ROYAL CENTER FOR DISEASES CONTROL

QUARTERLY BULLETIN: First Quarter 2024

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

Immediate reports:

- Total of 20 samples of suspected Measles/Rubella cases were tested, among them one sample tested positive for Rubella IgM. A positive samples were from Thimphu dzongkhag.
- One suspected cases of Pertussis was reported from JDWNRH, it tested negative for *Bordetella pertussis.*
- Two suspected cases of Acute Encephalitis Syndrome were reported from JDWNRH, both were negative for JE.
- One suspected cases of malaria, was reported from JDWNRH

Events reports:

- During the quarter, six disease outbreaks were reported. Among them four were the outbreak of Influenza-like Illness, one was of Glossitis, and one of conjunctivitis. All outbreaks were responded to by the respective health centers upon the recommendations of RCDC.

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

1.1 Reporting status of health centres under 20 Dzongkhag

In the first quarter 2024, a total of 3445 weekly reports were expected from 265 health centers across the country. The reporting was in consistence with the fourth quarter of 2023. Overall , 91.0% of reports were received in the NEWARS information system of which 82.0% were reported on time, 9.0% were reported late and the rest were not reported (**Figure 1**).

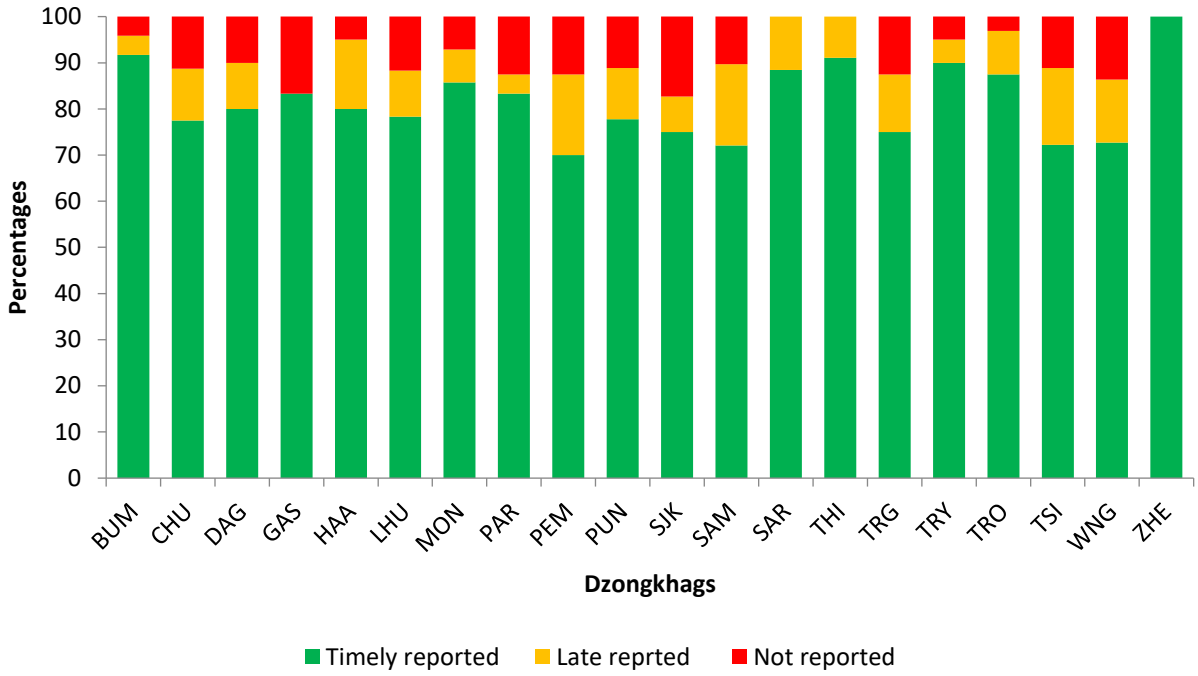


Figure 1: Dzongkhag-wise weekly reporting status for 3rd Quarter 2023

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

Among 11 weekly reportable diseases/syndromes, the highest number of cases reported were respiratory illnesses (ARI & SARI). In total, 29841 (88.0%) cases of respiratory illnesses and 5743 (11.0%) diarrheal cases were reported **(Table 1).** The total cases reported were lower than that of fourth quarter 2024.

Table 1: Notifiable diseases/syndromes reported by Dzonkhag

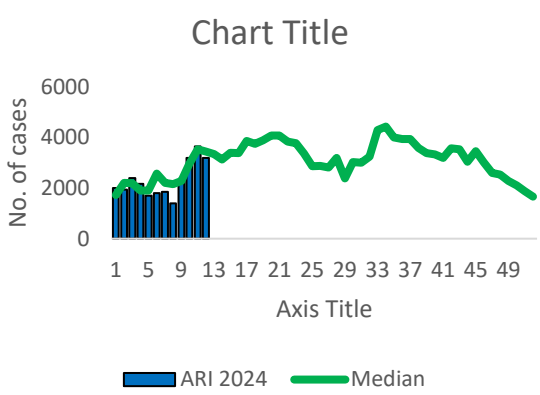
Dzongkhag	ABD	AWD	AJS	ARI	DGF	MUM	FWR	FDP	TPF	SAR	RKS
BUM	12	47	0	608	0	0	0	0	0	14	4
CHU	29	510	34	3339	2	0	27	7	1	20	3
DAG	9	294	8	2523	0	1	1	3	0	19	2
GAS	1	24	0	73	0	0	0	0	0	1	0
HAA	3	38	0	206	32	0	0	6	0	2	0
LHU	16	96	0	1010	0	0	0	0	0	0	0
MON	17	225	18	1441	27	0	1	0	0	6	1
PAR	15	396	24	1142	0	0	0	0	0	0	0
PEM	9	166	0	1313	0	0	0	0	0	4	2
PUN	16	200	2	556	7	0	0	0	0	38	0
SJK	9	304	5	2501	0	26	0	1	1	18	0
SAM	23	584	0	2381	0	0	5	2	0	30	0
SAR	14	578	2	2922	4	1	16	1	1	34	6
THI	36	739	1	2777	0	3	9	0	1	32	0
TRG	19	382	13	674	0	0	1	0	45	34	11
TRY	5	135	0	997	6	0	0	0	21	10	14
TRO	10	154	50	1018	0	2	0	0	0	2	0
TSI	15	123	0	574	0	0	0	0	0	2	0
WNG	18	356	3	2360	0	0	12	1	0	24	0
ZHE	16	98	0	1124	0	2	0	0	3	1	8
	292	**5451**	**160**	**29550**	**78**	**35**	**72**	**21**	**73**	**291**	**51**

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).

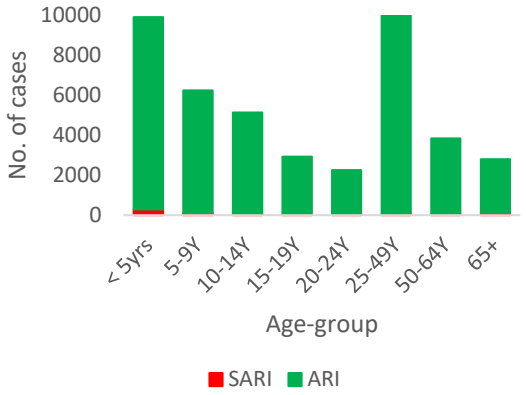
1.3 Descriptive analysis of most common notifiable diseases

