from Gelephu having 1.6 times higher than guideline value of zinc in drinking water. While Zinc is an essential mineral, higher level of this metal can impart an undesirable taste to water and may develop greasy film on boiling. They can enter into water system through reservoirs and pipelines made of zinc material (8).

These results emphasize the need for continuous monitoring and periodic assessment of these heavy metals to provide appropriate remedial measures to ensure safe water supply.

Conclusion

This report highlights the quality of drinking water in urban and rural areas specifically focusing on microbial, physical, and chemical parameter, providing crucial insights into the state of drinking water in the region for 2023.

One critical concern is the presence of *E. coli* in significant proportion of the water samples. Moreover, some of the samples were grossly contaminated, thereby posing serious health risks. In addition, the inadequacy of residual chlorine levels in most of the treated water supply is cause of a concern. The absence of *E. coli* even at a minimum concentration of residual chlorine indicates the crucial role of disinfection emphasizing, the urgent need for improvement in water treatment practices.

While most of the physical and chemical parameters were within acceptable limits, seasonal variations of physical parameters and elevated levels of chemicals (Cd and Zn) in some samples indicate potential health risks associated with chronic exposure to heavy metals. There is need for continuous monitoring of these parameters to mitigate remedial measures and ensure safe water supply.

Collaborative efforts from Local government, Thromdes, Municipalities, surveillance bodies and other relevant authorities is important in addressing the identified challenges and implementing sustainable solutions.

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VII Medical Product Quality Monitoring Report 2023

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1. Background

The world health organization (WHO) had reported an estimated 10% (1 out of 10) of medical products circulating among the low- and middle-income group countries (LMICs) to be substandard and/or falsified (SF). This implies that the people in these countries are taking medicines that do not meet the standard requirements of being able to treat or prevent diseases [1]. Use of these SF medicines not only lead to increased rates of morbidity and mortality, but they also incur huge financial losses to the public health systems and individuals [1,2]. The situation was further worsened due to the impact of the COVID-19 pandemic. The issue of unavailability of essential medicines increased in many countries. As countries in the LMIC groups struggled to maintain the availability of essential medicines, the amount of SF medicines in these markets further multiplied. This in turn increased the threat to public health, thereby impacting the health programs [3]. The only effective solution to prevent consumption of SF medicines and provide quality medicines for the mitigation of illnesses of the general public is through quality monitoring of the medicines imported into the country or are manufactured within the country [4].

The National Medical Product Testing Laboratory (NMPTL) under the Royal Centre for Disease Control (RCDC) is recognized as the national quality control laboratory for medical products. It is mandated to carry out quality testing of all the medicines that are available in the country.

2. Methodology

Category of samples

The samples of medical products collected by the NMPTL are broadly categorized into 3 categories namely:

a) Surveillance samples: these samples were collected by the NMPTL on a quarterly basis from identified sentinel sites. The identified sentinel sites are Jigme Dorji Wangchuck National Referral Hospital in Thimphu, Eastern Regional Referral Hospital in Mongar, Central Regional Referral Hospital in Gelephu, Sarpang, Medical Supply & Distribution Division (MSDD) in Phuentsholing, Chukha, Samtse Hospital, Wangdue Phodrang Hospital, and Samdrup Jongkhar Hospital. The Medical Product Division under the Bhutan Food and Drug Authority (BFDA) collected samples from other health centers and retail pharmacies and sent to NMPTL for testing.

b) Compliant samples: these are samples that were referred to the NMPTL by the health centers and other relevant agencies based on complaints received/encountered with regard to the quality of medicines and/or adverse drug reactions.

c) Market authorization samples: these are the samples of products that have been submitted to the BFDA for the purpose of registration for market authorization in Bhutan.

The details of the samples collected/received were entered into the Medicinal Product Quality Monitoring System (MPQMS). The test results were also updated into the system which can be accessed by the focal person of each health center. Complaint samples from health centers were directly submitted to the Substandard and Falsified Medical Product Reporting System (SFPRS) by the focal persons.

Testing

The samples were analyzed as per their pharmacopeial claims and availability of reference standards. If the prescribed pharmacopeial methods were not achievable, the selection of the method followed the following order: International Pharmacopeia, British Pharmacopeia, United States Pharmacopeia and Indian Pharmacopeia. Primary reference standards from Indian Pharmacopeia Commission, United States Pharmacopeia, French Reference Standard, European Pharmacopoeia were used for the analysis of samples. Samples were tested for the parameters listed in **Table 1**.

Table 1: List of parameters for testing of medical products

Physical parameters	**Chemical parameters**
Identification	Assay
Average weight	Dissolution
Average volume	Microbiological assay
Uniformity of weight	Uniformity of content
Friability	
Disintegration	
pH	
Sterility	

Data analysis

Data generated from the MPQMS was imported into the MS excel 2019. Data was analysed using Epi Info v.7 (CDC, USA) and descriptive analysis is presented as frequency and percentage.

3. Results

Overall, a total of 469 samples of medicines were collected from various health centers and sentinel sites in the year 2023. Quarterly data are given in **Figure 1**. The samples collected can be differentiated into various groups based on the category of samples, collection site, types of formulation, and pharmacological category.

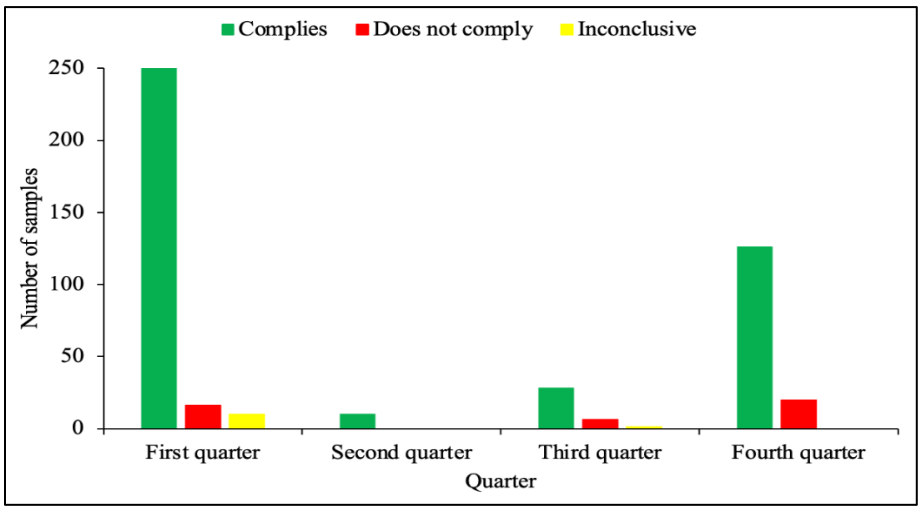

Figure 1: Number of samples collected on quarterly basis

Out of the 469 samples collected, 91.5% (429/469) were collected through the surveillance, 3.8% (18/469) were received through the complaint system and 4.7% (22/469) were received for the purpose of market authorization.

In the surveillance category, 383 out of 429 samples complied to the test parameters, while 36 samples did not comply and 10 had inconclusive results. The rate of non-compliance was 8.4% (36/429). Under the complaint category, 13 of the 18 samples complied to standards, whereas 5 of them did not comply providing a non-compliance rate of 27.8% (5/18) and in the samples for market authorization, 20 out of 22 samples complied to standards, while 1 did not comply and 1 showed inconclusive result. The rate of non-compliance was 4.6% (1/22), **(Table 2)**

Table 2: Distribution of samples collected by category of samples

Category	Complies	Does not comply	Inconclusive	Total in each category
Surveillance	383	36	10	429
Complaint	13	5	0	18
Market authorization	20	1	1	22
Total samples	416	42	11	469

Samples were collected from a total of 26 sites including the identified sentinel sites. The top five sites with the highest number of samples collected were 1) Medical Supply & Distribution Division with 35.8% (168/469); 2) Others (4 sites) with 15.1% (71/469); 3) BFDA with 11.9% (56/469); 4) JDWNRH with 7.7% (36/469); and 5) Mongar ERRH with 5.1% (24/469) samples. Rate of non-compliance of the samples collected from various sites ranged from 0% upto 22.2%, with Phuntsholing Hospital scoring the highest non-compliance rate of 22.2% (2/9) and sites consisting of Deothang Hospital, Gonpa Singma PHC, Jomotsangkha Hospital, Medical Supply & Procurement Division, Menjong Sorig Pharmaceuticals Corporation Ltd., Nganglam Hospital, Pemagatshel Hospital, Punakha Hospital, and RCDC scoring nill, **(Table 3)**.

Table 3: Distribution of samples collected by collection site

Site	Complies	Does not comply	Inconclusive	Total of each site
Medical Supply & Distribution Division	160	8.0	0	168
Others	61	10.0	0	71
BFDA	41.0	5.0	10.0	56
JDWNRH	33	2.0	1.0	36
Mongar ERRH	21	3.0	0	24
Gelephu CRRH	15	3.0	0	18
Samtse Hospital	11	3.0	0	14
Medical Supply & Procurement Division	13	0	0	13
SamdrupJongkhar Hospital	11	2.0	0	13
Tsirang Hospital	11	2.0	0	13
WangduePhodrang Hospital	10	2.0	0	12
Nganglam Hospital	9	0	0	9
Phuntsholing Hospital	7	2.0	0	9

Menjong Sorig Pharmaceuticals Corporation Ltd.	3	0	0	3
Pemagatshel Hospital	3	0	0	3
Deothang Hospital	2	0	0	2
GonpaSingma PHC	2	0	0	2
Jomotsangkha Hospital	2	0	0	2
Punakha Hospital	1	0	0	1
RCDC	1	0	0	1

Types of formulation

The samples tested consisted of many formulations. However, the top five formulations tested consisted of tablets amounting to 93.8% (440/469), capsules 7.2% (34/469), miscellaneous 6.4% (30/469), medical devices 5.5% (26/469), and creams/gels/lotions/ointments 2.3% (11/469).

