ROYAL CENTERS FOR DISEASE CONTROL

QUARTERLY BULLETIN: 2ND QUARTER 2023

WEEK 14 TO 26

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Highlights on National Early Warning and Alerts Response Surveillance(NEWARS)

- Overall reporting rate for notifiable diseases had increased compared to the previous quarter
- A majority of the immediately notifiable diseases/syndromes reported were suspected measles/rubella cases.
- Three patient samples tested positive for measles IgM, one sample tested positive for measles PCR and two tested positive for rubella IgM.
- Eight suspected bacterial meningitis, two suspected cholera cases, five malaria, five suspected pertussis, eight severe dengue, two rabies, one anthrax case was reported during the quarter
- There were 28 diseases outbreaks/ events were reported , of which 12 were of suspected chickenpox, eight were of influenza like illness, three events of food poisoning, one chilblain, two AGE, one suspected typhoid, one conjunctivitis

1. National Early Warning Alert and Response Surveillance (NEWARS)

1.1 Reporting status of health centres under 20 Dzongkhags

In the second quarter 2023, a total of 3710 weekly reports were expected from 265 health centers across the country. Compared to the first quarter, there was an overall improvement of (2.0%) in total reports submitted. Overall 94.9% of reports were received in the NEWARS of which 86.3% were reported on time, 8.6% were reported late and the rest were not reported (**Figure 1**).

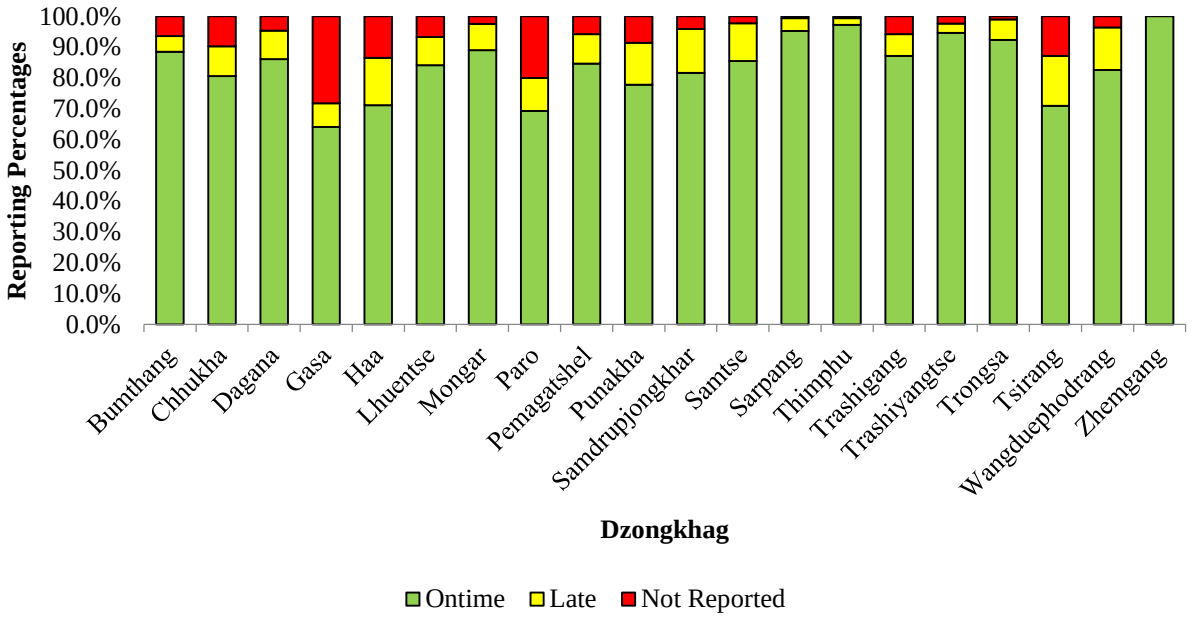


Figure 1: Dzongkhag-wise weekly reporting status for2nd Quarter 2023

1.2 Status of Weekly Notifiable Diseases/Syndromes reported by health centers

Among 11 weekly reportable diseases/syndromes, the highest number of cases was reported were ARI- 49294(83.6%) followed by AWD-7289 (12.4%), (**Table 1**). The total number of cases reported was higher in this quarter compared to the first quarter 2023.

Table 1: Notifiable diseases/syndromes reported by Dzongkhags

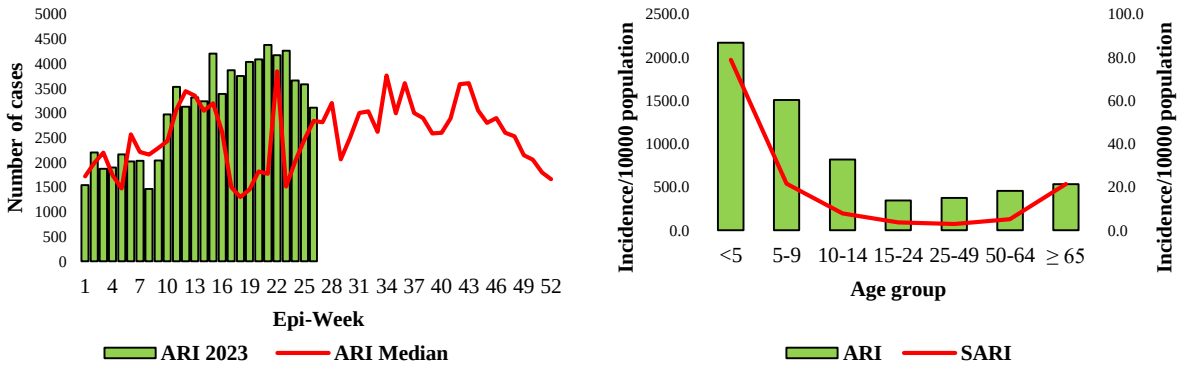
Dzongkhag	ABD	AWD	AJS	ARI	DGF	MUM	FWR	FDP	TPF	SAR	RKS
Bumthang	4	54	0	670	0	0	1	0	0	3	0
Chhukha	60	575	8	5379	8	1	39	6	11	91	13
Dagana	13	440	0	2634	37	0	15	2	10	11	17
Gasa	2	26	0	45	0	0	0	0	0	1	0
Haa	2	90	0	503	0	0	0	3	2	1	1
Lhuentse	16	80	1	850	3	0	0	0	2	1	0
Mongar	152	369	4	2383	4	0	6	6	0	55	1
Paro	7	344	4	562	0	0	0	0	0	0	0
Pemagatshel	3	206	0	1572	5	0	0	3	11	7	5
Punakha	38	303	3	1328	1	4	30	3	0	18	0
Samdrupjongkhar	47	408	4	3398	3	1	6	35	1	124	0
Samtse	24	607	0	4353	2	2	18	13	1	72	2
Sarpang	19	605	9	6882	14	2	27	3	18	249	18
Thimphu	37	924	0	8373	0	6	9	0	0	154	0
Trashigang	46	588	20	1313	3	0	25	8	98	45	12
Trashiyangtse	29	513	24	1446	0	2	6	12	0	22	3
Trongsa	15	312	25	2268	2	0	1	0	0	10	0
Tsirang	21	115	0	1153	0	0	0	0	0	16	0
Wangduephodrang	90	611	28	2660	0	21	31	1	0	16	0
Zhemgang	7	119	0	1522	5	1	1	9	1	7	8
Total	**632**	**7289**	**130**	**49294**	**87**	**40**	**215**	**104**	**155**	**903**	**80**

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).

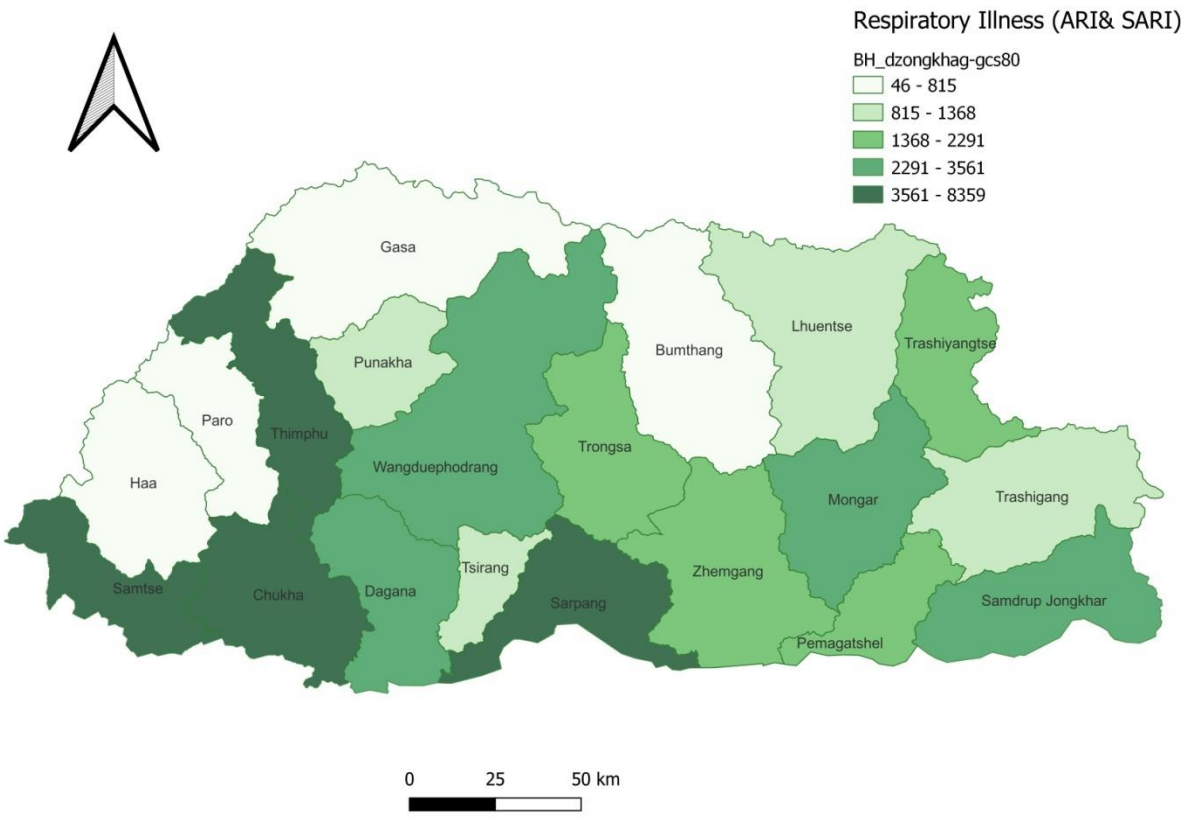
1.3 Descriptive analysis of most common notifiable diseases:

1.3.1 Respiratory Illness (ARI and SARI) syndrome

A total of 50197cases of respiratory illness were reported (ARI (98.0%)and rest was SARI cases). The trend of ARI cases was found slightly higher compared with the median of the last three years of the same quarter **(Figure 2A).** The most commonly affected age group by respiratory illness was observed in the younger age-group **(Figure 2B)**. By district, Thimphureported the maximum number of ARI cases **(Figure 2C).**



A: Cases by Epi-weekB: Incidence by age group

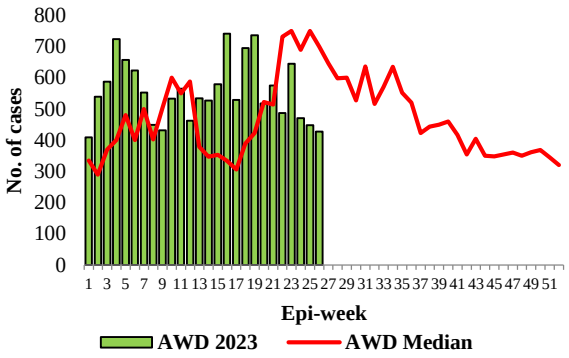


C: Respiratory illness cases by district

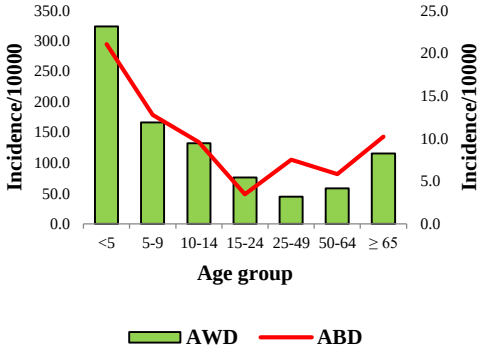
Figure 2: Respiratory illness incidence by epidemiological weeks, age groups and place.