1.3.1 Respiratory Illness (ARI and SARI) syndrome

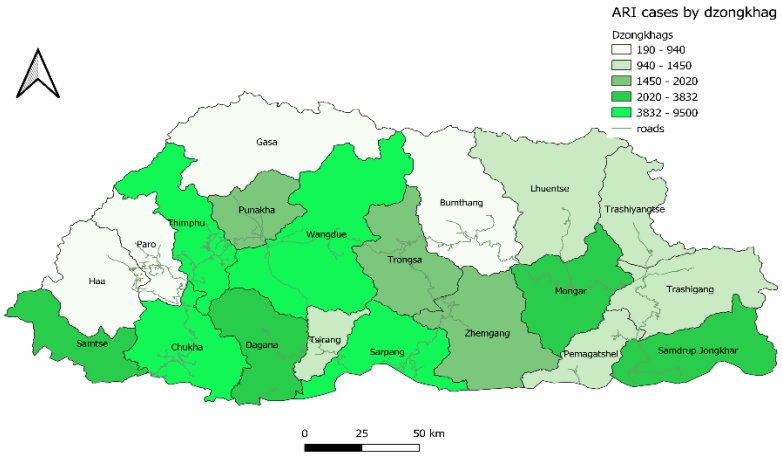
A total of 29841 cases of respiratory illness were reported, of which majority of cases were ARI (99.0%) and rest was SARI cases. The trend of ARI cases was found slightly higher compared with the median of the last three years of the same quarter **(Figure 2A).** The most commonly affected age group by respiratory illness was observed in the younger age-group **(Figure 2B)**. By dzonkhag, Thimphu Chhukha and Sarpang reported the maximum number of ARI cases **(Figure 2C).**



A: Cases by Epi-week



B: Incidence by age group

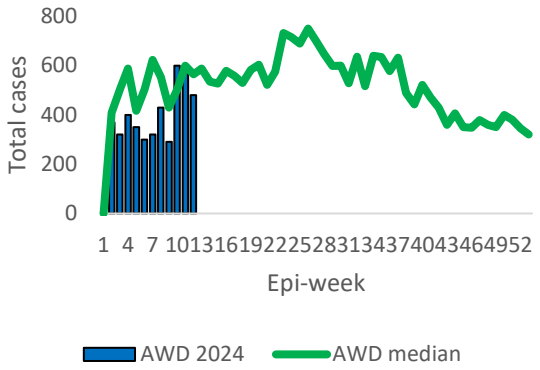


C: Respiratory illness cases by dzongkhag

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

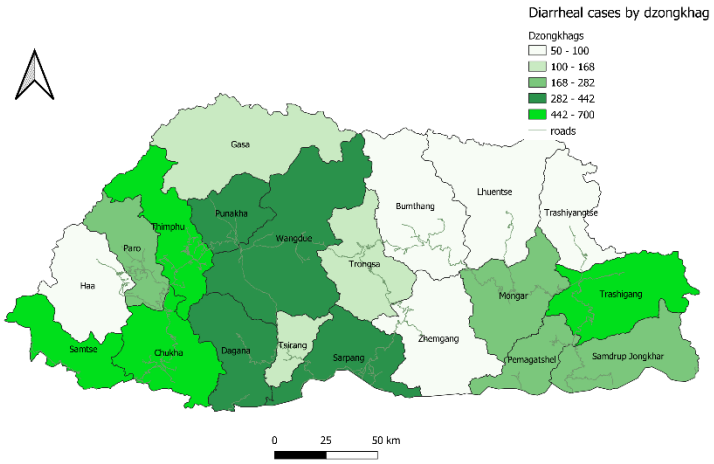
1.3.2 Diarrheal syndrome: (Acute Watery Diarrhea [AWD] and Acute Bloody Diarrhea [ABD])

Of the total 5743 diarrheal cases reported, 95.0% were AWD and rest were ABD. Compared with the median for the last three years, the trend for AWD was found mostly similar **(Figure 3A).** A high incidence of diarrheal diseases was observed in children 0-4 years **(Figure 3B)**. By dzongkhag, Thimphu, Chhukha, Sarpang and Trashigang reported the maximum number of cases **(Figure 3C).**



A: Cases by Epi-week

B: Incidence by age group

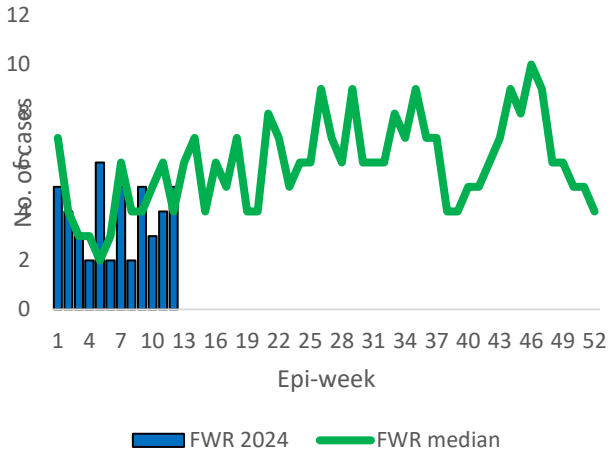


C: Diarrheal Cases by dzongkhag

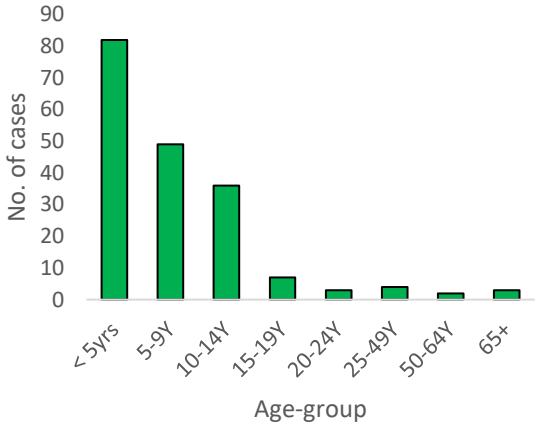
Figure 3: Diarrheal disease incidence by epidemiological weeks, age groups and place.

1.3.3 Fever with Rashes syndrome

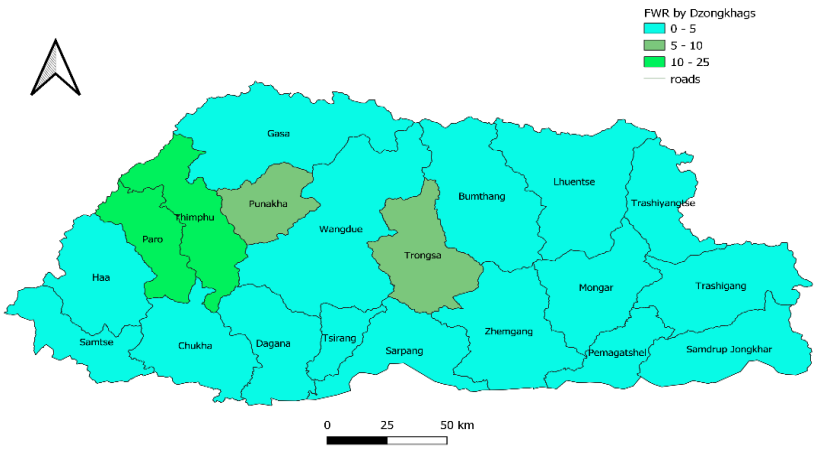
A total of 62 cases of fever with rash (FWR) syndrome were reported in the quarter **(Figure 4A)**.The trend of FWR was found consistently lower compared to the median of last three years. A majority of FWR were reported in the age group < 14 years **(Figure 4B).** As usual Thimphu and Paro reported maximum number of the fever with rashes cases **(Figure 4C).**



A: Cases by Epi-week



B: Incidence by age group



C: Fever with rash cases by dzongkhag

Figure 4: 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 samples tested, one samples from JDWNRH tested was positive for rubella IgM and none were positive for measles IgM. Additionally, one suspected pertussis, and one suspected bacterial meningitis were reported from JDWNRH. One malaria cases were reported during the quarter from JDWNRH.

1.5 Events

During the quarter, six disease outbreaks were reported. Among them four were the outbreak of Influenza-like Illness, one was of Glossitis, and one of conjunctivitis. All outbreaks were responded to by the respective health centers upon the recommendations of RCDC. The brief description of key events are described as follows.