Out of the top five formulations, creams/gels/lotions/ointments had the highest proportion of non-compliance with 18.2% (2/11), while capsules did not show any non-compliance, **(Table 4).**

Table 4: List of formulation of samples

Formulation	**Complies**	**Does not comply**	**Inconclusive**	**Total of each formulation**
Tablets	401	37	2	440
Capsules	34	0	0	34
Miscellaneous	25	5	0	30
Devices	23	3	0	26
Creams/Gels/Lotions/ Ointments	9	2	0	11

Samples belonging to more than 50 pharmacological categories have been tested in the year 2023. Out of that, the top ten categories with the highest number of samples are 1) Anti-biotics with 15.1% (71/469); 2) Anti-diabetics with 10.5% (49/469); 3) Anti-pyretic & analgesics with 7.7% (36/469); 4) NSAIDs with 6.8% (32/469); 5) Anti-TB drugs with 6.4% (30/469); 9) Anti-hypertensives with 4.1% (19/469); 6) Corticosteroids with 3.8% (18/469); 8) Anti-convulsants with 3.8% (18/469); 7) Anti-histamines with 3.6% (17/469); and 10) Vitamins with 3.6% (17/469) samples.

Of the top ten categories, anti-histamines showed the highest proportion of non-compliance with 52.9% (9/17), followed by vitamins with 11.8% (2/17). Anti-pyretic & analgesics, NSAIDs and Corticosteroids did not show any non-compliances, **(Figure 2).**

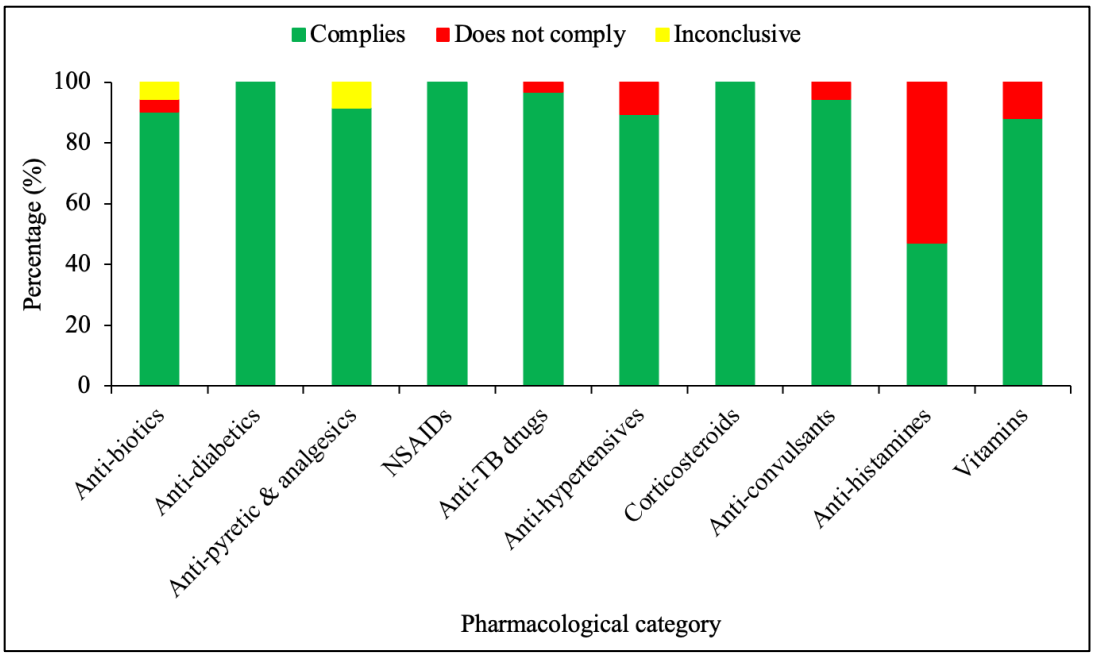

Figure 2: Rate of compliance according to pharmacological category

3.2 Five-year trends of compliances and non-compliances

Looking at the rate of non-compliances of the medicine samples to the standard requirements over the past five years, the year 2020 reported the highest rate of non-compliance [10.6% (18/170)]. In

2021, the rate of non-compliance had dropped by half compared to the previous year, however, the rate increased from 2022 and almost doubled in 2023, **(Figure 3).**

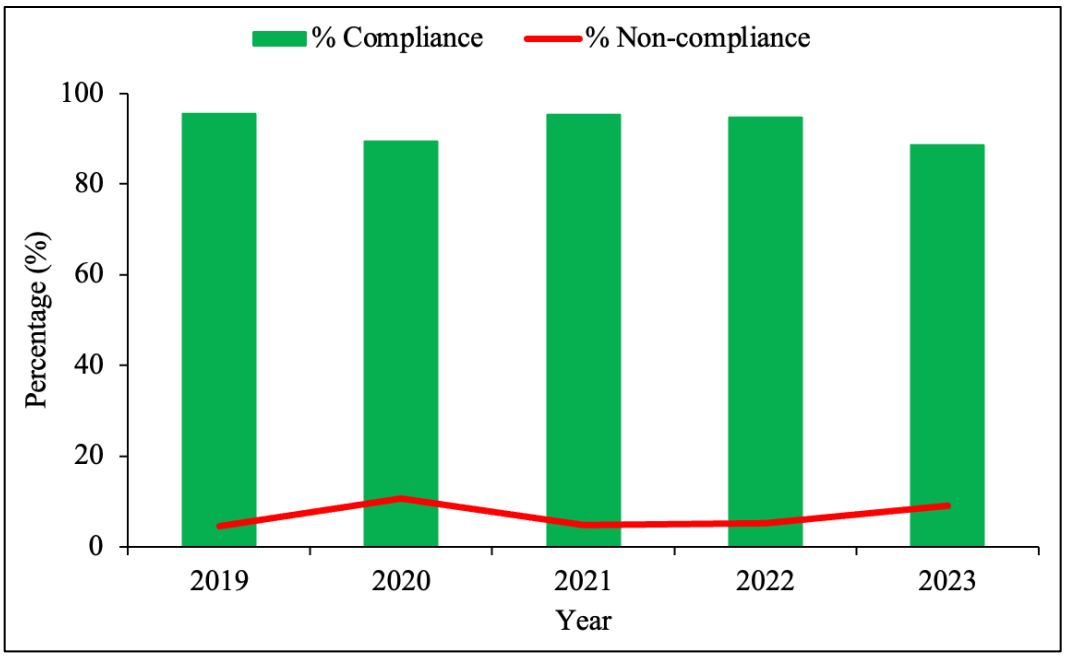

Figure 3: Five-year trends of compliances and non-compliances

4. Discussion

The NMPTL had tested a total of 469 medicine samples for quality in the year 2023. The number of samples tested had increased by 17.5% compared to the year 2022 (399 samples were tested in 2022). This increase in numbers is mainly due to the continuation of the surveillance sample collection activity by the NMPTL after it was kept on hold due to the COVID-19 pandemic. Majority of the samples tested were collected through the surveillance activities, although some were received through the SFPRS and some were tested for the purpose of market authorization, submitted by the BFDA. By virtue of being complaint samples, this category had the highest rate of non-compliance [27.8% (5/18)] compared to the other two categories of samples.

Samples collected from different sites showed varying rates of non-compliances. This could possibly be due the differences in the storage conditions in different sites. However, whether the storage conditions of the respective sites have any direct correlation with the rate of non-compliance remains to be determined.

Of the various types of formulations tested, the creams/gels/lotions/ointments showed the highest rate of non-compliance with 18.2% (2/11), while capsules did not show any non-compliances. This could be explained based on the fact that the stability of the creams/gels/lotions/ointments are inferior compared to other formulations like capsules and tablets.

Medicines in the anti-histamine category showed the highest rate of non-compliance [52.9% (9/17)] compared to other pharmacological categories. Considering the number of samples in this category being not the highest, it calls for detailed investigation into the main cause of the high rate of non-compliance. Anti-biotics showed a non-compliance of 4.2% (3/71), consisting of anti-TB drugs showing anon-compliance rate of 3.3% (1/30). Although the figure might not seem large, it is noteworthy that these could contribute to the increasing issues of antimicrobial resistances in the country.

Studying the five-year trend of non-compliances, the year 2020 reported the highest number of non-complaint medicine samples [10.6% (18/170)]. Although the rate of non-compliance had dropped starting 2021, it kept increasing after that till 2023.One possible reason behind the high rate of non-compliance in 2023 could be the increased number of samples being tested after the continuation of the surveillance activities by the NMPTL.

Conclusion and way forward

Considering the presence of consistent proportion of non-complaint medicines for the past five years, there is a possibility of the prevalence of SF medicines circulating among the health centers. The straightforward strategy to control the SF medicines from reaching the general public is the quality monitoring and surveillance. Therefore, the quality testing and related activities need to be further strengthened. Ideally, the medical products need to be tested for quality prior to distribution to the health centers in order to prevent adverse events and curb the waste of resources involved in recalling SF products later on.