1.3.2 Diarrheal syndrome

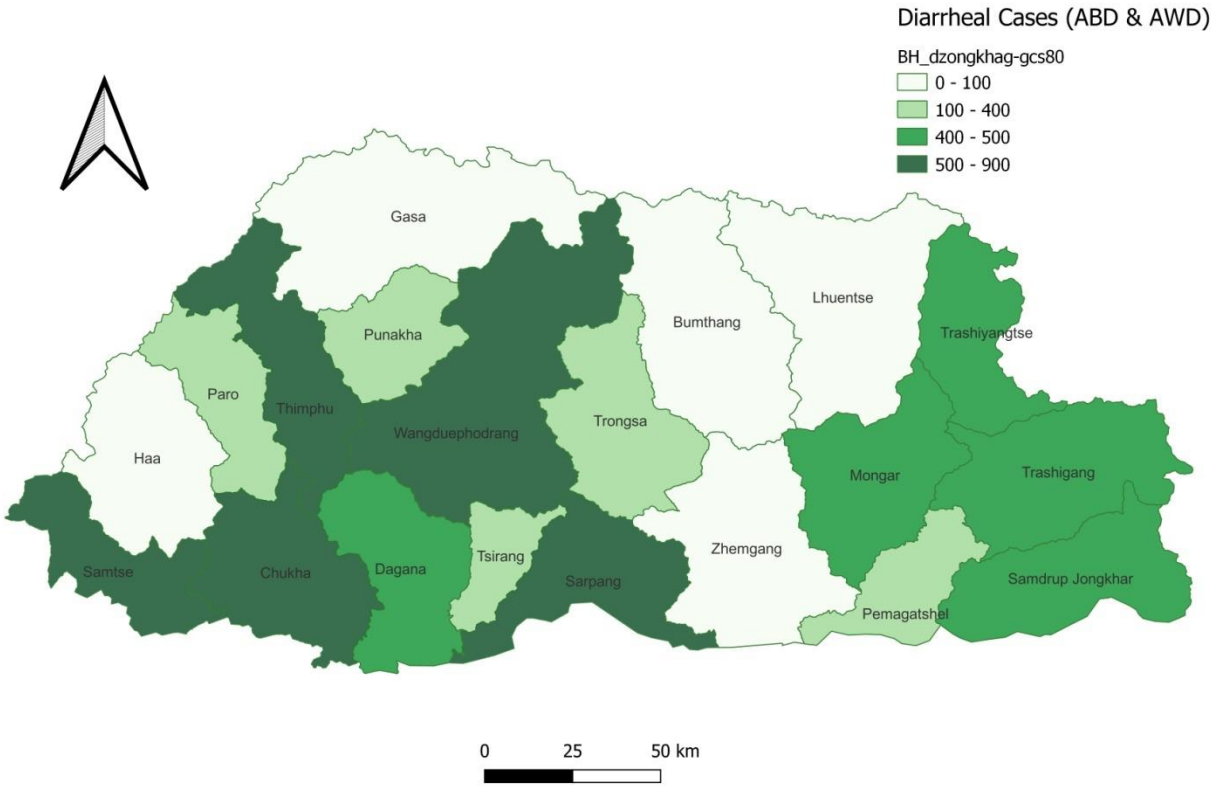
A total of 7921 diarrheal cases were reported (AWD: [7289] 92.0% and rest were ABD). Compared with the median for the last three years, the trend for diarrheal diseases was found slightly higher from week 14 until week 20 and slightly lower from week 21 onwards (**Figure 3A**). A high incidence of diarrheal diseases was observed in children 0-4 years (**Figure 3B**). Thimphu and Wangduephodrang reported maximum diarrheal cases **(Figure 3C)**.



A: Cases by Epi-week



B: Incidence by age group

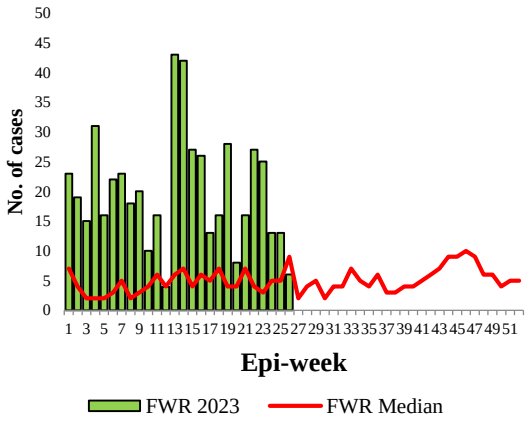


C: Diarrheal Cases by districts

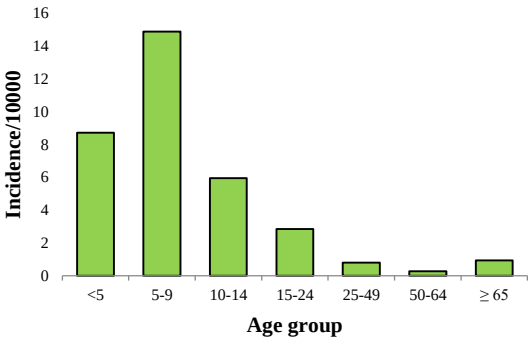
Figure 3: Diarrheal disease incidence by epidemiological weeks, age groups and place.

1.3.3 Fever with Rashes syndrome

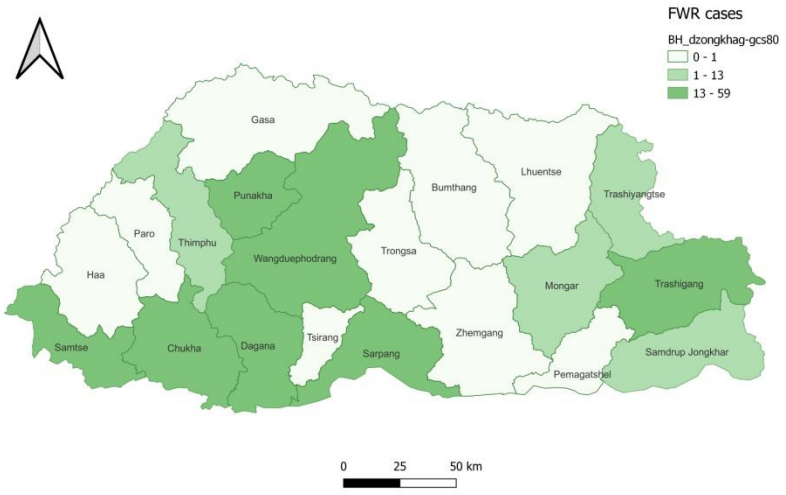
A total of 226 cases of fever with rash (FWR) syndrome were reported in the quarter **(Figure 4A).**The trend of FWR was found consistently higher compared to the median of last three years. A majority of FWR were reported in the age group < 14 years **(Figure 4B)**. Among the dzongkhag,Chukhaand Wangduephodrang reported maximum number of the cases **(Figure 4C).**



A: Cases by Epi-week



B: Incidence by age group



C**:** Fever with rash cases by districts

Figure 3: Fever with rash incidence by epidemiological weeks, age groups and place.

1.4 Immediately Notifiable Diseases/syndromes

A majority of the immediately notifiable diseases/syndromes reported were suspected measles/rubella cases (n=20). Of the total, three samples were positive for measles IgM and one sample was positive for measles PCR. Four samples were positive for Rubella IgM. Additionally, eight suspected bacterial meningitis, two suspected cholera cases, five malaria, five suspected pertussis, eight severe dengue, one anthrax, two cases of rabies were reported during the quarter **(Figure 4).** The respective samples tested negative for cholera and anthrax.

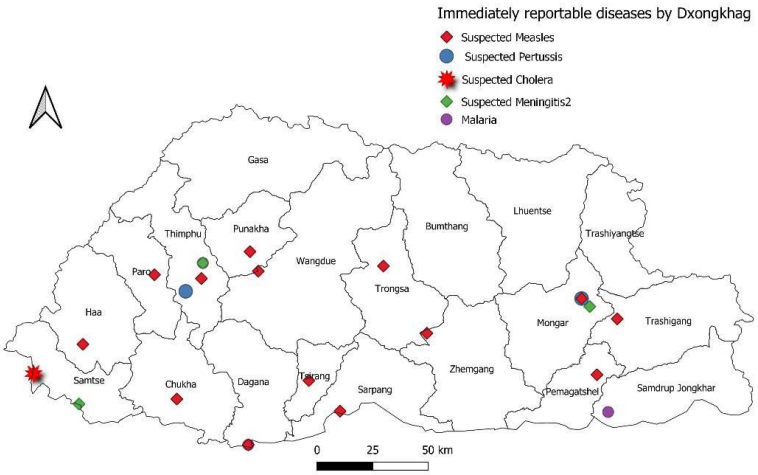


Figure 4: Distribution of immediately notifiable diseases/syndrome by dzongkhag

1.5 Events

In the second quarter, 28 diseases outbreaks/ events were reported , of which 12 were of suspected chickenpox, eight were of influenza like illness, three food poisoning, one chilblain, two AGE, one suspected typhoid, one conjunctivitis. Specimen from suspected typhoid was tested negative. Influenza subtype FLU A/H3 was detected from the outbreak that occurred in Punakha. All outbreaks were responded to by the respective health centers and the District Health Rapid Response Team (DHRRT) upon the recommendations of RCDC. There was no mortality following the outbreak.