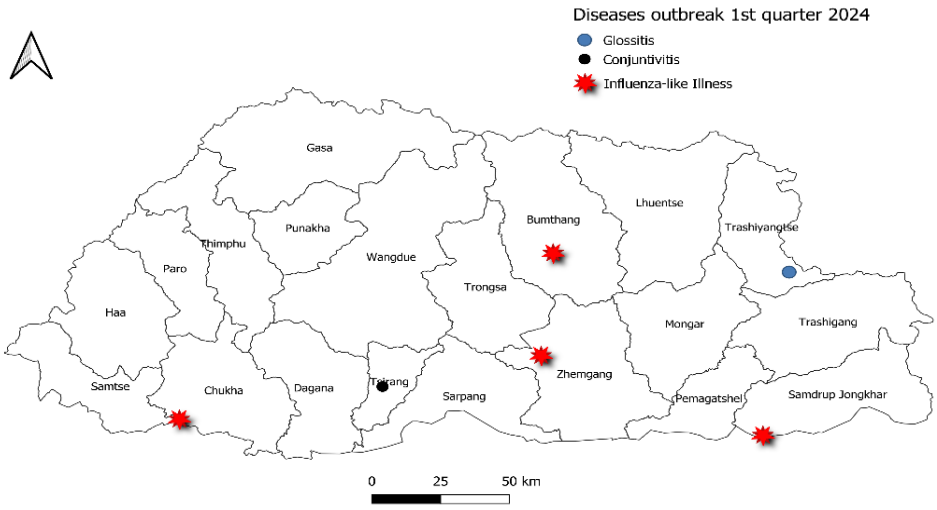


Figure 5: Diseases outbreak reported in first quarter, 2024

Description of Key events

1.5.1 Influenza-like Illness:

Between 1st January and 31 March 2024, a total of four ILI events/outbreaks were reported in the event-based surveillance NEWARS. The cumulative number of cases affected was a total of 276 cases, with no ILI-related mortality. A total of 11 samples were received by National Influenza Centre for confirmation. The samples were tested and detected Influenza subtype A/H1pdm09 and A/H3 as well as a few with COVID-19 **(Figure 5).**

A significant decrease in the number of ILI cases was observed, as compared to those reported in the fourth quarter of 2023. During this reporting period, all events were reported from Schools under four dzonkhag (Chhukha, Punakha Wangduephodrang and Samdrupjongkhar).

1.5.2 Glossitis:

A glossitis outbreaks was reported from Tongzhang PHC, Trashiyangtshe during the quarter. The cumulative number of students affected was 20 from the Kuenzangling Center School, Trashiyangtse. The samples could not be collected because of the lack of logistic and testing facilities. There was no any complication or mortality following the outbreaks.

This is the first outbreak of Glossitis outbreak reported at the start of School academic session which is very uncommon. In the past outbreak was usually observed during the winter, especially in the end of the School's academic year.

1.5.4 Conjunctivitis:

An outbreak of conjunctivitis was reported from Tsirang during the quarter. Total of 25 students from Damphu central School, Tsirang were detected of having conjunctivitis. The samples could not be collected because of the lack of testing facilities. Outbreaks was investigated by the respective health centers upon the recommendations of RCDC

2. Laboratory Based Surveillance

2.1 Drug Resistant Surveillance for Tuberculosis

A total of 418 suspected tuberculosis patient samples were received at the National Tuberculosis Reference Laboratory (NTRL) for culture and drug susceptibility testing for anti-tuberculosis drugs. Of those 187 (44.7%) were pulmonary tuberculosis (PTB) cases, 120 (28.8%) were culture follow-up samples from MDR-TB patients under treatment, 69 (16.5%) were pulmonary samples received for TB screening for VISA purposes and 42 (10.0%) were extra-pulmonary tuberculosis cases.

Among the PTB cases, new smear-negative (NSN) constituted 36.9% (n=69) of the cases, followed by 47.6% (n=89) of new smear-positive (NSP) cases, 12.8% (n=24) were cases with uncertain treatment history, 2.7% (n=5) with history of previous TB treatment and 0% (n=0) was received for re-culture (**Figure 6**).

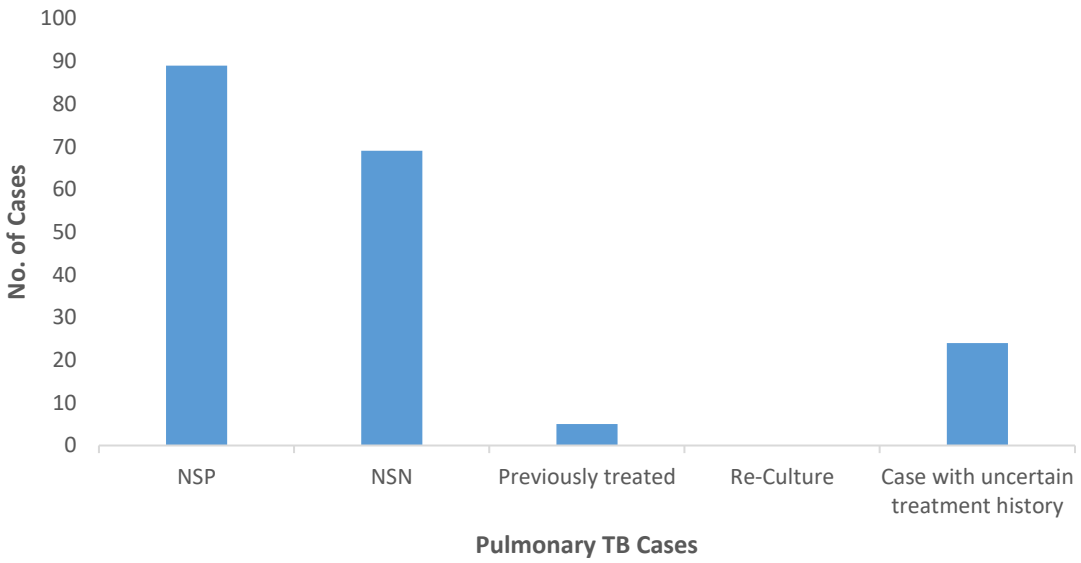


Figure 6: Classification of Pulmonary TB samples

2.1.1 Drug Sensitivity Test

A total of 75 out of 418 (17.9%) patients had complete drug susceptibility testing (DST) reports using rapid molecular line probe assay. A total of six multi-drug resistant tuberculosis (MDR-TB) cases were detected among patients with complete drug susceptibility reports. Among the MDR-TB cases, five were new cases and one was an extra-pulmonary tuberculosis case. MDR-TB cases were highest in the age group of 20-29 years (**Figure 7**). Four MDR-TB cases had 2nd line DST reports tested using rapid molecular line probe assay and three were sensitive to both Fluoroquinolones and aminoglycoside, while one was resistant to Fluoroquinolones and Rifampicin only.

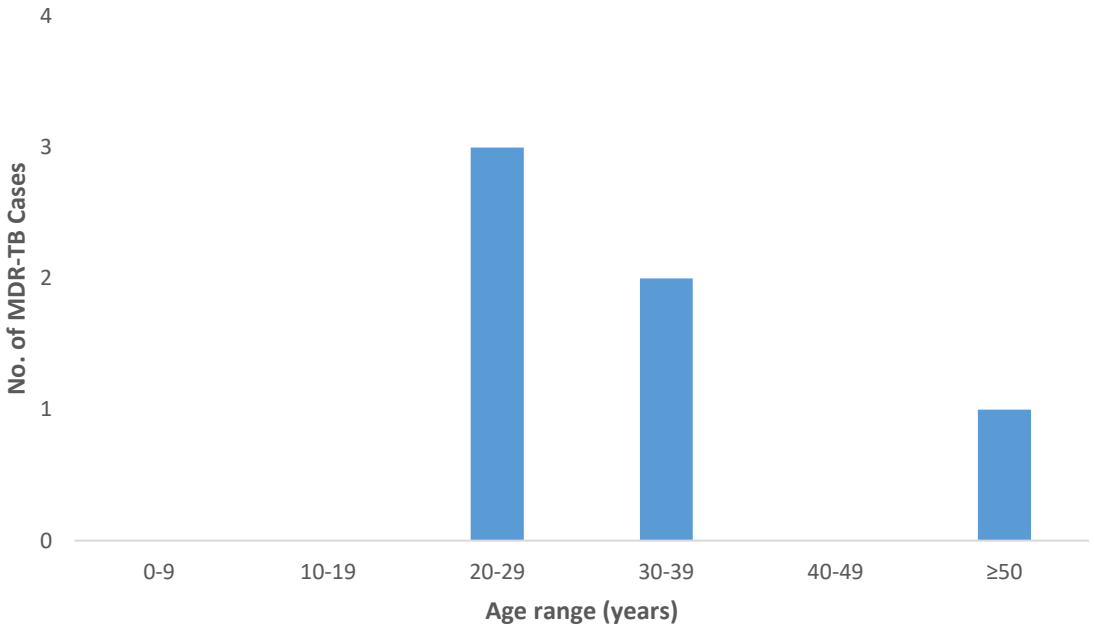


Figure 7: Distribution of MDR-TB cases by age group

2.2 COVID-19 Integrated Influenza surveillance

2.2.1 Epidemiological Surveillance

Influenza-like illness (ILI) and severe acute respiratory infection (SARI) cases were collated and compiled for the first quarter from January to March (Week 1 to 13) (**Figure 8**). A total of 1,723 ILI cases and 241 SARI cases were reported. On average the weekly ILI and SARI proportion was 18.5 per 1000 patients and 17.1 per 1000 patients respectively. The ILI cases dropped in week 8, however, it gradually increased over the week. The most affected age group for ILI was 30 – 64 years (534) followed by 5 – 14 years (333) while the most affected age group for SARI was > 65 years (73) followed by 30 – 64 years (57) (**Figure 9**).