Way forward

- Increase and strengthen the surveillance and testing activities;
- Encourage active participation from health centers in reporting defective medicines through SFPRS;
- collaboration with National Medical Services to ensure timely testing of medicines to guarantee distribution of only quality medicines;

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VIII Food Safety surveillance in Bhutan, 2023

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1. Introduction

Foodborne disease (FBD) or food poisoning is any illness that is often caused by the consumption of food contaminated with microorganisms (pathogenic bacteria, viruses, or parasites). Globally, World Health Organization (WHO) estimates that FBDs cause 600 million illnesses with 420,000 deaths and 33 million disability adjusted life-years (DALYs) annually (Chhetri and Dorji, 2021). The highest burden was recorded in the Africa region (1,300 DALYs per 100,000 population) followed by South East Asia (1,200 DALYs per 100,000 population) with the second highest burden of FBD incidences per population among WHO regions (Organization, 2015). In the South East Asian region approximately 50% of FBDs are caused by diarrheal agents (*E. coli, Salmonella, Campylobactera, Norovirus*). In Bhutan diarrhea causes significant morbidity and FBD is mostly associated with diarrheal symptoms. More than 60 events of food borne disease have been recorded with the Royal Centre for Disease Control (RCDC) from 2012 to 2020. The common pathogen causing FBD was *Salmonella* species accounting for 42.9% of cases, followed by *Shigella* species (21.4%). Foodborne diseases represent a threat to public health and hinder socioeconomic development (Botelho et al.). Therefore, food safety surveillance is an important public health activity to identify risk and mitigate necessary action to prevent FBD.

2. Methodology

The current surveillance results present on the samples collected between January to December 2023 from the five sites (Paro, Thimphu, Phuentsholing, Gelephu and Monggar) (**Figure 1**). The samples were collected from hotels/restaurants, bakery and canteens by the trained food inspectors (BAFRA).

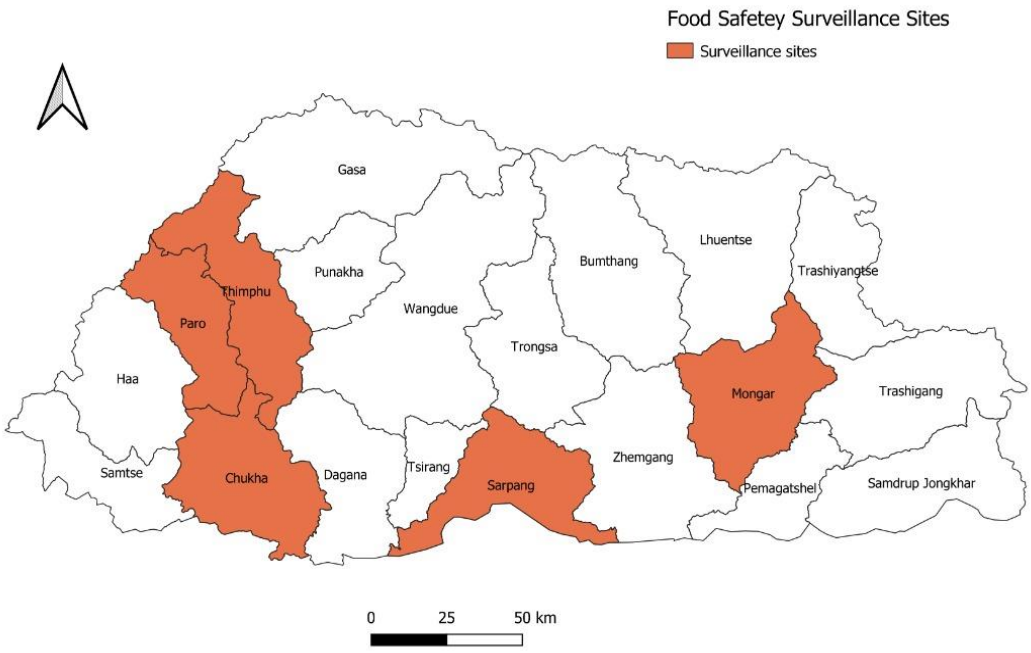

Figure 1: Food safety surveillance sites (highlighted) in Bhutan (2023)

Samples collection

The food samples were aseptically collected (five samples per month) from randomly selected restaurants/hotels/bakery by the Food Inspectors. Any ready to eat food samples; including both vegetables and meats and bakery items were collected. The samples were coded with unique sample identification number generated through Food Safety Surveillance Information Management System (FoodSIMS). Samples were packed separately and transported in cold chain to the Food and Nutrition Laboratory, Royal Centre for Disease Control, Thimphu for laboratory analysis.

Sample preparation and Laboratory Analysis

Samples were prepared according to the Association of Official Analytical Chemists (AOAC) method (Feldsine et al., 2002). Twenty-five grams of each sample was weighed and homogenized for 30 seconds at 230 rotation per minute (RPM) by stomacher in 225 mL of sterile buffered peptone water (BD, USA). Furthermore, the serially dilution to three more tubes was prepared. The indicator organism test; total plate count, total *Enterobacteriaceae* count, total coliform, Environmental

Listeria and yeast mould count was performed from the diluted samples using specific culture media (3M™ Petrifilm™ Plates). The plates were incubated aerobically for 24 h at 37 °C except for yeast mould at 25 °C for 48 h.

All discrete colonies were counted where possible and expressed as the $\log^{10}$ of colony forming units per gram (CFU g−1). To improve recovery and detection, pre-enrichment media was used for *Campylobacter, Salmonella, Shigella, Listeria monocytogenes*, the tubes were incubated aerobically at 37 °C for 12–24 h, except for *Campylobacter* at 35ºC ±2ºC for 48 hours with 5 % CO_2. After which a loopful of enrichment broth was cultured on *Salmonella/Shigella* (Sigma, India), Mac Conkey (Criterion, USA), Modified Charcoal Cefoperazone Deoxycholate Agar (mCCD) (Sigma, India), Hektone (Sigma, India), Baird Parker media (Sigma, India), Mannitol-egg-yolk-polymyxin agar (Criterion, USA). The plates were incubated aerobically for 24–48 h at 37 °C, except mCCD was incubated at 35ºC ±2ºC for 48 hours with 5 % CO_2.

Isolate identification

Colonies were presumptively identified by colony pigmentation and Gram staining characteristics. Pure cultures were obtained by streaking a portion of an isolated colony on nutrient agar and incubated aerobically at 37 °C for 24 h (Gilbert et al., 2000). Biochemical identification test and furthermore API 20E was performed on isolated with discordant results.

Polymerase chain reaction (PCR)

The PCR test was performed onto the *E. coli* isolates to study the presence of any pathogenic strains. The primers used were manufactured by Macrogen with following primer sequences; *Enterotoxigenic E. coli* (ETTC) F 5’ CAG ACG GAG CTC CTC AGT 3’ and R 5’ CCC CCA GCC TAG CTT AGT TT 3’, *Enterohemorrhagic E. coli* (EHEC) F 5’ CAG TTA ATG TGG TGG CGA AGG 3’ and R 5’ CAC CGA ACA ATG TAA CCG 3’, *Enteroinvasive E. coli* (EIEC) F 5’ TGG AAA AAC TCA GTG CCT CT 3’ and R 5’ CCA GTC CGT AAA TTC ATT CT 3’ and *Enteroaggragative E. coli* (EAEC) F 5’ CTG GCG AAA GAC TGT ATC AT 3’ and R 5’ ACG ACA CCC CTG ATC AAC AA 3’.

Statistical Analysis

Statistical analysis was performed using Micro Soft excel and descriptive analysis are presented as percentages and ratio.

3. Results

In 2023 a total of 262 ready to eat food samples were collected from five Dzongkhags, as shown in figure 2. Of the total ready to eat food samples collected 100% of the samples were solid and low acid foods. Overall, 13.74% of food samples were unacceptable due to pathogenic organism growth and 10.69% due to indicator test organism growth. The dzongkhag wise distribution of food samples contaminated either due to pathogenic and indicator test organism is presented in the **figure 2** below.