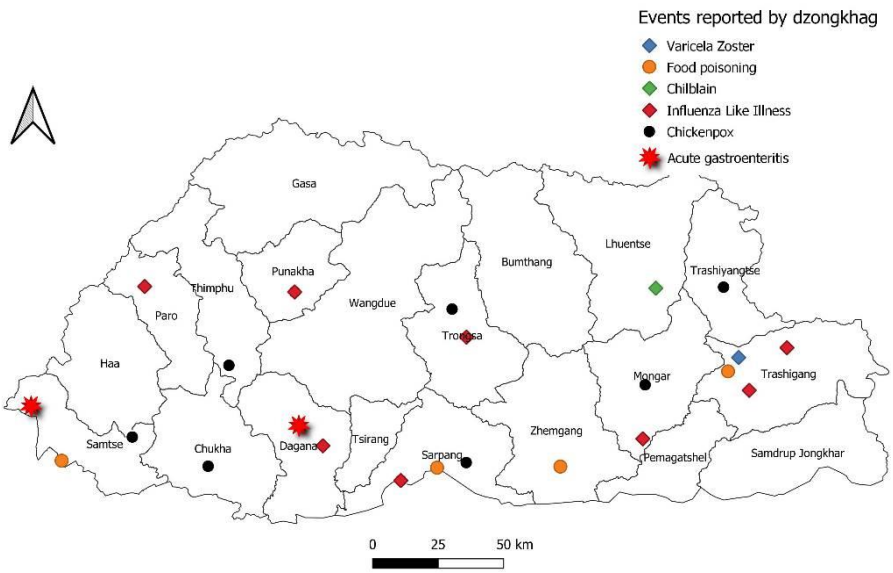


Figure 5: Distribution of events by dzongkhag

2. Laboratory Based Surveillance

2.1 Drug Resistant Surveillance for Tuberculosis

A total of 539 patient samples were received at National Tuberculosis Reference Laboratory (NTRL) for culture and drug susceptibility testing (DST) for anti-tuberculosis drugs. Of the total, 200 (37.1%) were pulmonary samples, 57 (10.6%) were extra-pulmonary samples and 151(28.0%) were pulmonary samples received for TB screening for VISA. In addition, 131(24.3%) follow-up samples were received for culture for MDR-TB patients under treatment.

Among the pulmonary samples, new smear positive (NSP) constituted 44.0% (n=88) of the total samples, and 0.1% (n=2) were previously treated cases (**Figure 6**).

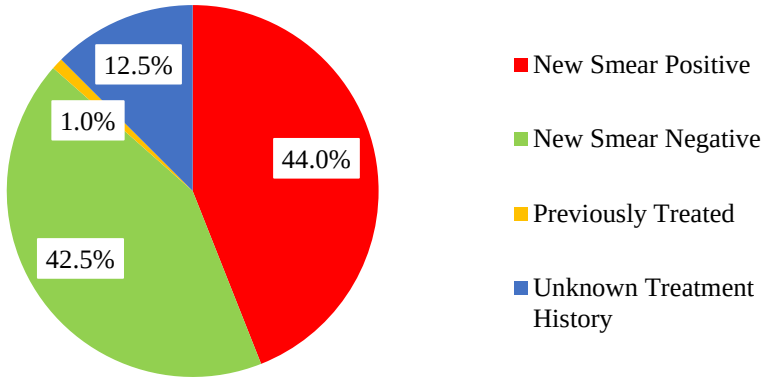


Figure 6: Classification of Pulmonary TB samples

2.1.1 Drug Sensitivity Test

Drug sensitivity test report was available for 91 samples using Line Probe Assay and seven samples had reports for Liquid DST for first line drugs. A total of seven multi-drug resistant tuberculosis (MDR-TB) cases were detected among patients with complete DST report. Of the seven samples, two of the MDR-TB cases were detected among new smear positive pulmonary samples, one case was detected from new smear negative sample, three MDR-TB cases was detected from EPTB samples and one from sample screened for VISA. MDR-TB cases were highest in the age group of 20-29 years (85.7%) (**Figure 7**). Of the seven MDR-TB samples, second line DST was available for five MDR-TB cases. Four samples were sensitive to both Fluoroquinolones and aminoglycoside. One sample was resistant to fluroquinolone.

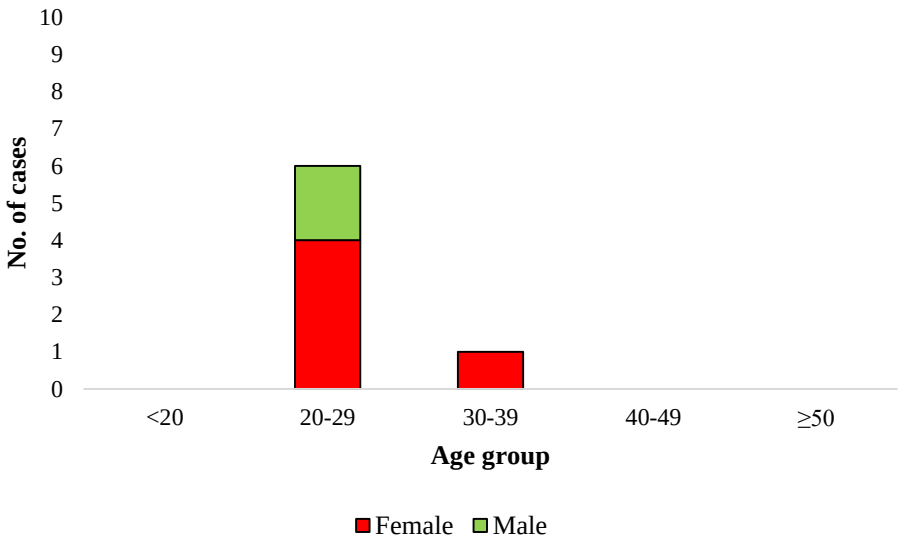


Figure 7: Distribution of MDR-TB cases by age group and gender

2.2 COVID-19 Integrated Influenza surveillance

2.2.1 Epidemiological Surveillance

Influenza-like illness (ILI) and severe acute respiratory infection (SARI) activity increased during the second quarter (April to June 2023). A total of 2196 ILI cases and 377 SARI cases were reported by respective sentinel hospitals. The surge in ILI cases were observed by 57.7 % compared to first quarter and 44.3 % SARI cases (**Figure 8**).

The most affected age group for ILI was 5 – 14 years (31.0 %) followed by 15 – 29 years (18.0 %), while for SARI the most affected age group was from 0 – 1 years (32.0 %) followed by 2 – 4 years (23.0 %) (**Figure 9A & 9B**).

The cases were reported from all the respective sentinel sites, though few sites have not reported and few sites have reported as zero reporting. Paro (n=454) and SamdrupJongkhar Hospital (n=454) has reported highest ILI cases and Gelephu Hospital (105) has reported highest SARI cases during the second quarter of 2023 (**Table 2&3**).

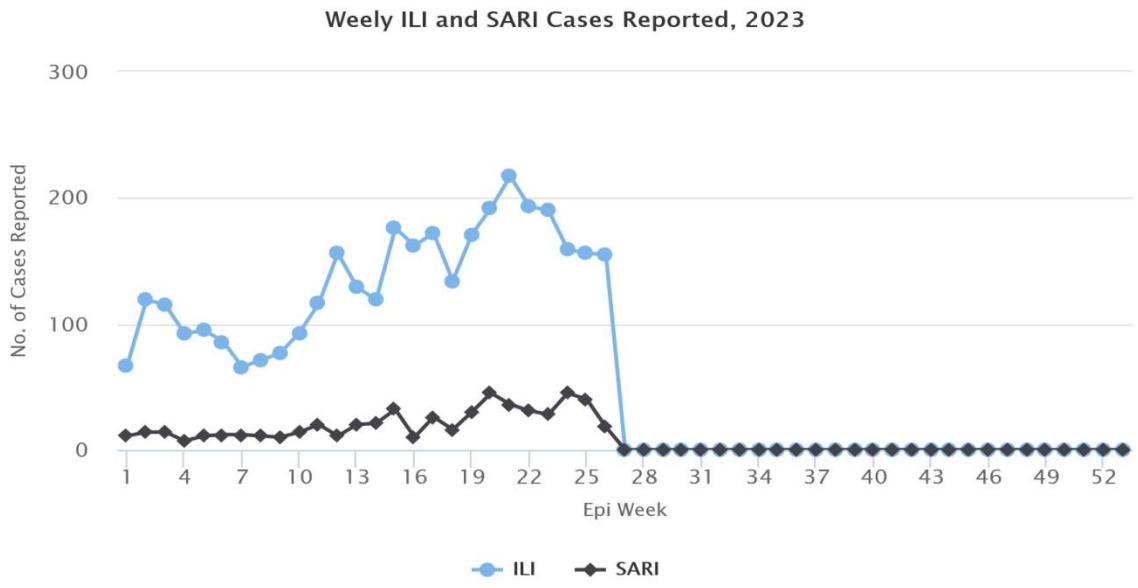
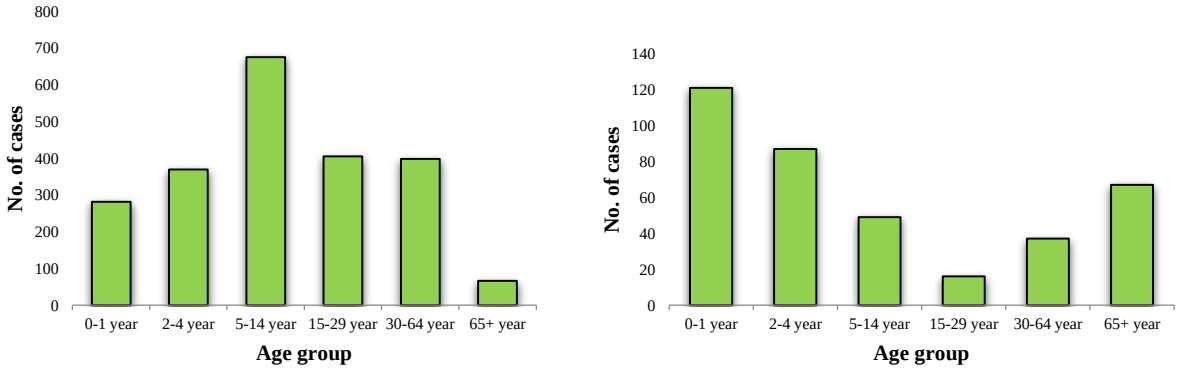