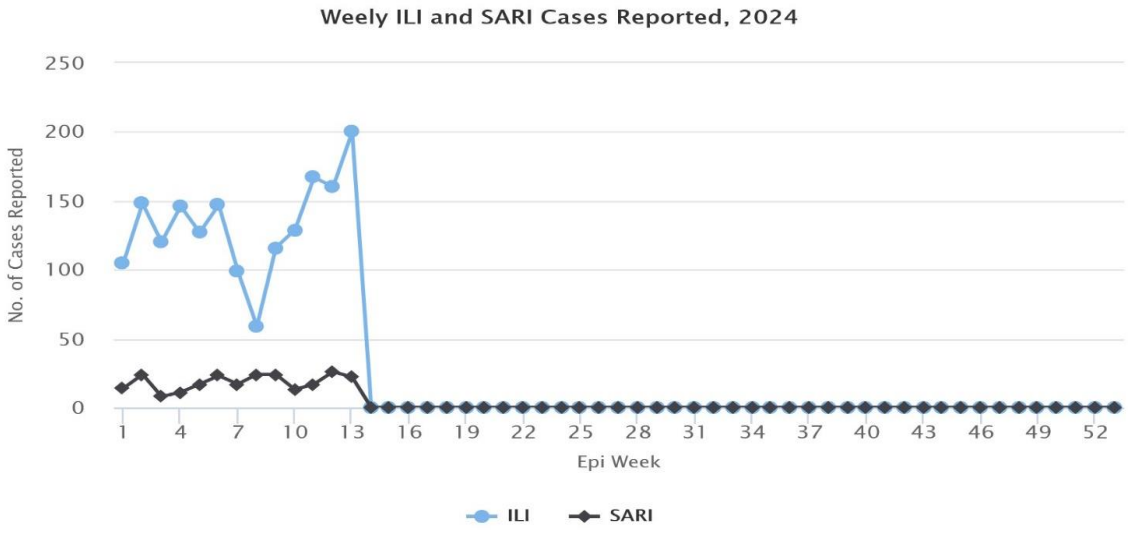


Figure 8: Weekly trend of ILI and SARI cases reported from Sentinel sites (Source RCDC).

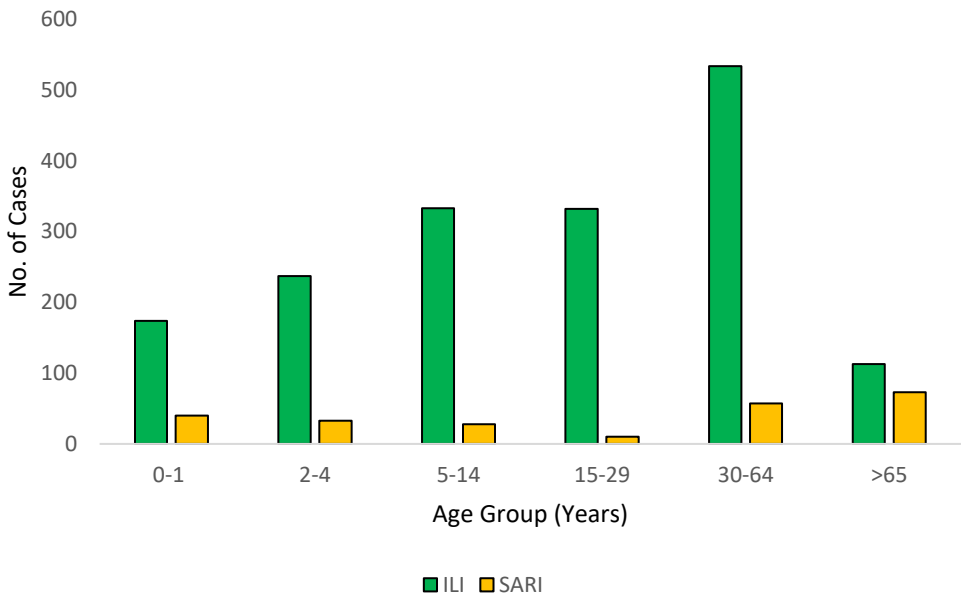


Figure 9: Aggregate ILI and SARI cases by age group

The ILI and SARI cases were reported from all the respective sentinel hospitals. Punakha (396) and Samdrup Jongkhar Hospital (359) have reported more ILI cases. Similarly, Gelephu (67) and Phuentsholing Hospitals (66) have reported more SARI cases during the quarter (**Table 2**).

Table 2: ILI and SARI cases reported in first quarter

Sentinel Sites	Total ILI	Total SARI
Gelephu CRRH	NA	67
JDWNRH	NA	14
Monggar ERRH	NA	9
Phuentsholing Hospital	NA	66
Paro Hospital	225	32
Punakha Hospital	396	15
Samdrup Jongkhar Hospital	359	8
Samtse Hospital	140	3
Trashigang Hospital	285	3
Trongsa Hospital	155	2
Tsirang Hospital	163	22
Grand Total	**1723**	**241**

2.1. Laboratory-Confirmed Influenza and COVID-19

A total of 905 specimens (ILI- 678, SARI- 227 were received and tested for Influenza and SARS-CoV-2 through multiplex RT-PCR (Flu SC2) and detected 8.8% (83) Influenza positives, 16.4% (147) SARS-CoV-2 positives, and 0.4% (4) co-infection of SARS-CoV-2 and Influenza. Influenza subtype A/H1pdm09 (38.6%) was the most predominant strain, followed by Influenza A/H3 (20.5%) and Influenza B/Victoria lineage (19.3%) (**Figure 10).**

The mean age for influenza was 25.7 years (range: 6 months – 81 years) and the median age was 28.5 years (IQR: 7.7 – 51 years). The most affected age group for Influenza was 30 – 64 years (32.9%) followed by 5 – 14 years (31.4%), while the most affected age group for SARS-CoV-2 was 30 – 64 years (43.3%). The males were affected more compared to the females in both Influenza and SARS-CoV-2 (**Figure 11**).