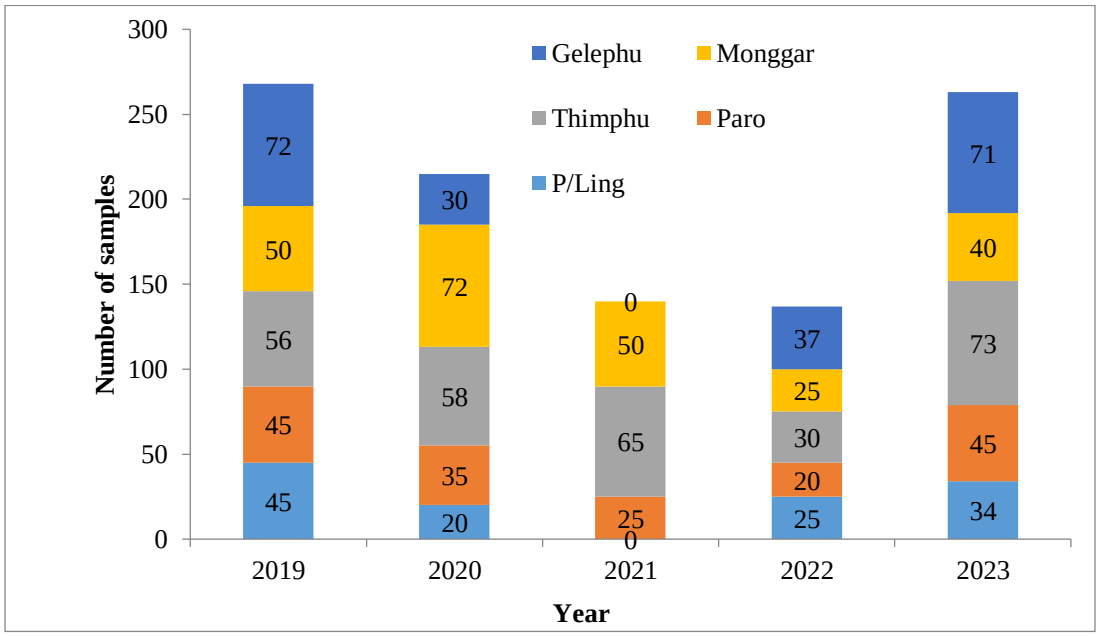

Figure 2: Total samples collected for food safety surveillance

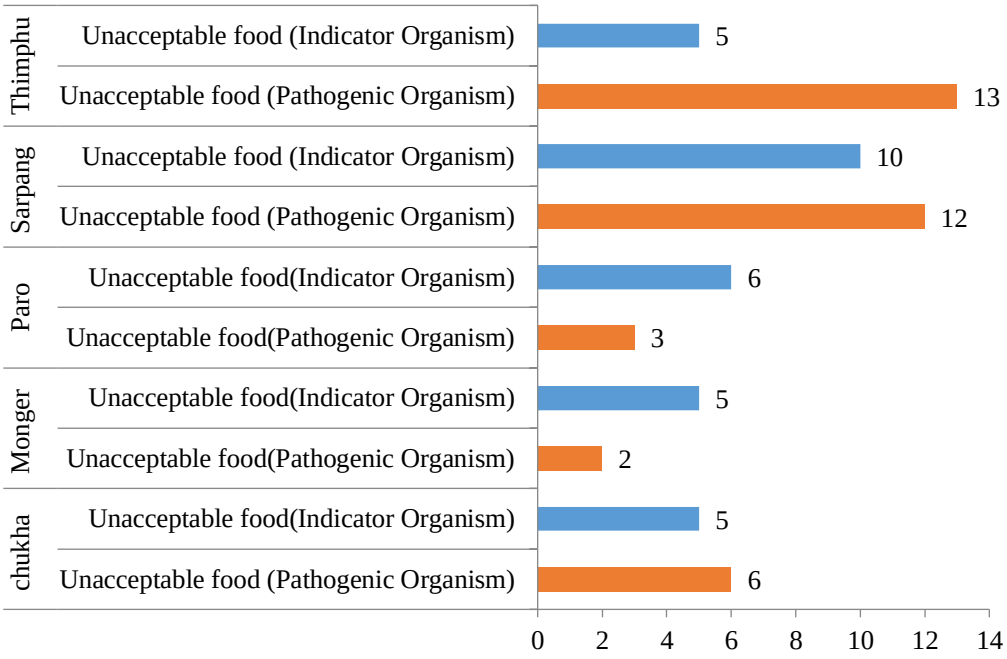

Figure 3: Number of food samples un-acceptable due to indicator and pathogenic organism from five Dzongkhags (Food safety surveillance 2023)

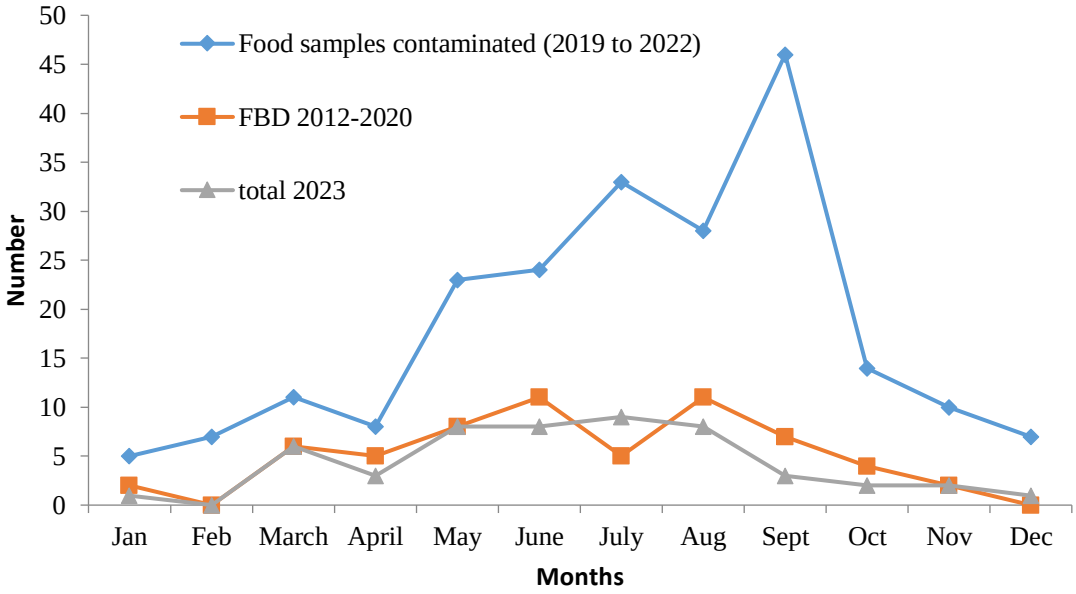

Figure 4: 2023 monthly surveillance unacceptable food samples compared with 2019-2022 Food safety surveillance and Foodborne disease outbreaks (2011 to 2020)

4. Discussion

Food safety surveillance is routinely conducted by the Royal Centre for Disease Control (RCDC) and in collaboration with Bhutan Food and Drug Authority (BFDA). The surveillance was initiated in 2019 and the current report presents the data for January to December 2023. Since the initiation of food safety surveillance from June 2019 to December 2023, 1029 ready to eat food (RTE) samples has been laboratory analyzed.

Bhutan has recorded several events of FBD outbreak (figure 4) and comparison was made between monthly FBD (2011-2020) records and food safety surveillance report (2019-2021). It is noticeable that the most of the food contamination occurs during the hot and wet seasons.

Indicator Test Organism

Foods were classified as acceptable or hygiene based on the inference from the microbial count based on the **table 1**. The common indicator test includes; total bacterial count or total plate count, Yeast mould count, Total *Enterobacteriaceae* count and *E.coli* count (Chhetri and Dorji, 2021). Total Plate count of aerobic microorganisms and yeast mould count found in food is one of the microbiological indicators used as surrogacy for hygiene indicator (Nyenje et al., 2012).

Table 1: Percentage of samples with un-acceptable level of indicator and pathogenic organism, Food safety surveillance 2023

Place	**TPC**	***E.coli***	***Entero-bacteriaceae***	**Y/M count**	***B. cereus***	***S. aureus***	***B. cereus and S. aureus***
Chukha	8.8%	2.9%	5.9%	0.0%	14.7%	8.8%	5.9%
Paro	11.0%	4.4%	4.4%	2.2%	2.2%	6.7%	2.2%
Thimphu	2.7%	2.7%	2.7%	0.0%	8.2%	12.3%	2.7%
Mongar	5.0%	2.5%	0.0%	7.5%	2.5%	5.0%	2.5%
Sarpang	9.9%	2.8%	4.2%	5.6%	5.6%	12.6%	2.8%

Table 2: Microbiology limits for indicator organism in ready to eat food

Indicator test	Limits
Total plate count	$<10^5$ CFU/g
E. coli count	$<10^2$ CFU/g
Enterobacteriaceae count	$<10^2$ CFU/g
Listeria (other than *Listeria monocytogenes*)	$<10^2$ CFU/g
Yeast/Mold	$<10^2$ CFU/g

During the current food safety surveillance period it was found that indicator test (total plate count) un-acceptability was slightly higher, these findings are in concordance with the earlier findings from 2022 surveillance. The highest number of indicator test unacceptable was due to $>\log^5$ growth of total plate count/total bacterial count 11.11% (Paro samples) followed by total *Enterobacteriaceae* count 5.88% (Chukha samples).

Pathogenic Organism

The common type of pathogenic organism isolated remains similar to the previous report of 2019-2021 and of the total 19.2% of samples in 2022 was contaminated with pathogenic organism as compared to 13.7% in 2023. Both *Bacillus cereus* and *Staphylococcus aureus* were isolated from several foods with maximum from 14.7% *Bacillus cereus* from Chhukha and 12.7% *Staphylococcus aureus* from Sarpang samples (**Table 3**).

Also, during the current study period, it was observed that 5.9% of food samples collected from Chhukha had the growth of both *B. cereus* and *S. aureus*. This also indicates the need for proper adherence to good manufacturing process, which is preventable. Failure to comply could lead to widespread food contamination, posing significant public health risk and loss to business.

Table 3: Microbiology limits of pathogenic organism in ready to eat food

Pathogenic organism	Limits
Bacillus cereus	$<10^2$ CFU/g
Clostridium perfrigenes	Not detected in 25g of Food
Coagulase positive *Staphylococcus aureus*	$<10^2$ CFU/g

Listeria monocytogenes	Not detected in 25g of Food
Vibrio spp	Not detected in 25g of Food
Salmonella spp	Not detected in 25g of Food
Shigella spp	Not detected in 25g of Food
Campylobacter	Not detected in 25g of Food
Aeromonas	200 CFU/100g
Plesiomonas	200 CFU/100g
Pathogenic *E coli*	Not detected in 25g of Food

The most common type of food in which pathogenic organisms is isolated were from channa (n=6) and chowmein (n=5), similar to the earlier findings in 2022. The other food groups contaminated is presented in the figure 6. During the current surveillance period no pathogenic strains of *E.coli* was detected.