Figure 8: Weekly ILI and SARI cases reported from Sentinel sites



A: ILI cases by age group

B: SARI cases by age group

Figure 9: ILI and SARI cases by age group

Table 2: Weekly ILI cases reporting Status from week 14 – 26, 2023

	EPI-WEEK													
ILI Sentinel Hospitals	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**Total**
Paro Hospital	4	7	7	4	11	44	70	97	78	87	60	88	71	628
Punakha Hospital	7	14	13	9	20	8	9	8	6	6	4	6	0	110
SamdrupJongkhar Hospital	38	93	69	86	55	46	39	45	50	28	30	27	22	628
Samtse Hospital	10	14	11	15	9	16	14	10	18	14	7	9	20	167
Trashigang Hospital	10	14	11	16	12	11	14	16	16	12	21	12	10	175
Trongsa Hospital	26	21	34	30	21	31	33	15	18	16	12	9	17	283
Tsirang Hospital	24	13	17	12	6	15	13	26	7	27	25	5	15	205
Grand Total	**119**	**176**	**162**	**172**	**134**	**171**	**192**	**217**	**193**	**190**	**159**	**156**	**155**	**2196**

Table 3: Weekly SARI cases reporting Status from week 14 – 26, 2023

	EPI-WEEK													
SARI Sentinel Hospitals	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**Total**
Gelephu CRRH	7	11	5	8	2	8	10	7	7	9	15	16	0	105
JDWNRH	2	0	0	2	1	2	9	2	2	0	6	5	1	32
Monggar ERRH	0	0	0	1	3	0	0	0	0	0	0	0	0	4
Paro Hospital	3	3	0	5	6	0	9	4	6	7	7	6	4	60
Phuentsholing Hospital	7	8	1	7	3	10	10	8	6	1	5	4	5	75
Punakha Hospital	1	5	3	1	0	8	7	10	7	9	10	3	6	70
SamdrupJongkhar Hospital	0	NR	0	1	1	2	0	2	1	0	1	0	0	8
Samtse Hospital	0	0	0	0	0	0	NR	0	0	1	1	3	0	5
Trashigang Hospital	0	1	0	0	0	0	0	3	0	0	0	0	0	4
Trongsa Hospital	1	2	1	0	0	0	0	0	0	1	0	0	1	6
Tsirang Hospital	0	2	0	1	0	0	0	0	2	0	0	3	0	8
Grand Total	**21**	**32**	**10**	**26**	**16**	**30**	**45**	**36**	**31**	**28**	**45**	**40**	**17**	**377**

Note: NR- Note Reported

2.2.2 Virological Surveillance

Influenza positivity has increased during the second quarter of the year 2023 (epi week 14 – 26). The overall Influenza positivity rate was 20.7 % (211/1021), which is more than the previous quarter (19.9 %). A total of 1021 flu specimens including ARI outbreak samples (ILI- 715, SARI-265, Outbreak- 41) were received and tested for Influenza and SARS-CoV-2 through multiplex RT-PCR (Flu SC2) and detected 20.7 % (211/1021) Influenza positives,2.4 % (24/1021) SARS-CoV-2 and 0.1 % (1/1021) Co-infection. Influenza subtype A(H3N2) (62.1 %) was found most predominating strain, followed by Flu B (37.9 %) (**Figure 10, Table 4**).

Influenza positivity rate was more among ILI patients (25.6 %) compared to SARI patients (4.5%). Similarly COVID-19 positive percent was more among ILI (3.1 %) than SARI (0.8 %) (**Table 4**). The Influenza positivity rate was higher in Trongsa and Mongar Districts (44.4 – 53.1 %) followed by Punakha and Tsirang Districts (**Figure 11**).

The median age for Influenza positive was 13 years (IQR: 8 – 27 years). The most affected age group for Influenza was 5 – 14 years (47.3 %), followed by 15 – 29 years (23.2 %). Males (55.9 %) were affected more than the females (44.1 %) (**Figure 12**). Almost all the cases had cough (88.6 %) and fever (88.2 %), followed by sore throat (66.3 %) and headache (55.3 %).

Samtse Hospital (182) and Trongsa Hospital (152) followed by Phuentsholing (141) and Gelephu (133) have collected more samples compared to rest of the sentinel hospitals (**Figure 13**&**Table 4**).

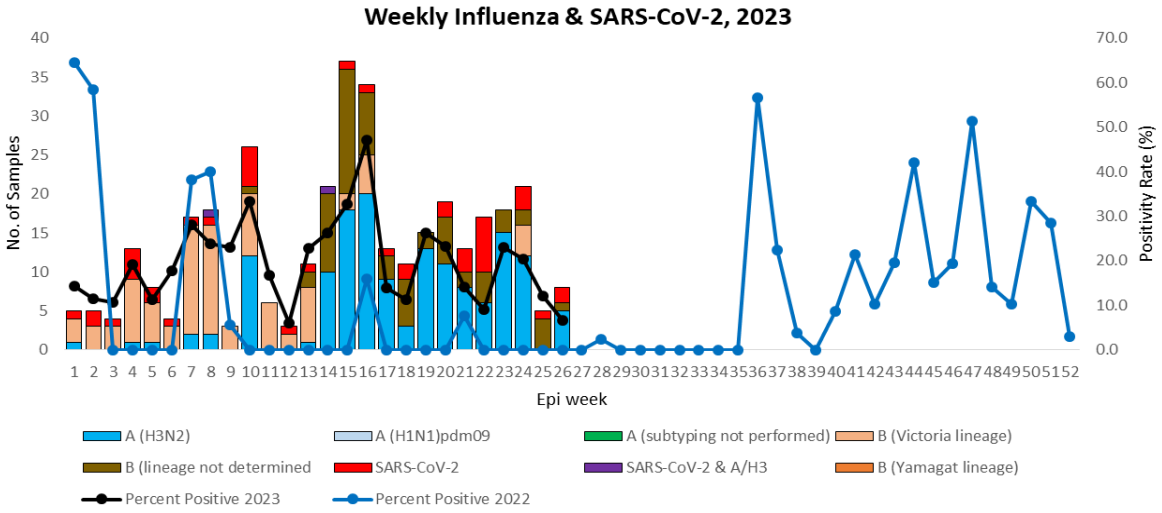


Figure 10: Weekly Influenza subtypes and SARS-CoV-2

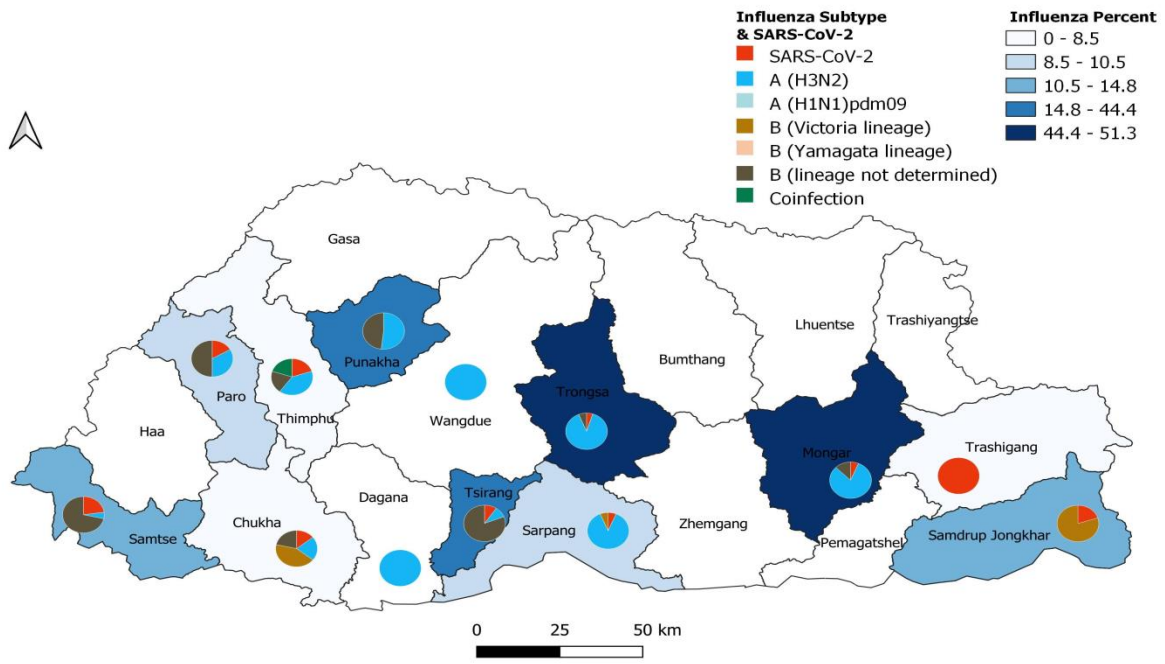


Figure 11: Influenza subtypes and SARS-CoV-2 with Influenza positive percent in Sentinel Hospitals