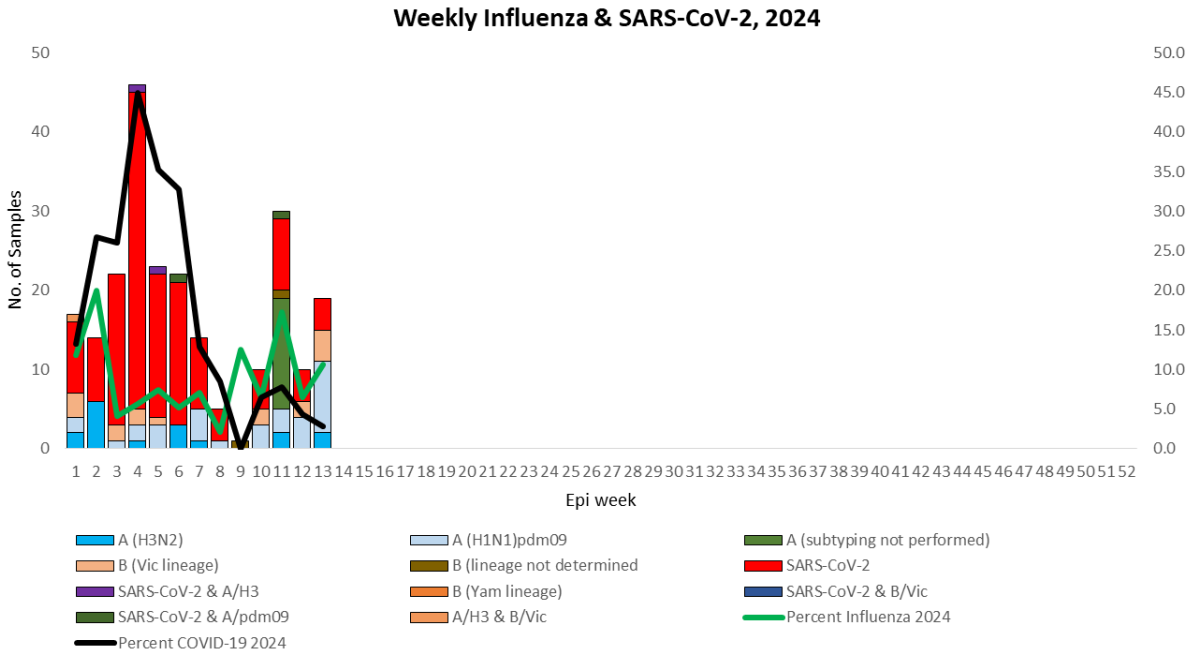


Figure 10: Weekly Influenza subtypes and SARS-CoV-2 (Source: RCDC)

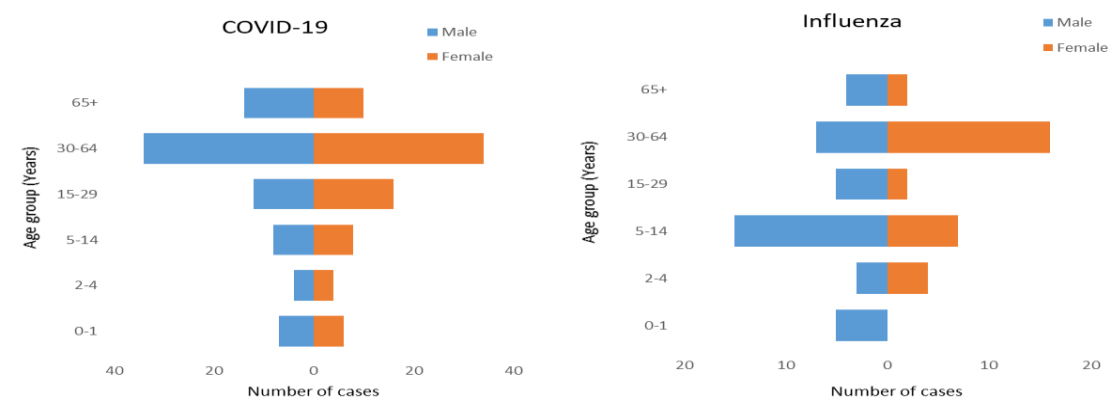


Figure 11: Influenza and SARS-CoV-2 by age group and sex

A total of 905 ILI and SARI specimens were received from the Sentinel and non-sentinel sites. RCDC recommends 10 – 15 ILI samples per week from each sentinel site. At the same time, all the SARI cases are recommended to collect specimens. Trongsa Hospital (131) and Samtse Hospital (119) have collected more ILI samples (**Table 3**). Likewise Phuentsholing (61) and Gelephu Hospitals (44) have collected more SARI samples compared to other sentinel hospitals (**Table 3**).

Table 3: Summary table for ILI and SARI samples received and tested in first quarter

Sentinel sites/ Hospitals	ILI	SARI	Grand Total
Gelephu CRRH	5	44	49
JDWNRH	4	38	42
Monggar ERRH	8	10	18
Paro Hospital	68	28	96
Phuentsholing Hospital	36	61	97
Punakha Hospital	96	10	106
Samdrup Jongkhar Hospital	77	9	86
Samtse Hospital	119	3	122
Trashigang Hospital	19	4	23
Trongsa Hospital	131	2	133
Tsirang Hospital	42	18	60
Dewathang Hospital	19		19
Lungtenphu Military Hospital	24		24
Royal Centre for Disease Control	30		30
Grand Total	**678**	**227**	**905**

2.3 Surveillance for Acute Encephalitic Syndrome (AES), Measles and rubella (MR) and Pertussis:

Vaccine Preventable and Venereal Disease Laboratory (VPVDL) carries out nation-wide laboratory –based surveillance for some vaccine preventable diseases. During the first quarter of the year a total of 20 samples were received for testing of Measles and Rubella. There was no sample for Pertussis in this quarter.

One sample collected from a baby of 10 months old was positive for Rubella IgM. No throat swab was collected for the same case. After a thorough verification and deliberation, the case was classified as "rubella positive induced by vaccine" Seven (7) samples were subjected to RT-PCR for measles and rubella and all were tested negative (**Table 4 & Figure 12).**

Most affected age group was <4year of age (80.0%). Overall fever with rash syndromes was more common in male (60.0%) than female (40.0%) with rate ratio 2:1 (**Figure 13**)

Table 4. Number of MR samples received from health center's for surveillances

Sl.no	District	Total sample	Rubella IgM Positive	Measles IgM Positive	RT PCR Measles	RT PCR Rubella
1	**Haa Hospital**	1	0	0	Undetermined	Undetermined
2	**JDWNRH**	10	1	0	Undetermined (2)	Undetermined (2)
3	**Paro Hospital**	3	0	0	Undetermined	Undetermined
4	**Punakha Hospital**	1	0	0	Undetermined	Undetermined
5	**Nganglam Hospital**	1	0	0		
6	**Sarpang Hospital**	2	0	0	Undetermined	Undetermined
7	**Trongsa Hospital**	1	0	0	Undetermined	Undetermined
8	**Bumthang**	1	0	0		
	Total	20				

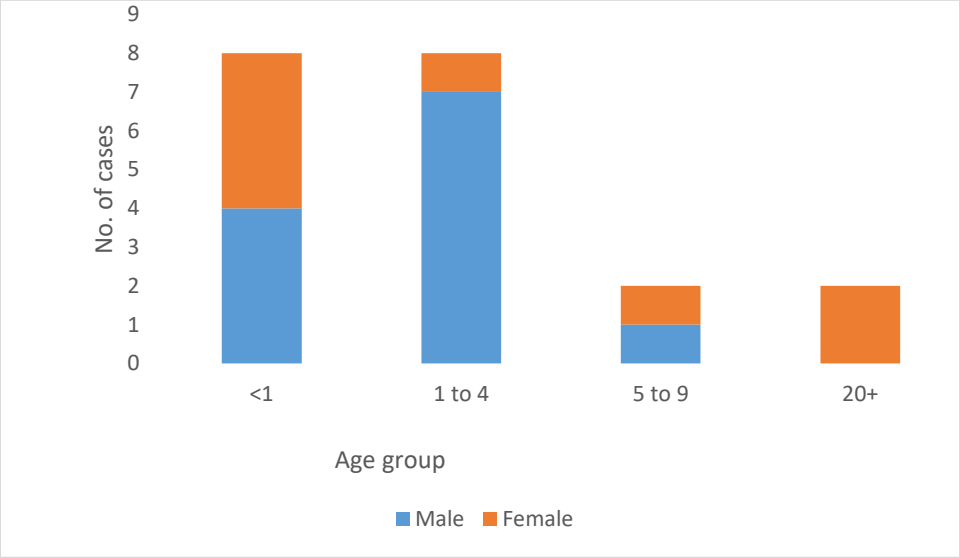


Figure 13. Age distrubition of suspected age group

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

A total of 12 samples were received from the sentinel site for AUFI testing. Of the 12 samples, two were sent for Japanese encephalitis and Leptospirosis confirmation respectively. The AUFI parameters include Dengue, Chikungunya, Scrub typhus, Japanese encephalitis, leptospirosis, and Brucellosis. All tests were conducted using the ELISA method.