Conclusion

Our food is diverse and therefore to ensure it is safe for consumption it requires a systematic, proactive method to reduce contamination. Moreover, access to safe running water plays a paramount role in food safety. The findings demonstrate that ready-to-eat food is likely hazard to human health. The isolation of both the indicator (10.7%) and pathogenic micro-organism 13.7%) indicates the risk of FBD outbreaks during the hot and wet seasons.

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IX A report of Poisoning Surveillance and identification of toxicants / chemicals /drugs of abuse- 2023

- *Adeep Monger and Pooja Mongar*

1. Introduction

Poisoning is a public health concern in low and middle income countries that has resulted to loss of over 7.4 million years of healthy life based on the disability adjusted life years (DALYs) (1). Toxicants or poisons pose significant risks to human health, as well as wildlife, and the environment.

Globally, different countries have different characteristics of poisoning. For instance, the major burden of poisoning in the developed countries like the United States and United Kingdom are from unintentional carbon monoxide poisoning and household products (2, 3). In contrary, in the countries in South East Asia regions such as India, Nepal and Thailand, the majority of the reported incidence of poisoning cases are intentional involving pesticides and pharmaceuticals (4, 5). However, the true burden of poisoning in this regions is thought to be grossly under-reported due to scant poisoning surveillance.

Bhutan is a not an exception to be undeniably affected by this public health threat. The country is known for its pristine environment and a rich bio-diversity committed to environmental conservation. This leads to the high incidence of poisoning from snakebite envenomation and wild plants. Nevertheless, poisoning can stem from various sources, including accidental exposure to agricultural chemicals and intentional ingestion of toxic substances (4).

Agriculture is a key sector in Bhutan's economy, with a significant portion of the population engaged in farming. The use of pesticides and fertilizers to enhance the crop yields, improper handling or application of agricultural chemicals can result in unintended poisoning among farmers and agricultural workers (6). It is very pertinent that the nature of poisoning be explored by analyzing the population at risk, major toxic agents and the poisoning outcomes to ultimately decrease the incidence and effects of poisoning.

Therefore, the Poison Information and Toxicology Laboratory unit (PITL) conducts poisoning surveillance via the National Toxic Information Surveillance aimed at providing scientific evidence

for policy decisions and implementing public health interventions. Furthermore, the current data on poisoning must be systematically assessed to identify the needs and goals for the development of poisoning control in Bhutan.

2. Methodology

The National Toxic Exposure Information and Surveillance System (NTESIS) is a surveillance program designed to collect information on poisoning related cases. It is a part of immediately notifiable diseases under the category of event based surveillance in NEWARIS. Any life-threatening cases visiting the hospitals and primary health care centers related to toxic/poisons are enrolled for poison investigation and notified in the NEWARIS. Analytical toxicology laboratory cases related to narcotics/drug of abuse from Bhutan Food and Drug Authority (BFDA) are directly processed for analysis for confirmation.

Specimen collection and shipment

Biological samples (blood and urine) are collected from a suspected case for confirmation of toxins/drugs of abuse either at the respective hospitals/PHC or by BFDA and are shipped to PITL at RCDC within 24hrs of collection. Non-biological samples are also collected in the same manner and are shipped to PITL in RCDC within 5 days from the date of collection.

Laboratory testing

Samples are prepared for analysis using various extraction methods including Liquid-liquid or Solid-phase extraction methods. Analysis of the samples is performed using various analytical techniques:

1. GC/FID – Gas Chromatography – Flame Ionization Detector
2. GC/MS – Gas Chromatography – Mass Spectrometry
3. HPLC – High Performance Liquid Chromatography
4. ICP-OES – Inductively Coupled Plasma – Optical Emission Spectroscopy
5. CVAAS – Cold Vapour Atomic Absorption Spectrometry

Various compounds from the samples are accomplished by comparison of chromatographic retention times and mass spectra to certified reference standards analyzed under the same condition. Moreover, the results are also compared with the mass spectral from commercialized library of drug

standards using the NIST 11 library software (National Institute of Standards and Technology, USA).

3. Results

A total of 60 poison related cases were reported via NTESIS, of which 21 (38.33%) were females and 30 (61.7%) were males. The maximum cases were reported in the age group 25-49 (45.09%) years followed by 15-24 and 50-64 years with 17.64% each **(Figure 1)**

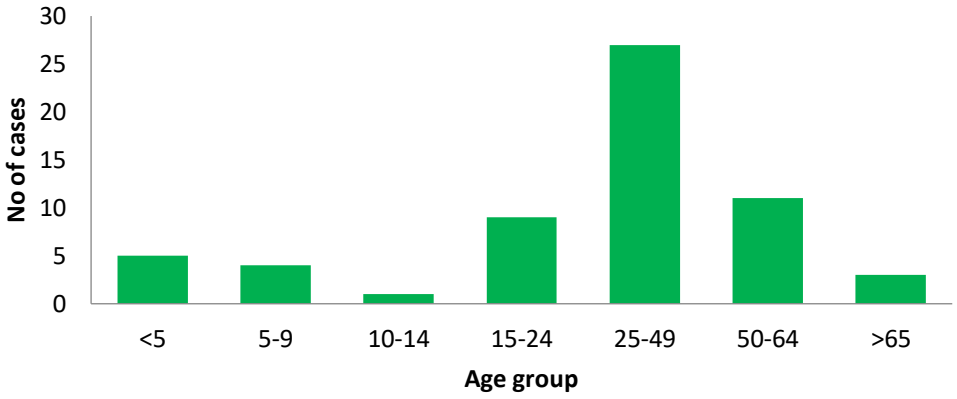

Figure 1: Distribution of poison cases among different age groups

Among the total poison cases reported, majority of the cases were snakebites (36.67%) followed by wild plants (15.0%) and pesticides ingestion (13.33%). Pesticides ingestion includes various forms like herbicides and insecticides (**Table 1**)

Table 1: Types of poison cases reported in 2023

SI.No	Types of poison	No. of cases	(%)
1	Food	4	6.67
2	Snakebite	22	36.67
3	Mushroom	4	6.67
4	Pesticides	8	13.33
5	Pharmaceutical	5	8.33
6	Alcohol	1	1.67

7	Wild plants	9	15.00
8	Others (Petroleum & insect bites)	7	11.67

Amongst the cases reported for the year (January to December 2023), the highest cases reported were from the month of May (16 cases), followed by June (10 cases) and July (6 cases). The data is presented in figure 2.

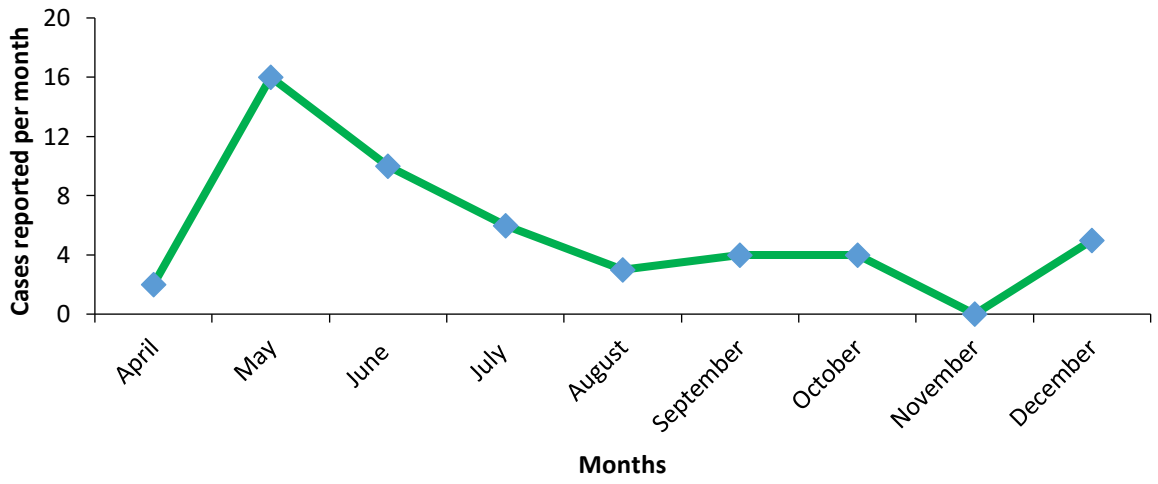

Figure 2: Distribution of poison cases among different months

As per the stratification of cases by dzongkhags, the highest number of cases were from Samtse (40%), Dagana (20%) and Chukha (0.15%). Notably, 16 cases out of 22 snakebite cases were reported from Samtse dzongkhag (**Figure 3**).