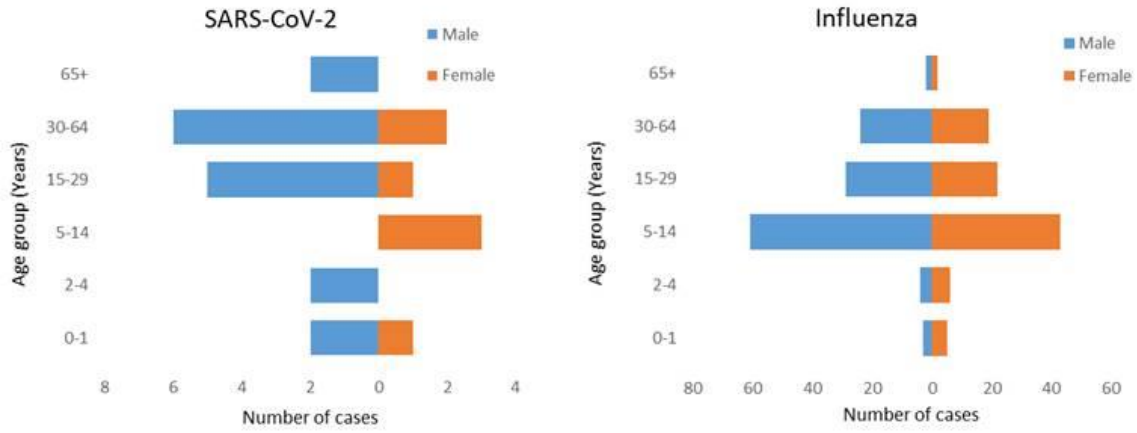


Figure 12: Influenza and SARS-CoV-2 by age group and gender in 2nd quarter 2023

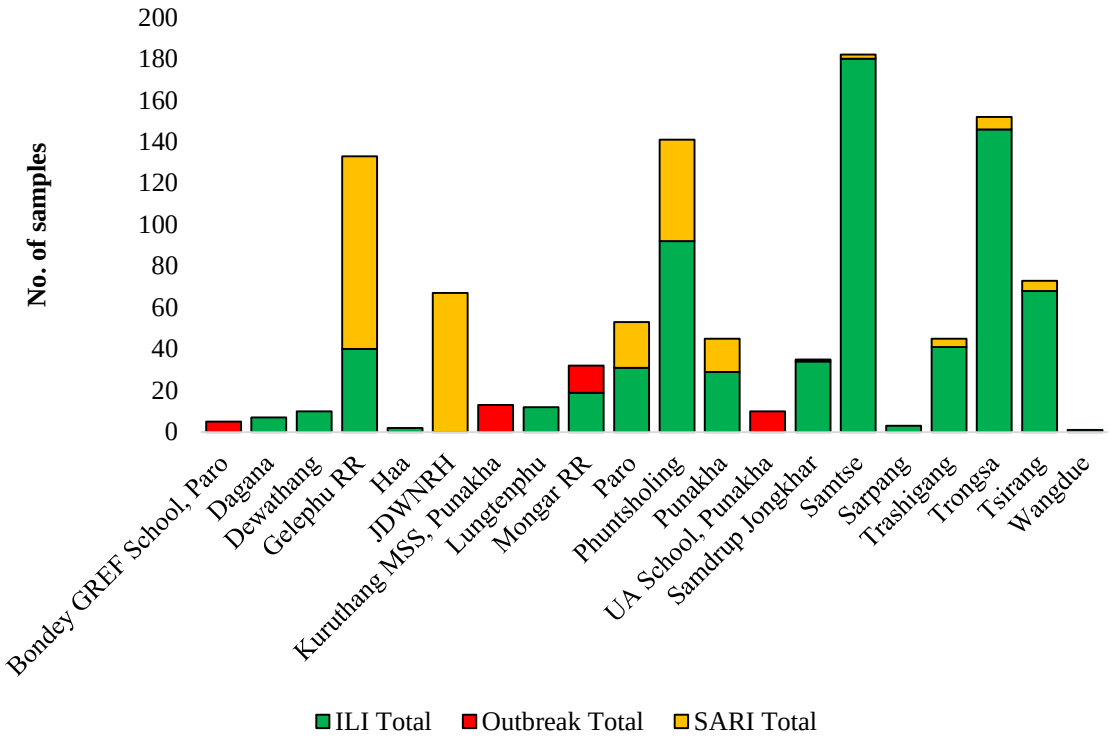


Figure 13: Number of ILI, SARI and Flu Outbreak Specimens received in 2nd quarter 2023

Table 4: Summary table for Influenza subtypes and sample tested in quarter, 2023

	ILI						Outbreak			SARI				
Sentinel Hospitals	A (H3N2)	B (lineage not determined)	B (Victoria lineage)	SARS-CoV-2	A/H3 & SARS-CoV-2	ILI Total	A (H3N2)	B (lineage not determined)	Outbreak Total	A (H3N2)	B (lineage not determined)	SARS-CoV-2	SARI Total	Grand Total
Bondey GREF School, Paro									5					5
Dagana	5					7								7
Dewathang						10								10
Gelephu RR	9		1			40				4		1	93	133
Haa						2								2
JDWNRH													67	67
Kuruthang MSS, Punakha							4		13					13
Lungtenphu	2	1		1	1	12								12
Mongar RR	4	2		1		19	9		13					32
Paro	1	3				31				1		1	22	53
Phuntsholing	3	3	6	2		92							49	141
Punakha	9	6				29				1	4		16	45
UA School, Punakha								3	10					10
Samdrup Jongkhar			4	1		34							1	35
Samtse	2	25		8		180							2	182
Sarpang	1					3								3
Trashigang				3		41							4	45
Trongsa	71	5		4		146				2			6	152
Tsirang	2	17		2		68							5	73
Wangdue	1					1								1
Grand Total	**110**	**62**	**11**	**22**		**715**	**13**	**3**	**41**	**8**	**4**	**2**	**265**	**1021**

2.3.1 Laboratory-based surveillance for vaccine-preventable diseases

During the second quarter of the year a total of 179 suspected measles and rubella samples were received. Out of these, three Measles IgM (two from Paro, one from and SamdrupJongkhar) and four Rubella IgM (one each from Paro, JDWNRH, Trongsa and SamdrupJongkhar) was positive by ELISA. (**Figure 14**). One sample was positive for Measles by RT PCR and sample has been sent to Regional Referral Laboratory (RRL) Thailand for further confirmation and genotyping sequencing. A maximum number of samples received from few dzongkhag like Paro, Samdrupjongkhar, Phuntsholing and Trongsa were attributed to the detection of Positive cases and their contact identified during the active case search (**Table 5**). All eight samples received for Japanese Encephalitis was negative. No samples were received for Pertussis.

Table 5: Number of samples received from health center's for MR and JE surveillances

Surveillance	Site/ Hospital	Number of samples received
MR	Giadakom Hospital	05
	Paro Hospital	66
	Trongsa Hospital	8
	Sarpang Hospital	5
	Bumthang	3
	JDWNRH	8
	Nganglam	1
	Trashigang	2
	Samdrupjongkhar	36
	Punakha	7
	Lungtenphu	1
	Chuzergang	1
	Tsirang	1
	Panbang	6
	Gelephu	1
	Lhamozingkha	1
	Chukha	2
	Haa	1
	Mongar	2
	Wangduephodrang	1
	Pling	21
AES (JE)	JDWNRH	08

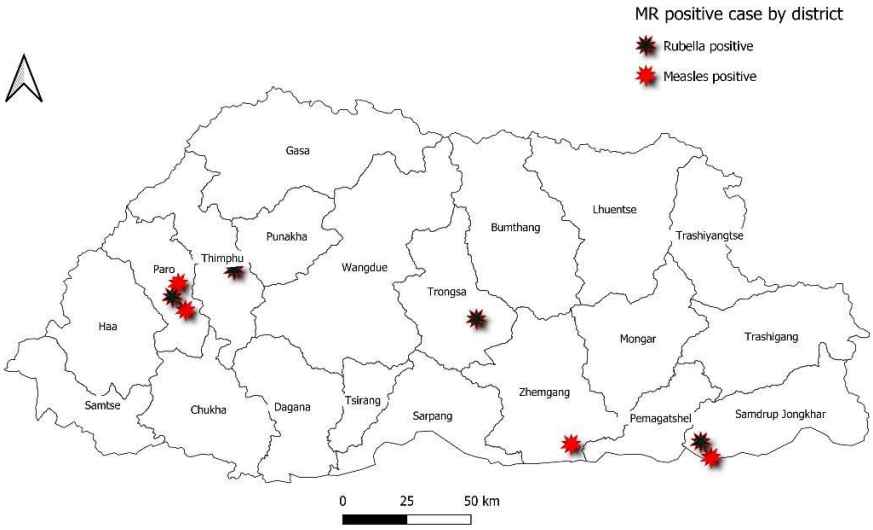


Figure 14: Map of Bhutan showing measles and rubella confirmed cases

2.3.2 Acute Undifferentiated Febrile Illness (AUFI) and other confirmatory tests

IDSL received 14 samples for AUFI surveillance, 15 samples for dengue and four samples for scrub typhus confirmation from 58 sentinel sites (**Table 6**). Some of the samples received were for diagnostic purpose.

Table 6: Samples received and tested for AUFI, dengue and scrub typhussurveillances

Surveillance/ Test requested	Site/ Hospital	No. of samples received	Positive test result						
			DENV NS1	**DEV IgM**	**Scrub typhus IgM**	**Lepto IgM**	**JE IgM**	**CHIKV IgM**	**Bru IgM**
AUFI	JDWNRH	2	0	0	0	1	0	0	0
	Tsirang	2	0	0	0	2	0	0	0
	Punakha	6	0	0	0	4	0	0	2
	Trongsa	2	0	0	0	1	0	1	0
	Sjongkhar	2	0	0	0	1	0	0	1
Dengue Confirmation	Pling	2	0	0	NA	NA	NA	NA	NA
	Sjongkhar	1	0	0	NA	NA	NA	NA	NA
	CRRH	2	0	0	NA	NA	NA	NA	NA
	Gedu	8	0	0	NA	NA	NA	NA	NA
	Dewathang	1	0	0	NA	NA	NA	NA	NA

DENV: Dengue virus, CHIKV: Chikungunya virus, Bru: Brucella NA: Not applicable

2.4. Sentinel Surveillance for Diarrheal Etiologic Agents

This quarter, 57 samples were received from seven sentinel sites (**Figure 15**). Most of the samples received were from the CRRH. Of these, 35 (61.0%) were male and 22 (39.0%) were females.

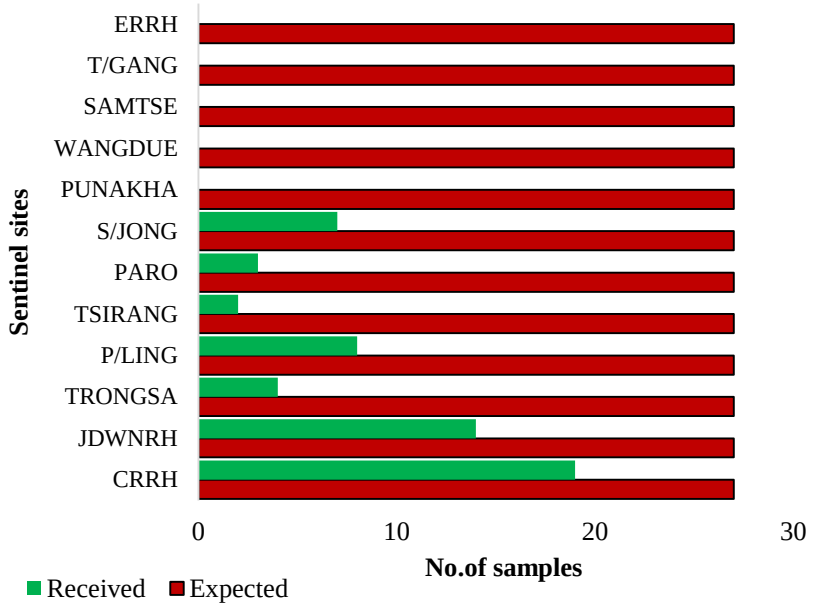


Figure 15: Number of fecal specimens collected from sentinel sites

The sample's character consists of loose (58.0%), watery (39.0%) and (4.0%) bloody. The mean age of the patients enrolled was 19 years. Of the 57 cases, 33.0% required hospital admission while the rest were treated on OPD basis. Of all, two diarrhea cases were found linked to having consumed food suspected of contamination. The most detected enteropathogens were Astrovirus (n=2), and DEC (n=5) (**Figure 16**). The antimicrobial-resistant pattern for the isolated bacterial pathogens is provided in (**Table 7**).