Out of the 12 samples, all tested positive for leptospirosis. Additionally, one sample tested positive for dengue, scrub typhus, and brucellosis. These samples were obtained for the purpose of surveillance through independent testing. (**Table 5**)

The Enteric Zoonotic Vector Borne Disease Laboratory (EZVBDL) received three samples for AES testing. All the samples tested negative.

Table 5. Number of AUFI samples received from health center's for surveillances

Surveillance site	Total samples	DEN (-)	DEN (+)	CKG (-)	CKG (+)	ST (-)	ST (+)	JE (-)	JE (+)	LPS (-)	LPS (+)	BRU (-)	BRU (+)
Samdrup Jongkhar Hospital	4	3	1	4	0	4	0	4	0	0	4	4	0
Tsirang Hospital	3	3	0	3	0	3	0	3	0	0	3	2	1
Trongsa Hospital	1	1	0	1	0	1	0	1	0	0	1	1	0
Punakha Hospital	2	2	0	2	0	1	1	2	0	0	2	2	0
CRRH	2							1	0	0	1		

DEN: Dengue, **CKG:** Chikungunya, **ST:** Scrub typus, **JE:** Japanese encephalitis, **LPS:** Leptospirosis; **Bru:** Brucellosis.

2.5. Sentinel Surveillance for Diarrheal Etiologic Agents

This quarter, a total of 24 samples were received from four sentinel sites **(Figure 14).** Most of the samples received were from the CRRH, JDWNRH and Tsirang. Of these, 13 (54.0%) were males and 11 (46.0%) were females.

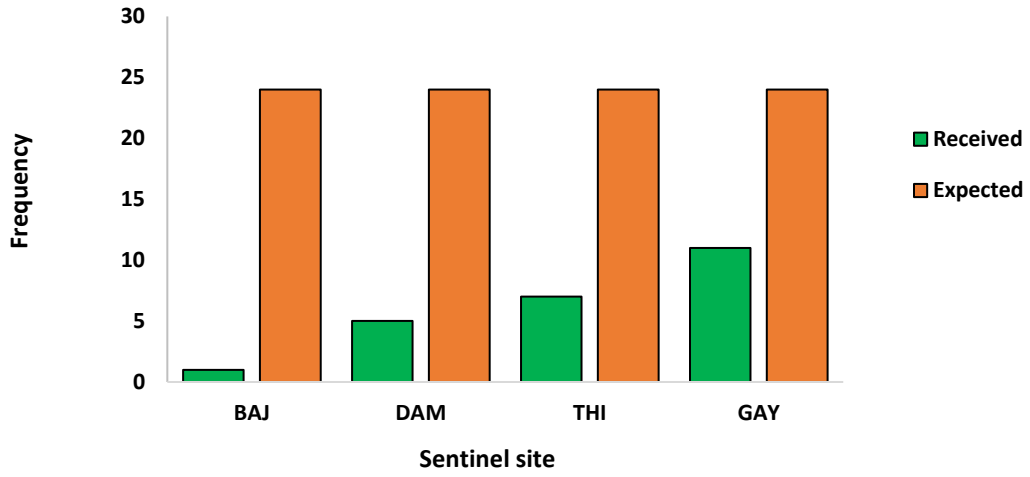


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Figure 14: Number of fecal specimens received from sentinel sites

The description of the sample shows that 50.0% of the individuals had loose and watery stools. The average age of the participants was 16 years and the average duration of diarrhea was 40 hours. Out of the total, 21.0% of the cases required hospitalization, while the remaining cases received treatment in the OPD. It was observed that four cases of diarrhea were linked to the consumption of suspected food items, such as momo, and two cases were found to have travelled to bordering areas. The most frequently detected enteropathogens were Diarrheagenic *E. coli* (n=3) and a single isolate of *Shigella sonnei* **(Figure 15).** The antimicrobial-resistant pattern of the isolated bacterial pathogens is provided in **Table 6**.

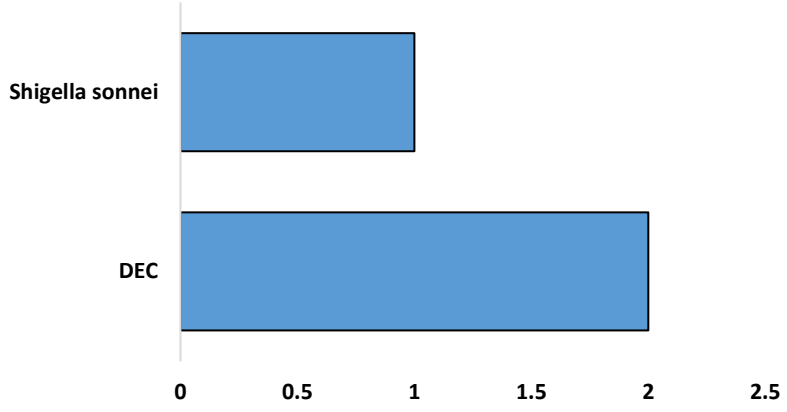

Figure 15: Proportion of pathogens isolated

Table 6. Antibiotic susceptibility pattern for bacterial pathogens

Antibiotics	*Shigella sonnei* (n=1)			DEC (n=2)		
	S	**I**	**R**	**S**	**I**	**R**
AMP	**1**				**2**	
CZO	**1**			**2**		
CRO	**1**			**2**		
LEX		**1**				**2**
CHL		**1**		**2**		
CIP			**1**	**2**		
GEN	**1**			**2**		
NAL			**1**	**2**		
TCY			**1**	**1**		**1**
SXT			**1**	**2**		

AMP (Ampicillin), CZO (Cephazolin), CRO (Ceftriaxone), LEX (Cephalexin), CHL (Chloramphenicol), CIP (Ciprofloxacin), GEN (Gentamycin), NAL Nalidixic Acid, TCY (Tetracycline), SXT (Trimethoprim and sulfamethoxazole), DEC (Diarrheagenic *E-coli*)

2.6 Food safety surveillance:

Total of 50 ready to eat food samples were received from January to March 2024 from five food safety surveillance sites **(Figure 16).**

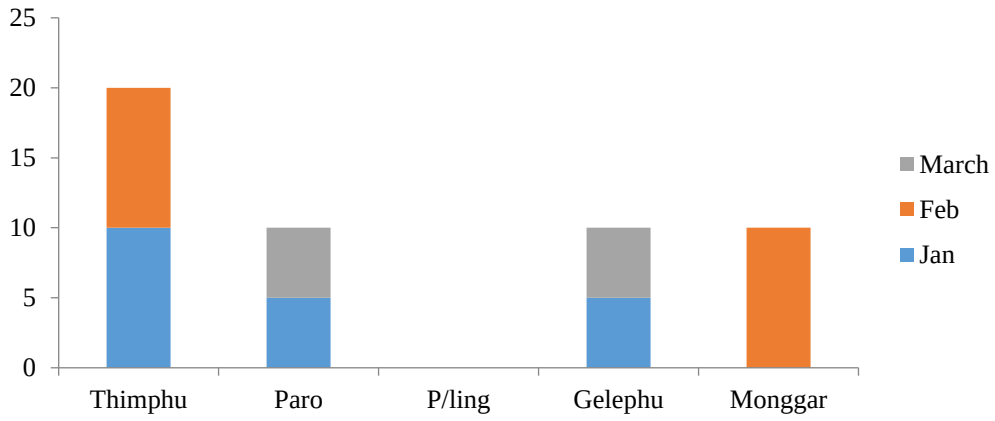

Figure 16: Number of Food safety surveillance samples received from sites (Q1 2024)

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 accessing food safety and surrogacy for hygiene practice. During the current period 12.0% (n=6) of food samples had been contaminated with pathogenic organism and 16.0% (n=8) with indicator organism. The common pathogen isolated is *Staphylococcus aureus*.During the sample period one event of foodborne disease was reported.