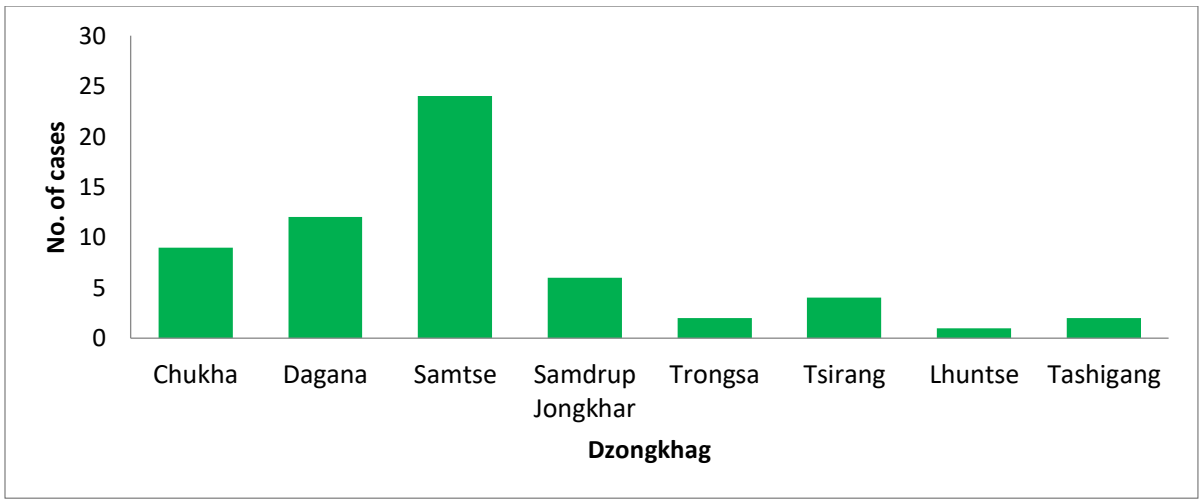

Figure 3: Patterns of poisoning among the different Dzongkhags

Laboratory identification of toxins/chemicals/drugs of abuse

A total of 420 samples were received in the laboratory for testing and identification of poisons/chemicals/drugs of abuse in the year 2023. Out of these, 61 (14.5 %) samples were for routine analysis and 359 (85.4 %) samples were for the purpose of National Health Survey (NHS) 2023. Figure 4 represents the types of samples received in the year 2023. Amongst the types of samples received for routine analysis and identification, the highest were urine samples (44.26%) followed by whole blood (31.15%).

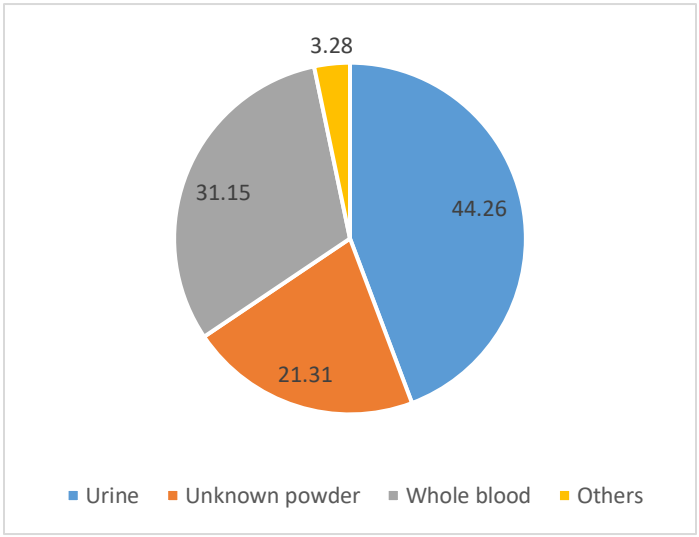

Figure 4: Types of sample received (%)

The figure 2 describes the types of tests conducted at the laboratory. The highest test that the laboratory conducted was for mercury in blood and urine, followed by delta-9 tetrahydrocannabinol (THC) and tramadol respectively. Furthermore, a total of 718 tests analysis were performed for the determination of concentration of ethanol and methanol in locally brewed alcohol samples for the purpose of NHS 2023.

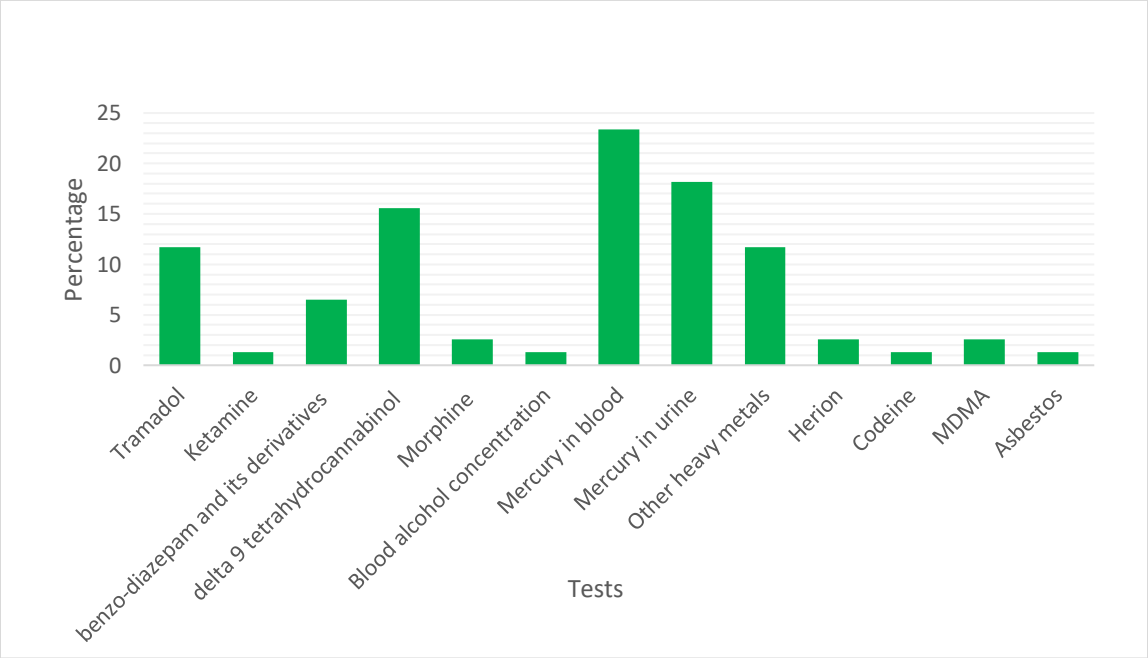

Figure 2: Frequency of types of tests conducted for the year 2023

The proportion of test results above the normal reference value of mercury in blood (i.e. >10 µg/L) was 83.3%. Similarly, for mercury in urine was 57.14%. Among the samples received from BFDA for the purpose of combating the illicit use of controlled substances in the country, the positive detection rate among the samples analyzed was 35.29%.

4. Discussion

This report presents the burden of poisoning in the country, highlighting its patterns and the distribution among the different dzongkhags of the country. The predominant cause of poisoning is from snake envenomation followed by wild plants and pesticides poisoning.

Snake envenomation is a global neglected disease. In the ASEAN countries it causes huge burden with an estimated 242,648 annual snakebite victims out of which 15909 died and 954 were amputated (7). Many of the victims are not treated with anti-venom. This is further aggravated with the limited knowledge of healthcare providers in proper management of the snakebites and the risk of life-threatening anaphylactic reactions (8). Similarly, our data here finds the high burden of snakebites in the country. The country currently imports the anti-venoms from the neighboring countries like India and Bangladesh which are produced specifically to the species of snakes found in their locality and are not specific to the indigenous snakes originating in the country. Therefore, we recommend that a mapping of venomous snakes including the identification of snakes, its venom profiles, and the toxicity assessment be conducted in the country to combat the disease burden attributed to snakebites.

Many plants are poisonous to human and animals. Toxicology centers in Australia, Thailand, Sweden, and Germany conducted surveys that showed that plant exposures were responsible for 1.8% to 8% of all injuries (9). The current study reported a total case percentage of 15 % which maybe attributable to the country's rich biodiversity of flora and fauna. Human poisoning by pesticides has been seen as a severe public health problem with about one million unintentional pesticide poisonings occurring annually leading to death of approximately 20000 (10). Studies conducted in India, Nepal and Thailand all reported poisoning by pesticides as the top most common type of poisoning recorded (1, 11, 12). Similarly, the present study also reported poisoning by pesticides as one of the top 3 causes of poisoning in the country.

Analytical toxicology laboratory plays a critical role in evaluation of suspected drug of abuse and also to detect, identify and measure drugs and other potentially harmful chemicals in body fluids for the diagnosis, treatment and prevention of poisoning (13, 14). The accuracy, precision and reliability of the analytical findings should be ensured by applying quality control assurance and monitoring of method development and validations.

The limited number of sample sizes are the limitation of the current report as many of the exposure goes under-reported. Some of the information such as types of snakes, outcome and patient history were either not available or limited. Overall, the report has managed to contribute substantial additional information regarding the epidemiology of poisoning in Bhutan. There is a need to further investigate on each poisoning cases and strengthen the surveillance system.

Conclusion

Snake envenomation, wild plant and pesticides poisoning remain a significant cause of poisoning in Bhutan. Poisoning incidences are mostly observed in the age group 25-45 years old and usually peak in the months of May to July. There is a need for strengthening and improving surveillance and research to develop interventions that will address various contributors to poisoning. In addition, tramadol and delta 9 THC are the most frequently involved drug of abuse that we encounter for testing in the laboratory.

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X National External Quality Assessment Scheme (NEQAS) of malaria microscopy among the participating laboratories in Bhutan

1. Background

Globally, malaria remains a major public health challenge. In 2022, there were an estimated 249 million cases of malaria worldwide with an increase of 5 million cases compared with 2021 (1). The World Health Organization (WHO) African Region continues to bear the highest burden, accounting for 95.0% of malaria cases and 96.0% of malaria deaths (1).