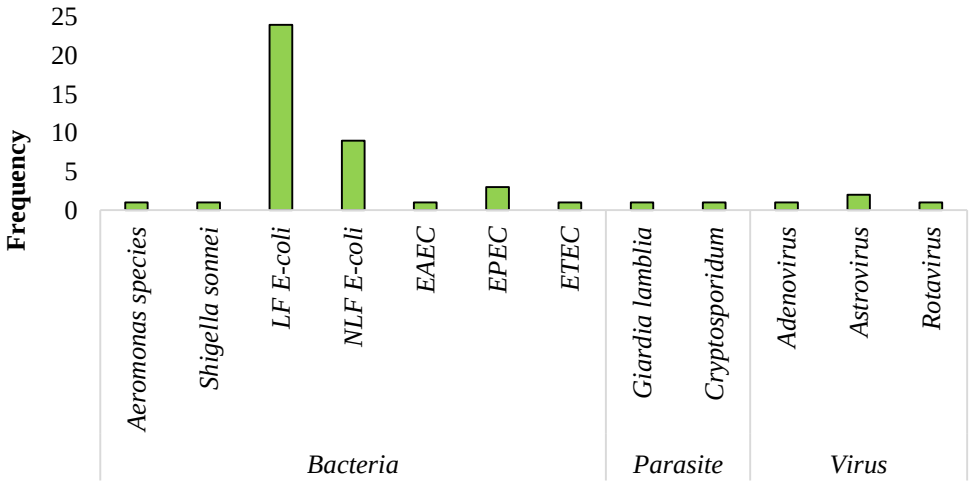


Figure 16: Proportion of pathogens isolated

Table 7: Anti-bio gram (Resistant pattern) for bacterial pathogens:

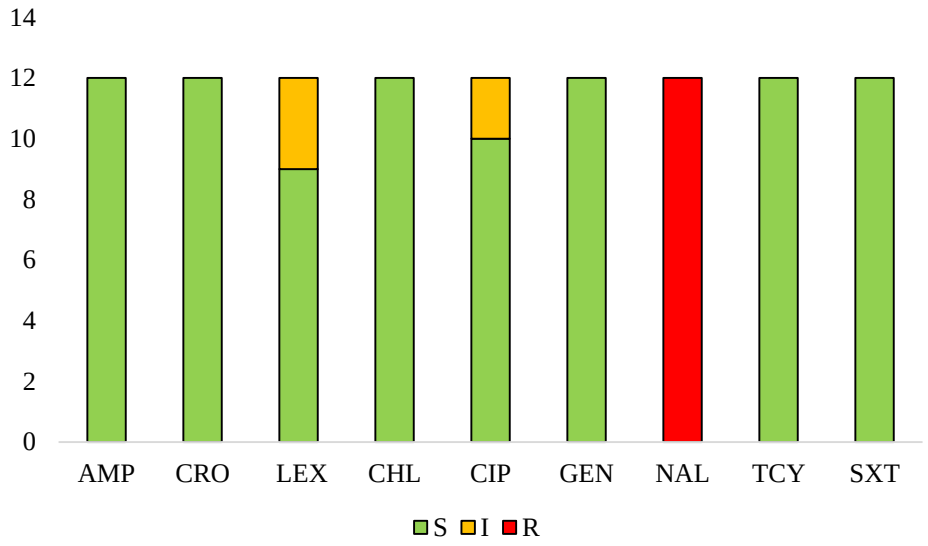
Pathogen	AMP	CZO	CRO	LEX	CHL	CIP	GEN	NAL	TCY	SXT
***Shigella sonnei* (n=1)**	0	1	0	0	0	1	0	1	0	0
***Aeromonas species* (n=1)**	1	1	0	1	1	0	0	1	0	1
***EAEC* (n=1)**	0	1	0	1	0	1	0	1	0	0
***EPEC* (n=3)**	3	3	1	3	0	3	0	3	0	1

AMX (Amoxicillin), CZO (Cephazolin), CRO (Ceftriaxone), LEX (Cephalaxin), CHL (Chloramphenical), CIP (Ciprofloxacin), GEN (Gentamycin), NAL Nalidic Acid, TCY (Tetracycline), SXT (Trimethoprim and sulfamethaxazole), EAEC (Enteroaggregative *E-coli*) EPEC (Enteropathogenic *E-coli*)

2.4.1 Diarrheal Outbreak in Sipsoo, Samtse

In 23rd May, 21 diarrheal outbreak samples were received for culture and identification from Sherabgatsel Middle Secondary School, Norgaygang (Bara), Tashichoeling Dungkhag, Samtse. Of these, 4 (19.0%) were male and 17 (81.0%) were females. Salmonella sp was detected in 12 cases and the rest were no growth. The antimicrobial-resistant pattern for the isolated bacterial pathogens is provided in (**Figure 17**).

On 29th June, 3 cases of suspected cholera samples were received from Sipso, Samtse. All the three samples were tested and confirmed negative for cholera.



S: Sensitive; I: Intermediate; R: Resistant

Figure17: Antibiotic susceptibility pattern

2.4.2 Suspected Anthrax outbreak, Wangduephodrang

A single case of suspected anthrax sample was received for culture and microscopy on 30th June. The gram stain results suggest observation of yeast cells and epithelial cells and no growth of suggestive anthrax colony in the culture.

2.5 Food safety surveillance

Total of 60 ready to eat food samples were received from three pilot Dzongkhangs from April to June 2023**(Figure 18).**

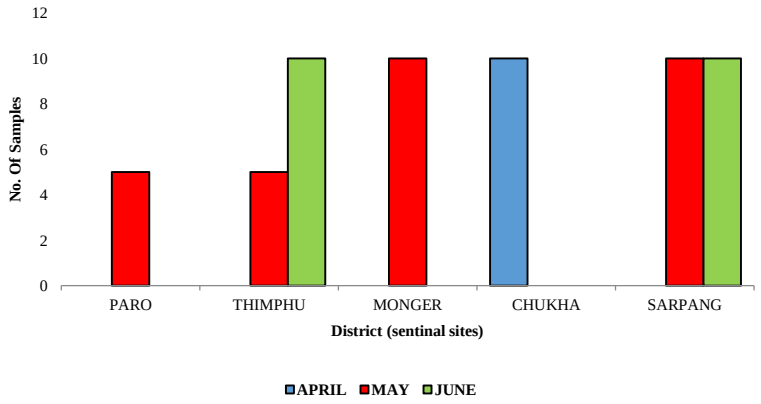


Figure 18: Total samples received for 2nd Quarter 2023 from five sites

The food samples were tested for physical parameters, indicator organisms and pathogenic organisms. The total plate count and *E. coli* counts are the two commonly used as indicator test for the foods. During the current period 15.0% of food samples had indicator organism test violation. The common pathogens isolated are *Staphylococcus aureus* and *Bacillus cereus*. During the same period four event of food-borne disease were reported through NEWARS.

2.6. Urban Drinking Water Quality Monitoring (UDWQM)

2.6.1 Bacteriology test Report

Total of 373 samples were tested by 27 Health Centers of urban areas. Out of this, 42.6% was found unsafe and 57.4%of the sample tested was found to be fit for consumption. Out of 38 health centers, 11 health centers have not reported for bacteriology test for this quarter **(Figure 19).**

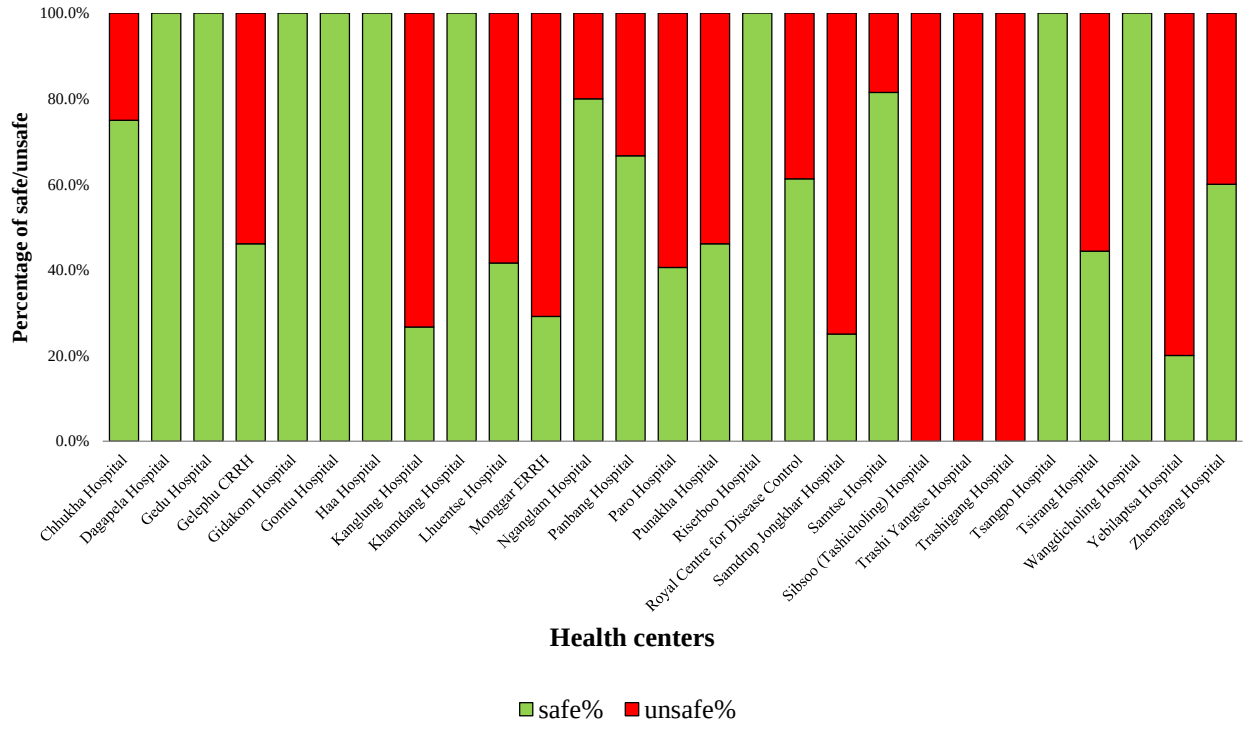


Figure 19: Bacteriology test report of 27 Hospitals/BHU-1 in urban area

2.6.2 Chlorination report

Out of 12 health centers monitoring free residual chlorine in drinking water sample Trongsa and Phuentsholing hospital has not reported for this quarter. Out of 223samples tested for the free residual chlorine, 81.2% were found to be inadequately chlorinated. Permissible limit of free residual chlorine is 0.2 to 0.5mg/L for safe water.