2.7 Urban Drinking Water Quality Monitoring (UDWQM)

2.7.1 Bacteriology test (Thermotolerant coliform) Report

A total of 352 samples were collected and tested for microbial analysis from 24 urban health centers for the 1st quarter 2024. The compliance rate was determined based on the guideline value given in Bhutan drinking water quality standard 2016. The water samples with no E. Coli contamination (0 CFU/100mL) are considered safe and is considered compliant with the standard. The result showed that the mean compliance rate across the health centers was 62.5% which is slightly higher than the previous quarter of 53.0%. **Figure 17** shows the microbial data compliance for 20 districts. As shown in the figure, urban water surveillance sites from Gasa, Haa, Wangduephodrang, Trongsa and Mongar failed to report for this quarter. **Figure 18** presents a breakdown of compliance data from all the reporting centers.

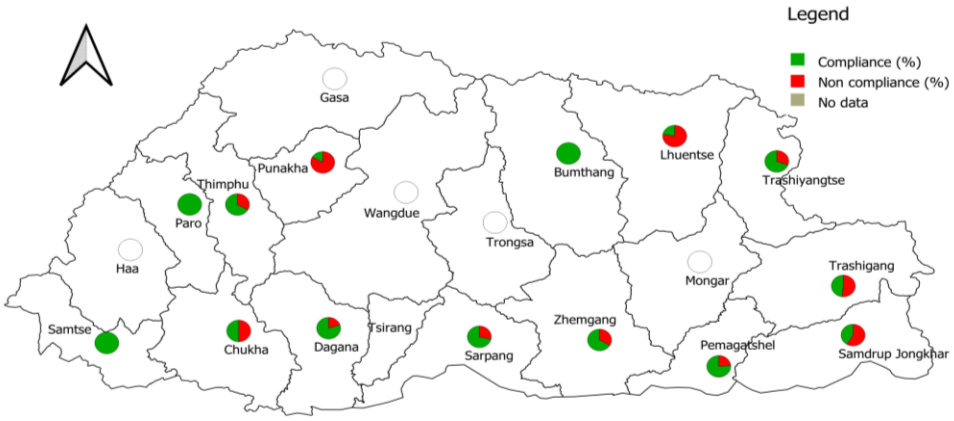

Figure 17: Microbial test result compliance of in urban area of 20 dzongkhags

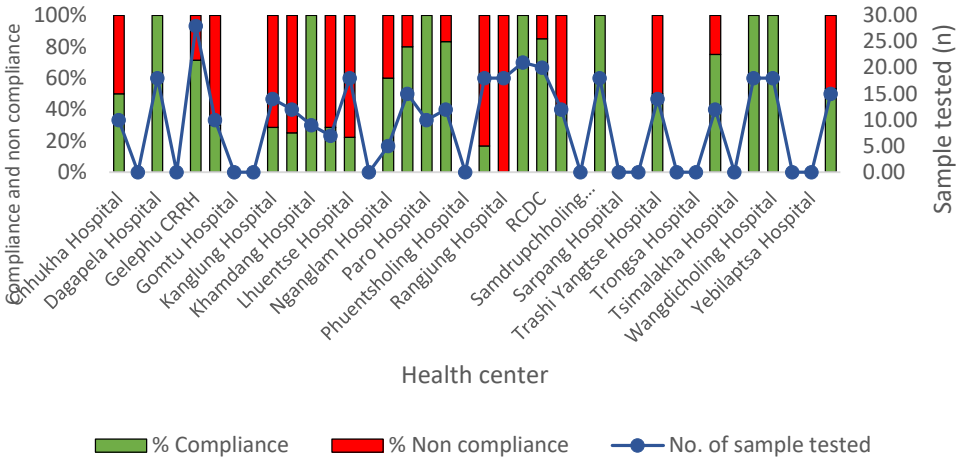

Figure 18: Microbial test result compliance of 24 health centers in urban area

Physical test

In regard to the physical test parameters, including pH and turbidity, data was received and analyzed from 15 dzongkhags (**Figure 19**). The compliance rate for physical parameter shows a comparatively better when compared with the microbial parameters, with the mean compliance rate of 94.0% and 96.0% for pH (recommended value; 6.5-8.5) and turbidity (recommended value; <5NTU), respectively.

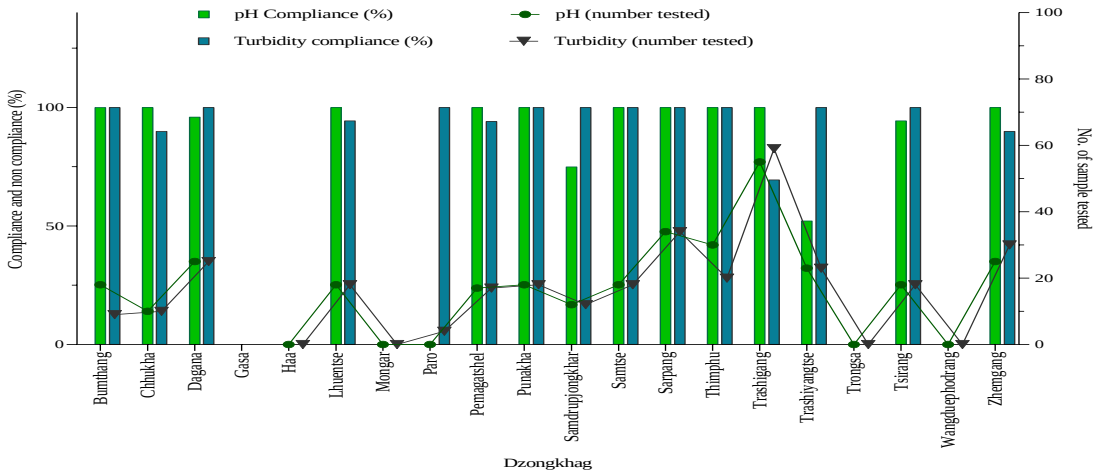

Figure 19: Physical test compliance rate from 20 dzongkhags

2.8. Drug Quality Monitoring:

A total of eight samples were tested at the National Drug Testing Laboratory in the 1st quarter of 2024 **(Table 7).** The samples were tested as per their pharmacopeial claim. All the samples complied with their specifications and accordingly, the test reports were communicated to BFDA for their necessary regulatory action. Also 3 oral liquids were screened for ethylene glycol and diethylene glycol.

Table 7: List of test parameters analyzed

Category	Assay	Disintegration	Friability	Identification Test	Sterility Test	Uniformity of Weight
January	7.00	6.00	6.00	0.00	0.00	7.00
February	1.00	0.00	1.00	0.00	0.00	0.00
March	7.00	0.00	0.00	1.00	3.00	0.00

The National Medical Product Testing Laboratory participated in External Quality Assurance Assessment Scheme Phase 11, 2024 for medicine quality control laboratories in Member States of the World Health Organization (WHO) coordinated by WHO.

2.9 NTESIS (National Toxic Exposure Surveillance Information System) Report

The Poison Information and Toxicology Laboratory at RCDC operate a real-time online web based poisoning surveillance system. The report provided here outlines the types of toxic exposures reported from the health centers between January to March, 2024.

Confirmation of drugs of abuse

A total of 13 samples were received in this quarter for confirmation of tramadol, benzodiazepines, codeine, heroin, morphine, blood alcohol and THC in both biological and non-biological forms (**Table 8).**

Table 8. Types of drugs of abuse samples received and tested

SI.No	Samples	Drugs of abuse	Result
1.	Unknown white crystalline powder	Test for drugs of abuse for heroin	Negative
2.	Unknown white crystalline powder	Test for drugs of abuse including heroin, morphine, codeine and tramadol	Positive for carisprodol (active metabolite is meprobamate)
3.	Blood alcohol level (4 samples)	Test for ethanol level in blood	3 samples detected with ethanol >LOD
4.	Urine	Test for tramadol	Negative
5.	Urine (3 samples)	Test for benzodiazepines and THC	1 sample positive for diazepam
6.	Unknown white powder (2 samples)	Test for heroin	Positive
7.	Urine	Test for THC and tramadol	Positive for tramdaol and meprobamate
8.	Capsules	Test for THC and tramadol	Positive for meprobamate

A total of 22 different sample tests were conducted for the mentioned drugs of abuse analyzed by Gas Chromatography Mass Spectrometry (GC-MS) (**Table 9 and Figure 20**). A unique finding during the test was determination of high concentration of meprobamate analyte in the SP plus capsule instead of tramadol. Spasmo-Proxyvon plus Capsule (SP plus) is a combination medicine used in the treatment of acute pain. However, SP plus remains the most abused pharmaceutical drug in the country. The drug combination of SP plus is paracetamol, dicyclomine and tramadol, however the drug combination in the recent analyzed SP plus was paracetamol, dicylomine and meprobamate. Meprobamate is a scheduled IV control substances that belongs to the class of drugs known as anxiolytics and sedatives. It is primarily used to treat anxiety disorders and to provide short-term relief from symptoms of anxiety.