In 2022, Bhutan has reported zero indigenous cases and proactively implementing strategies to prevent the introduction of indigenous malaria cases. The country is dedicated to achieving WHO certification for malaria elimination by 2025.

Bhutan, historically categorized as a low-endemic country for malaria, has primarily recorded cases in its southern districts bordering India. The nation initiated its malaria control efforts with support from both the Global Fund for AIDS, Tuberculosis, and Malaria (GFATM) and the WHO.

NMRL in collaboration with the Vector-borne Disease Control Program (VDCP) adopted a National strategic plan (NSP) aligned on Malaria Global technical strategy for malaria 2016-2023 to target the elimination of malaria.

Laboratory diagnosis is one of the main pillars of NSP for malaria surveillance ensuring every suspected case was tested and confirmed by parasitological-based techniques like microscopy or Quality assured malaria Rapid diagnostic tests (RDTs).

with these techniques available at the primary health care levels, quality assurance for malaria diagnosis is indispensable in delivering accurate and reliable laboratory results. Therefore, the panel testing serves as one component in assessing the performance of laboratory personnel skills on microscopy and provides room for NMRL and VDCP to strategies to overcome and improve the deficiencies pertaining to malaria diagnosis.

2. Method

The fifth round of the malaria panel was distributed to 60 district health facilities across Bhutan, encompassing a wide range of malaria regions, including both endemic and non-endemic transmission areas. Participants, consisting of laboratory technologists, laboratory technicians, and malaria technicians, were enrolled in the panel assessment to evaluate their competency in performing malaria microscopy.

Panel slide characteristics and timeline

The panel comprises of stained thick and thin blood film prepared on a single slide. It comprises a set of five slides that consists of three positive slides with varying parasite densities - low density (200-500 parasites/ul), moderate density (1000-1500 parasites/ul), and high parasitism (>15000 parasites/ul), as well as two negative slides. These slides were prepared to evaluate and compare results among the participating laboratories. The panel has been packed in a slide mailer box and distributed to the participating laboratories, along with an official letter and an instruction sheet for guidance.

The web-based system automatically accepts the results from participating laboratories within the designated time frame and promptly closes when the specified closing date is reached. Participating laboratories are unable to submit their results after the closing date has passed. The time frame for submission is fixed at 40 days from the date of shipment.

Data collection and management

The participating laboratories were requested to examine the panel slides maintaining proficiency and report the diagnosis as (Malaria “Yes” or “No”), identifying the infecting Plasmodium species (Plasmodium falciparum, Plasmodium vivax) and specific malaria blood stages- including asexual (trophozoite and schizont) and sexual (gametocytes), and count the parasite for each parasite species and enumerate the parasite density by counting infected RBCs and WBCs.

Data analysis

After the submission deadline, all examined data were recorded in the online database system (mNEQAS Performance levels on specificity, sensitivity, parasite detection; malaria species

identification, malaria stages identification and parasite density were assessed in comparison to the pre-determined results of the reference laboratory.

3. Results

The Panel testing consisted of five stained slides distributed to 60 participating laboratories. Of the total participating laboratories, 35 laboratories have examined and reported their report on time (58.3%) and 19 laboratories have examined the slides, but reported their results late (31.7%). The remaining six laboratories failed to participate in this round (**Figure 1)**

Of the 300 panel slides, 180(60.0%) were positive and 120 (40.0%) were no malaria parasite seen (NMPS) slides. 80 (60.0%) tested positive for malaria parasites, while 120 (40.0%) showed no malaria parasite seen (NMPS).Among the 180 positive slides, 120 (66.7%) were *Plasmodium vivax* and 60 (33.3%) were Plasmodium falciparum.

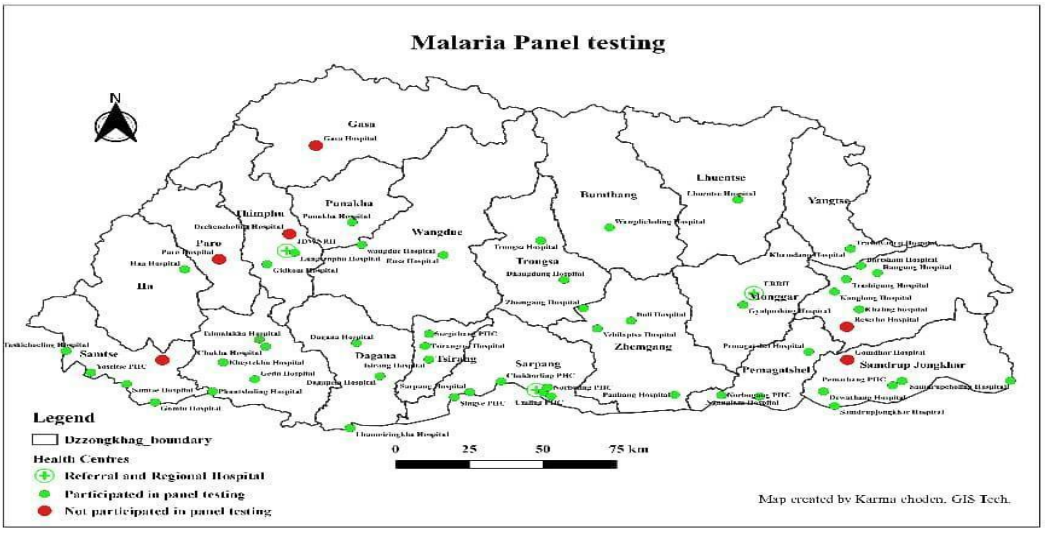

Figure 1. Participating laboratories for panel testing

A total of 123 laboratory staff including laboratory officers, laboratory technicians and malaria technicians have participated from various health facilities and reported their panel results. Out of 123, 76.42% (n=94) microscopists have examined and reported on time and the rest 23.58% (n=29) reported late **(Figure 2)**

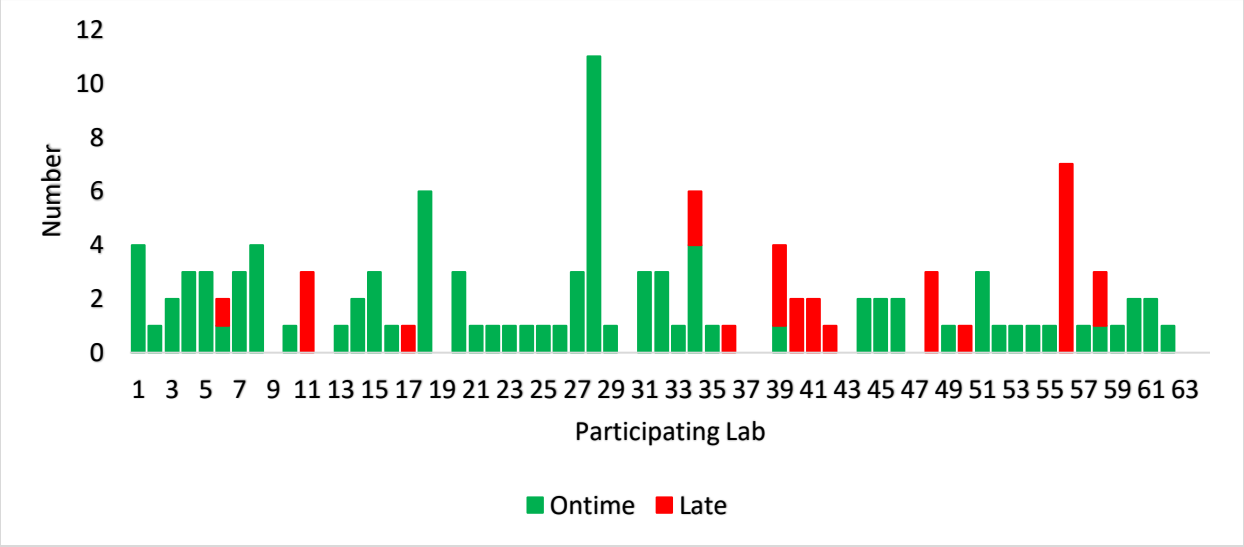

Figure 2: Panel reporting timeliness on malaria microscopy.

Accuracy of results for malaria parasite detection:

Malaria parasite detection refers to the assessment of the slides on the detection of malaria parasites in both positive and negative slides.