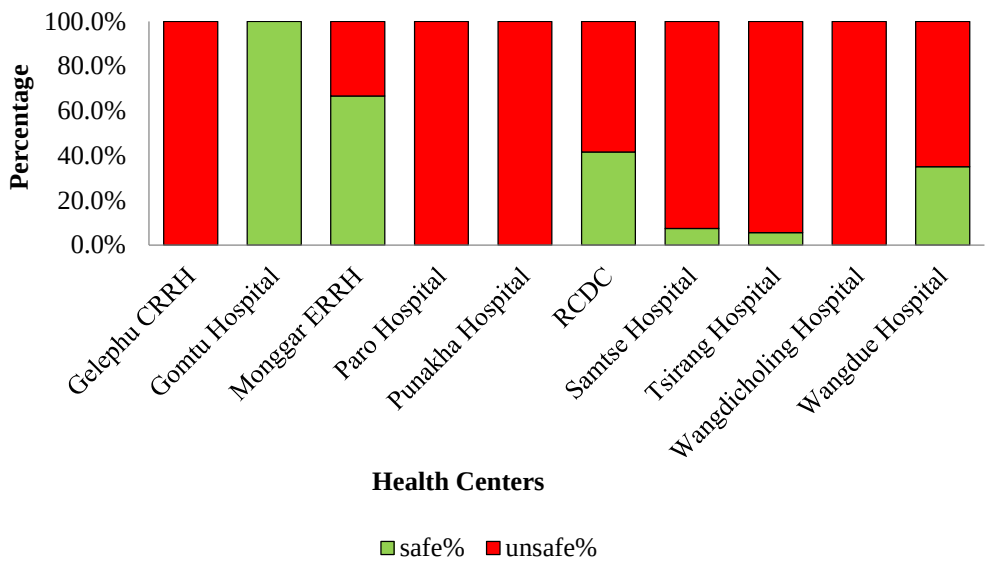


Figure 20: Free Residual Chlorine test report for 10 health centers in urban area

2.7. Drug Quality Monitoring

A total of 191 samples were tested. The samples were tested as per their pharmacopeial claim. Of the 191 samples tested, five samples were found to be non-compliant (**Table 8 & Figure 21**). Accordingly, the test reports were communicated to DRA for their necessary regulatory action

Table 8: Distribution of samples collected

Site	Complies	Does not comply	Total
Bumthang Hospital	16	0	16
Drug Regulatory Authority	9	1	10
Gelephu CRRH	11	0	11
JDWNRH	16	0	16
Medical Supply & Distribution Division	1	0	1
Monggar ERRH	24	0	24
Paro Hospital	16	1	17
Phuentsholing Hospital	7	0	7
Punakha Hospital	15	1	16
SamdrupJongkhar Hospital	12	0	12
Samtse Hospital	8	1	9
Trashigang Hospital	19	0	19
Trongsa Hospital	14	0	14
Tsimalakha Hospital	18	1	19
Total	186	5	191

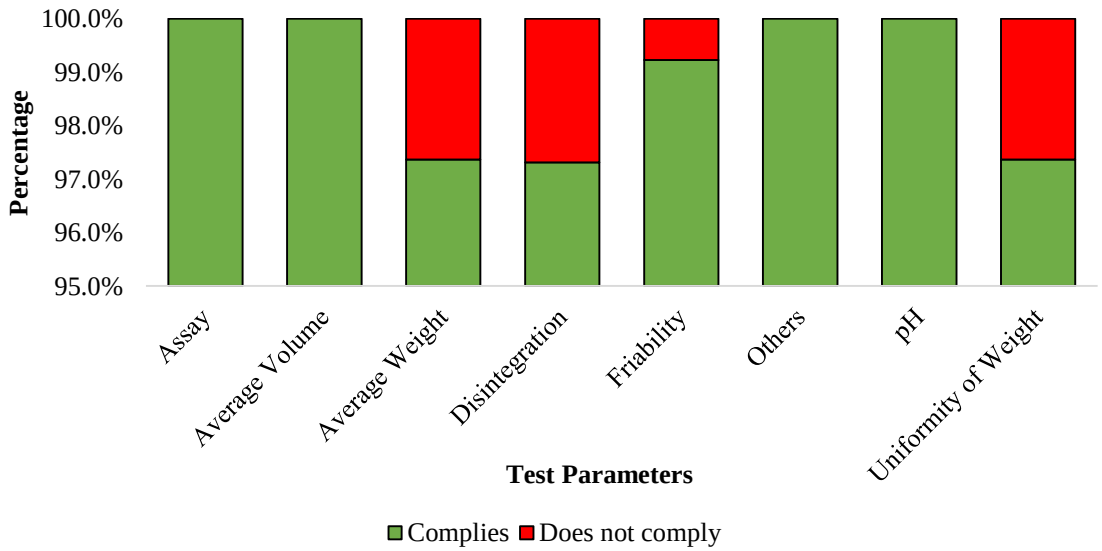


Figure 21: List of test parameters analysed

2.8 NTESIS (National Toxic Exposure Surveillance Information System) Report

2.8.1 NTESIS (National Toxic Exposure Surveillance Information System) Report

The poison information and toxicology laboratory, RCDC has a real time online web based poisoning surveillance that collects data on poisoning cases from different health centers of the country. The unit also provides information on case management through its clinical toxicology database. The report here describes the types of toxic exposure that were reported from health centers from April to June 2023.

A total of 29 cases were reported in NTESIS. The mean age of the cases was 31.86 ± 16.8 years. Male were the most exposed as compared to females (21 vs 8). Among the total cases, snake envenomation (41.3%) was the predominant exposure followed by poisoning by wild plants (31.03%). Poisoning with other agents constitute a total proportion of 27.5 % that includes ingestion of pesticides, pharmaceutical drugs and unintentional food poisoning **(Figure 22).**

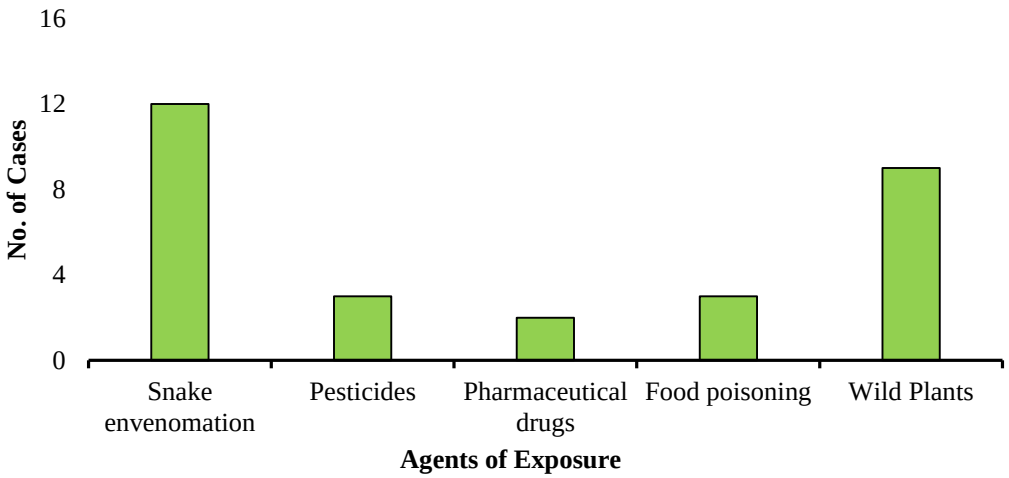


Figure 22: Common agents of exposure

Among the proportion of poisoning cases reported, highest were from Samtse dzongkhag (48.3%) followed by Dagana dzongkhag (31.0%). Samtse dzongkhag had the highest incident of snake bites cases as compared to other Dzongkhags with eight cases reported so far **(Figure 22**). Also,

majority of the poisoning cases via ingestion of pesticides and pharmaceutical drugs were also recorded.

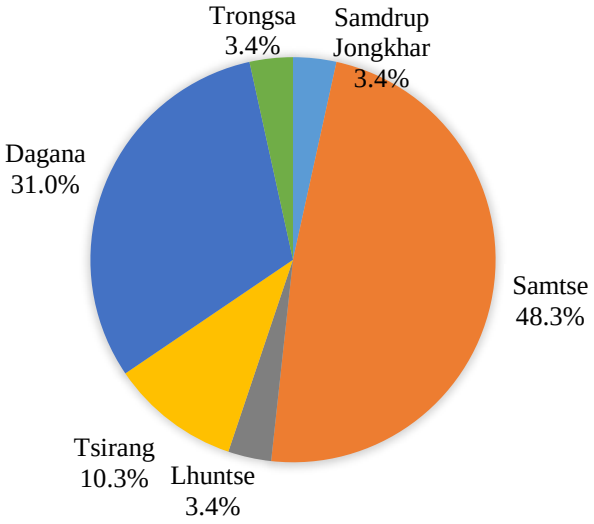


Figure 22: Proportion of poisoning cases reported from different Dzongkhags

2.8.2 Confirmation of drugs of abuse

A total of six samples were received in this quarter for confirmation of Tramadol, benzodiazepines and its derivatives, delta nine tetrahydrocannabinol, Ketamine, Heroine and other Opiates in urine samples **(Figure 23).** The samples were prepared using both liquid-liquid extraction and solid-phase extraction method.

The analysis method was developed for each type of drugs, validated using the certified reference standards, compared with the NIST 17.0 library and use of internal standards/spiking method

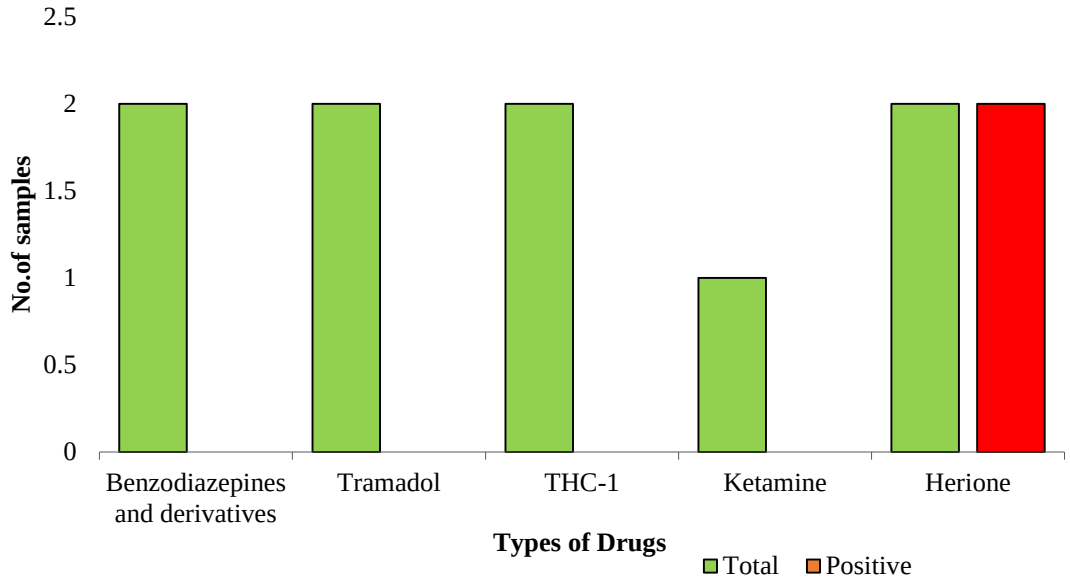


Figure 23: Types of drugs of abuse samples received and tested

2.8.3 Method Development and Validation for Tramadol using GC-MS

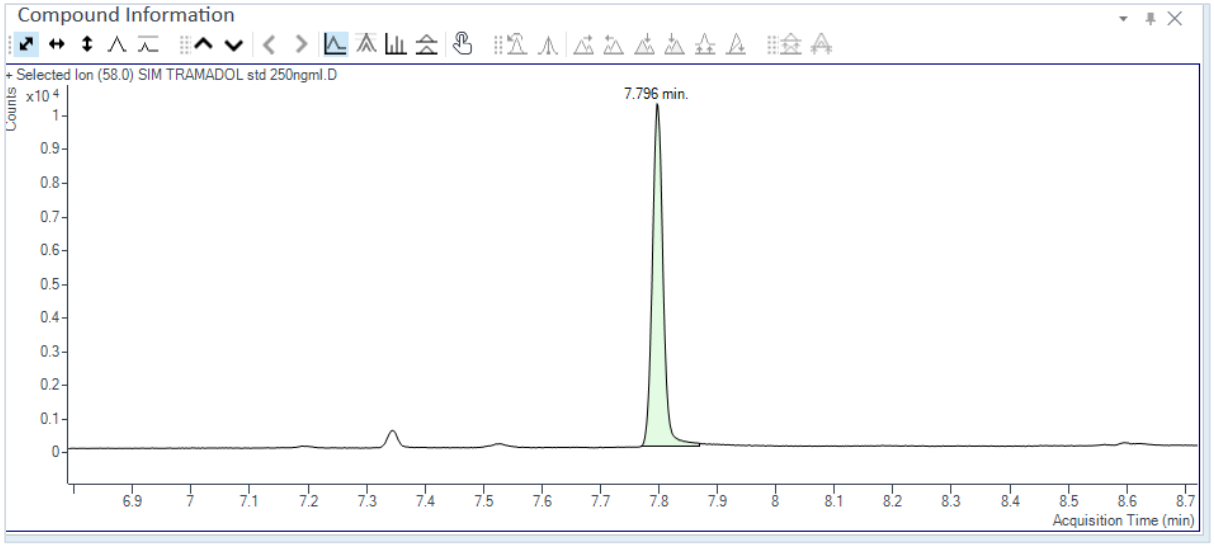
Tramadol is a synthetic opioid analgesic widely used for pain management that has the huge potential for abuse. Anecdotal reports of tramadol misuse and addiction is spawn among the youths and raises a public health concern in the country currently. This necessitates the requirement of a reliable analytical method that candetect and quantify the amount of tramadol and its metabolites in various sample matrices (urine/blood).

A valuable analytical method depends on the reliability, selectivity, sensitivity, and precision of the test methods to be deployed for true detection of tramadol in the sample matrices. The method development process primarily focuses on optimizing sample preparation, chromatographic separation, and mass spectrometric detection.

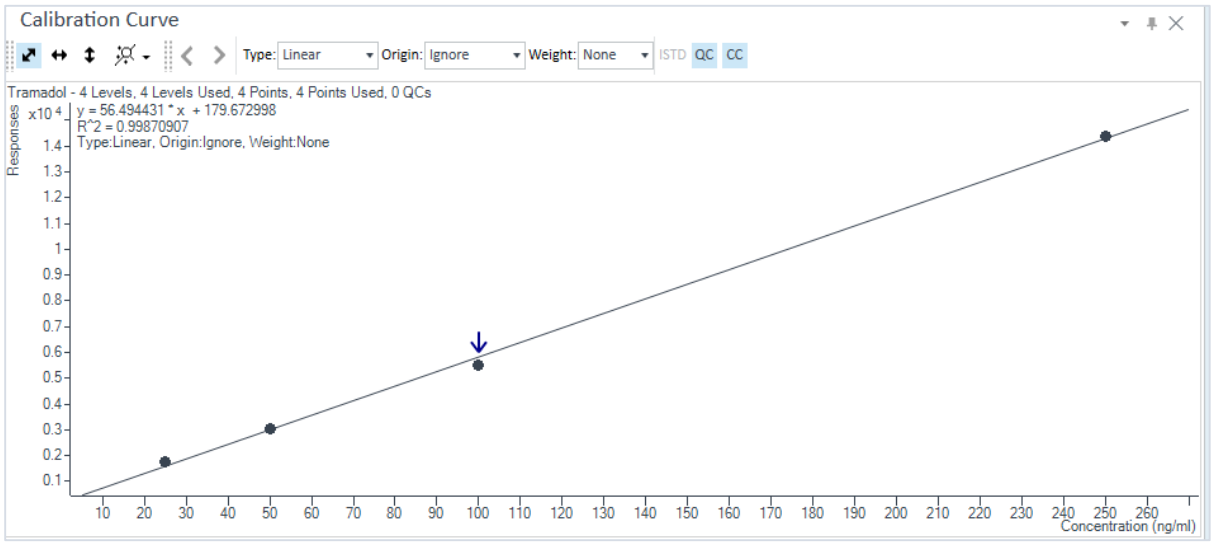
An attempt was made to develop and validate a Gas Chromatography-Mass Spectrometry (GC-MS) method for the analysis of tramadol from urine samples. A Solid-Phase Extraction (SPE) method was used in sample preparation and various validation parameters that focused on the method's robustness, sensitivity, selectivity, and accuracy were deployed accordingly. Altogether,

this method addresses the concerns associated with tramadol as a substance of abuse by enabling accurate identification and quantification in various matrices.

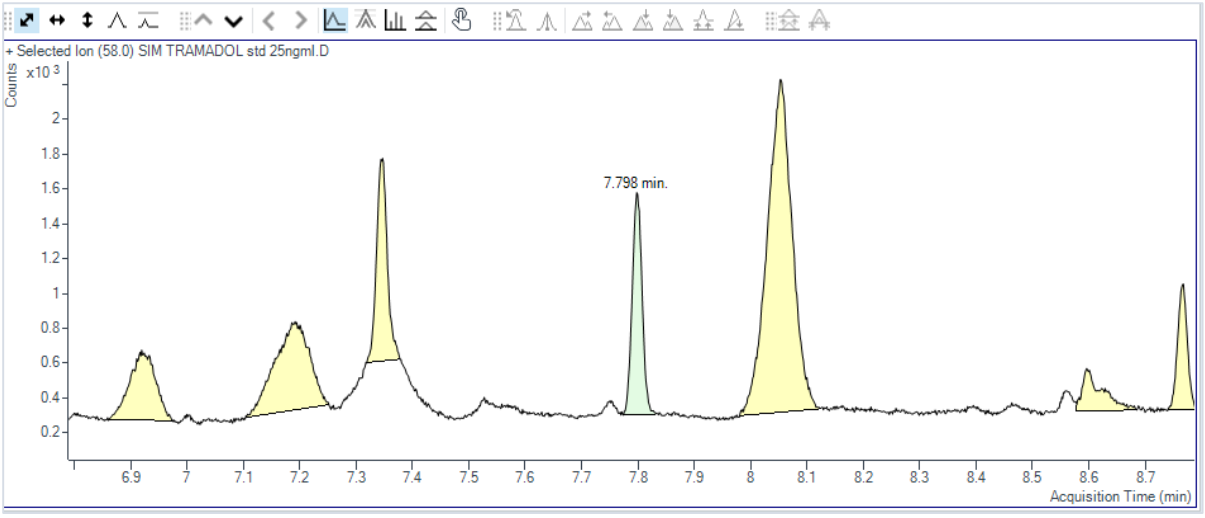
a) Sensitivity (Tramadol at retention time 7.7min)



b) Linearity (r^2=0.9987)



c) Selectivity



d) Accuracy

	Tramadol Concentration (ng/ml)	RT (min)	Final Conc.(ng/ml)	Accuracy (%)
1	25	7.798	27.62	110.5
2	50	7.798	51.05	102.1
3	100	7.797	94.65	94.7
4	250	7.796	251.66	100.7

2.9 Blinded rechecking of malaria slides

2.9.1 Reporting status of health centers

A total of 73 health centers have participated in malaria blinded rechecking. The overall average reporting rate with 33.9% were reported on time, 18.3% were zero reporting, 5.4% were reported late and rest were not reported (**Figure 24**)

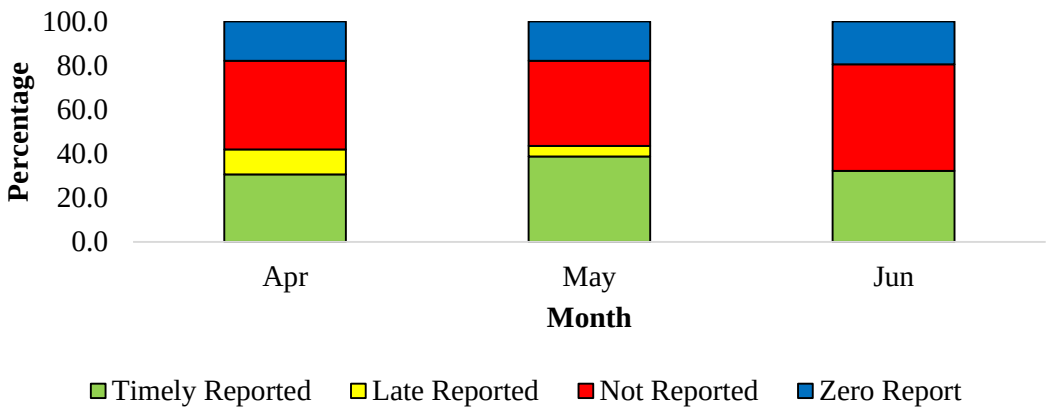


Figure 24: Monthly reporting status for 2nd quarter 2023

2.9.2 Blinded rechecking status

Total of 878 malaria slides were received at National Malaria ReferenceL aboratory for blinded rechecking. From the total slides examined, 5 malaria-positive slides were detected (0.6%). Of the total slides examined, all the slides were confirmed as negative slides.

All the slide received were evaluated on the following parameters and their performance score on sensitivity was 100.0%, specificity was 100.0%, malaria detection was100.0%, species identification was 100.0%, stages identification was 100.0% and parasite density determination was 100.0%. All the slide received were evaluated on the following parameters and their performance score on specificity was 100.0%, malaria detection was 100.0 %, quality of blood film was 72.4% and quality of stain was 82.5% **(Table 9 and 10)**

Table 9: Report on Malaria Blinded rechecking for 2nd quarter 2023

Third Quarterly report on Malaria Blinded Rechecking 2022				
Month	**Apr**	**May**	**Jun**	**Total**
Health center participated in blinded rechecking	38	40	37	115
Total slides received for blinded rechecking	269	332	277	878
Total positive detected	2	2	1	5
No malaria parasite seen	267	330	276	873
Total slide Examine				1871

Table 10: Report on performance score for Blinded rechecking

Performance score on blinded rechecking (%)				
Month	**Apr**	**May**	**Jun**	**Quarterly Score**
Sensitivity (True positive detection)	100.0	100.0	100.0	100.0
Specificity (True negative detection)	100.0	100.0	100.0	100.0
Malaria parasite detection	100.0	100.0	100.0	100.0
Mp Species Identification	100.0	100.0	100.0	100.0
Mp Stages Identification	66.0	100.0	100.0	86.66
Mp Parasite density	50.0	100.0	100.0	83.33
Stain Quality	63.9	67.0	65.9	65.6
Blood film Quality	87.5	88.1	93.4	89.7