Malaria parasite detection agreement = $\frac{TP + TN \times 100}{(TP+ FP+TN+FN)}$

Of the total 615 slides examined by microscopists,357 (58.05%) were correctly reported. Among the 369 positive slides, 222 (60.16%) were correctly detected as malaria-positive and the rest (39.84%) were reported as false negative. From 246 negative slides, 135 (54.88%) were correctly detected as No malaria parasite seen (NMPS) and the rest were reported as false positive (45.12%) (**Figure 3)**

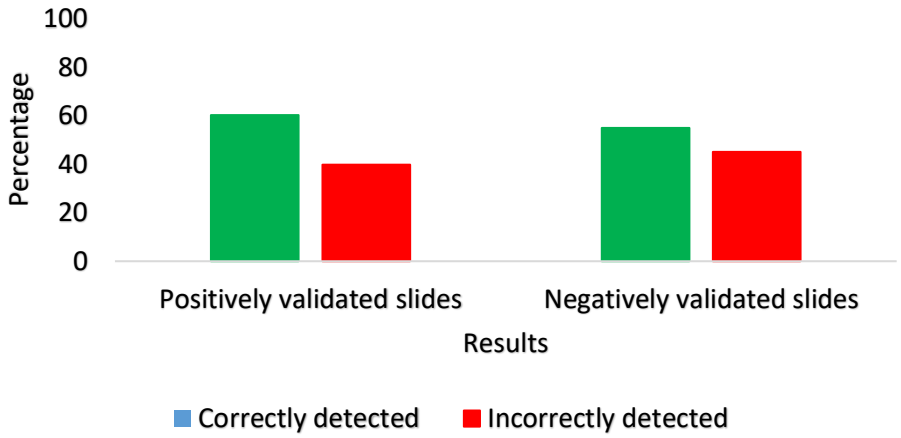

Figure 3: Proportion on malaria parasite detection

Plasmodium species identification

Out of 369 positive reported slides, the proportion of correct detection for Pf and Pv were 88 (71.54%) and 134 (54.47%) respectively. 20 microscopists failed to identify Pf and 49 microscopists failed to identify Pv and reported NMPS. Additionally, 15 Pf slides were misread as 14 Pv slides and one mixed infection as Pf+Pv. Likewise, 52 Pv slides were misread and identified as 49 Pf and three mixed infections of Pf- Pv respectively (**Figure 4**). The misreading of the positive slides as negative is considered a major error.

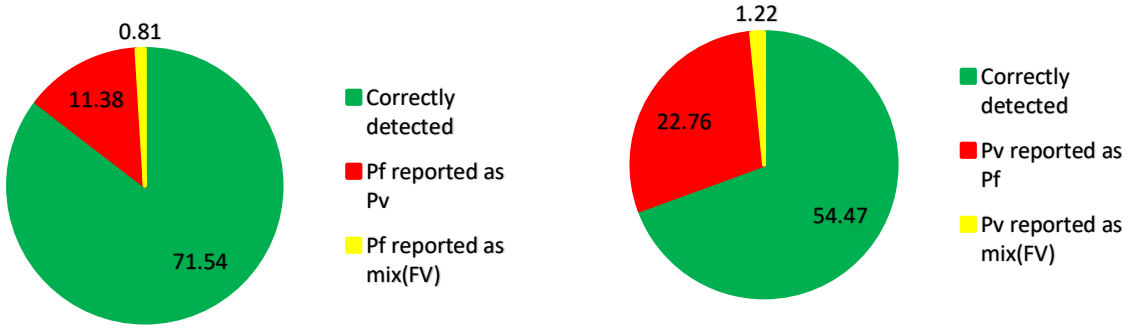

Figure 4: Percentage of plasmodium parasite identification and miss identification.

Out of the 126 Pf slides containing trophozoites, 76 (61.79%) were accurately identified as trophozoites and 13 (10.57%) were identified mis identification of parasite stage and the remaining results were discordant. Among the 47 Pf slides, the microscopists failed to identify parasite stages, resulting in 20 false negatives, and 15 identification errors reported. Out of the 246 Pv slides containing trophozoites and gametocytes, only 38 (15.45%) were correctly identified as all forms of malaria stages, and the remaining results were reported as discordant (Table 1).

Table 1: Comparison of plasmodium species stages reported by participating laboratories

Malaria stage results	**Reference Laboratory**		
Participating laboratories	**T (PF Slide)**	**T (PV Slide)**	**TG (PV Slide)**
T	76 [a]	23 [a]	49 [b]
S			7 [d]
G	1 [e]		
TS	5 [f]	2 [f]	21 [g]
TG	2 [f]	1 [f]	15 [a]
TSG	4 [f]	2 [f]	11 [j]
FN	20	43	6
IDE	15	48	11

T: Trophozoite; **S:** Schizont; **G:** Gametocyte; **FN:** False Negative; **IDE:** identification error

Codes: a: denote correct identification of all stages, b: denote gametocyte missed; c: denote only schizont and gametocyte reported in slide containing trophozoite; d: denote trophozoite & gametocyte missed; e: denote trophozoite missed; f: denote trophozoite/schizont/gametocyte reported in slide containing trophozoite; g: denote trophozoite and schizont reported in slide containing trophozoite and gametocyte; h: denote trophozoite and gametocyte reported in slide containing trophozoite; i: denote schizont and gametocyte reported in slide containing trophozoite and gametocyte; J: denote all stages reported in slide containing trophozoite and gametocyte

Parasite quantification

The panel includes one P. falciparum-positive and two P. vivax slides with parasite densities ranging from 234 to 32,197p/µL to test the parasite counting skills of the participating laboratories. The laboratories' counts were compared to the reference count. The reference count served as the basis for scoring in this category. To achieve a correct score, the responses had to be within ±50% of the reference count. Any other answers outside these ranges received zero scores.

For this round of panel test assessment, the accuracy range (±50% of the reference count) for the three positive slides was 101 (27.67%), while the remaining slides reported values outside the reference count range.

However, 121 slides (33.15%) were reported with parasite density values outside the reference count range. Of these, 67 slides (55.37%) had a lower parasite density compared to the reference count, 54 slides (44.63%) had a higher parasite density, 74 slides (20.27%) had parasite identification errors, and 69 slides (18.90%) were reported as false negatives (Figure 5).

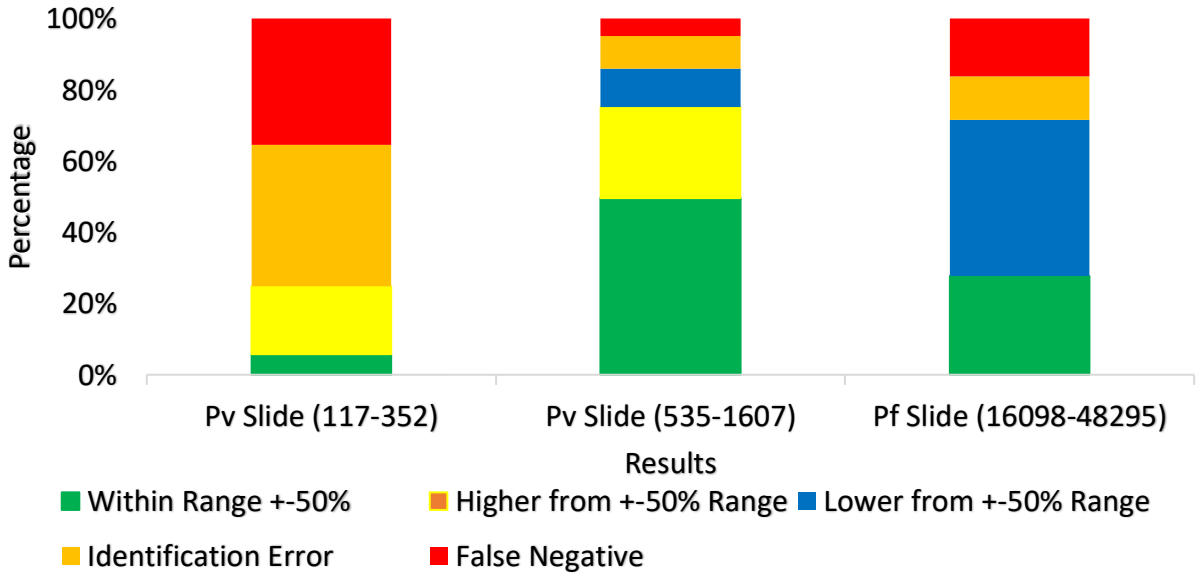

Figure 5: Parasite quantification performance score

4. Discussion

The current report shows poor performance of malaria microscopy in all levels of health facilities. However, data on the performance of panel testing is still limited due to inconsistent participation from the laboratories that took part in this activity.

Irregular Participation in panel testing. Among the 60 laboratories that were invited to participate, there was a lack of response from six of them. Various reasons were cited for this, including insufficient human resources and misplacement of slides.

Late submission of the panel report. Typically, the non-endemic malaria sites were the ones that often submitted their reports late.

False positive reporting. This type of error is considered a minor error, where the slides are misread as positive slides. This can lead to unnecessary admission and treatment of the patient, thereby increasing the risk of anti-malaria resistance and treatment failure.

False negative reporting. This is classified as a significant error wherein a positive slide is mistakenly identified as a negative slide. The under-diagnosis of malaria is a serious concern as it results in patients not receiving appropriate treatment. In cases of severe malaria, this can potentially lead to fatalities. Additionally, the continued transmission of the disease is not disrupted, increasing the risk of malaria outbreaks in the affected area.

Malaria parasite identification and stage concordance. Several participating laboratories have encountered challenges in accurately identifying and differentiating malaria parasite stages, specifically trophozoite, schizont, and gametocyte. It has come to our attention that in a majority of the panel slides, microscopists have failed to examine the schizont and gametocyte stages.

Based on the findings, the participating laboratories encountered challenges in quantifying the parasite density using the formula, as a significant portion of the parasite density values fell outside the ±50% range determined by the reference laboratory.

Conclusion

Overall, the present report demonstrates that laboratory professionals require improvement in malaria microscopy, as the majority scored below 90%. It is crucial to provide training and supervisory visits from a referral lab. Only a few participating laboratories demonstrated good performance in malaria parasite detection, species identification, and determination of parasite density. Therefore, to address these shortcomings, regular NEQAS activities should be conducted to ensure continuous assessment of quality performance. Additional NEQAS methods, such as blinded rechecking and supervisory visits, should complement the panel testing.