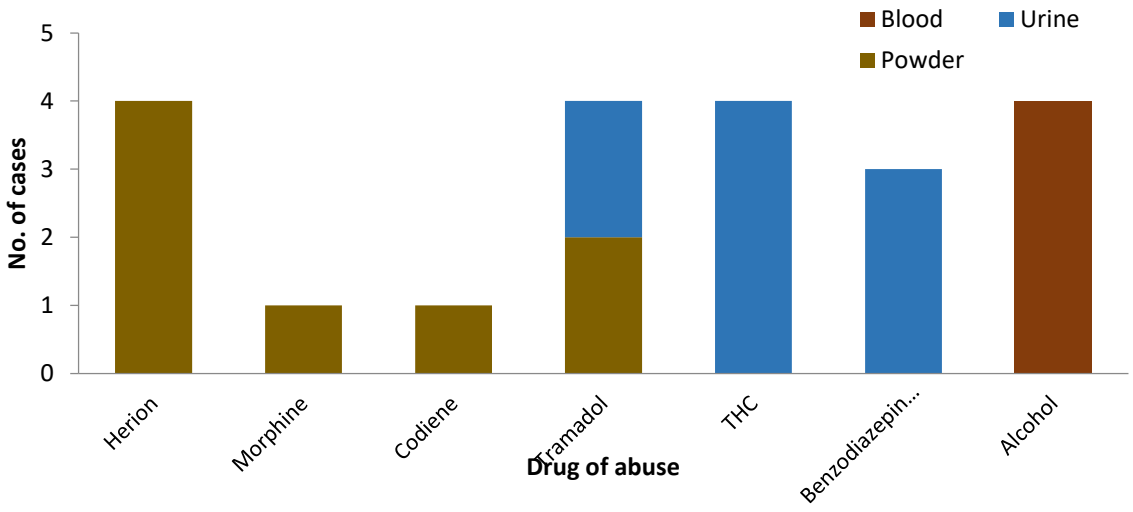

Figure 20. Types of samples received and tested

Highest number of test for heroin, tramadol, THC, benzodiazepines and blood alcohol level with four cases each were reported.

Method development for the analysis of pesticides in fruits and vegetables

Pesticides are widely used in agriculture to protect crops from pests and diseases. Residues of pesticides in fruits and vegetables pose potential health risks to consumers. Gas chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique for the identification and quantification of pesticides residues in food.

- Sample preparation:

  Representative samples of fruits and vegetables were selected from southern region of Bhutan. Initially, fruits and vegetables were washed and peeled to remove surface contaminants. Then, homogenized and extracted using methanol. QuEChERS method was used in the cleanup method.

- Instrumentation

  Gas Chromatography-Mass Spectrometry was used for the analysis of the samples **(Table 9).** 12 different pesticides were targeted in the study. Following are the chromatographic and mass spectrometric conditions developed:

Table 9. GCMS conditions for analysis of pesticides

Chromatographic conditions	
Initial oven temperature	70℃
Ramping program for separation	<table><tr><th></th><th>Rate ℃/ *min*</th><th>Value ℃</th><th>Hold time min</th><th>Run time min</th></tr><tr><td>(initial)</td><td></td><td>70</td><td>2</td><td>2</td></tr><tr><td>Ramp 1</td><td>10</td><td>150</td><td>2</td><td>12</td></tr><tr><td>Ramp 2</td><td>15</td><td>200</td><td>2</td><td>17.333</td></tr><tr><td>Ramp 3</td><td>15</td><td>280</td><td>5</td><td>27.667</td></tr></table>
Carrier gas flow rate and type	Helium, 1.5ml/min
Injection volume and temperature	1ul, 200℃
Split/ splitless injection mode	Spiltless mode
Mass spectrometric conditions	
Ionization mode (EI/CI)	EI
Ion source temperature	250℃
Mass range and scan speed	50-450, 1,526 (N=2)

- Method validation

  Calibration curve using standard pesticide solutions at different concentrations were evaluated along with the evaluation of linearity, sensitivity, accuracy, precision and limits of detection and quantification (LOD & LOQ) (**Figure 21, Table 10 &11**).

  Recoveries were determined by spiking known concentrations of pesticides into blank matrix samples. This method is developed for the analysis of a variety of fruits and vegetables obtained from different sources.

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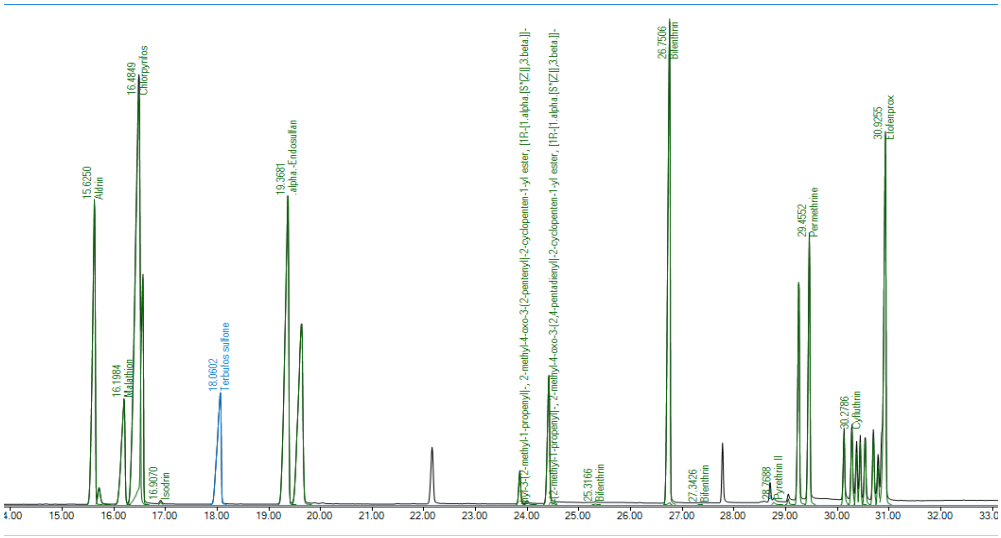

Figure 21. Chromatograms of 12 types of pesticides

Table 10. Calibration curve of 12 types of pesticides

Pesticides	Calibration curve equation	R^2
Aldrin	y = 1.610 + 49.611	R^2 = 0.99632
Malathion	y = 4.159 - 452.439	R^2 = 0.9999
Chlorphyrifos	y = 2.787 - 45.635	R^2 = 0.99995
Terbufos Sulfone	y = 5.161 - 947.666	R^2 = 0.99906
Bioallethrin	y = 18.638 - 2523.659	R^2 = 0.99897
Bifenthrin	y = 71.901 - 6968.137	R^2 = 0.99906
Amitraz	y = 7.136 - 1576.002	R^2 = 0.99971
Permethrin	y = 18.711 - 2369.082	R^2 = 0.99844
Cyfluthrin	y = 4.775 - 1031.506	R^2 = 0.99264
Cypermethrin	y = 4.919 - 778.868	R^2 = 0.99999
Etofenprox	y = 65.226 - 7977.808	R^2 = 0.99918
Deltamethrin	y = 4.479 - 1405.524	R^2 = 0.92963

For all the analyzed pesticides, r^2 > 0.990. Recovery (%) and accuracy (%) were all >90%) Recovery (%) and accuracy (%) were all >90%.

Table 11. Recovery and accuracy of 12 types of pesticides

	Recovery (%)			Accuracy (%)		
Pesticides	**250 ppb**	**500 ppb**	**1000 ppb**	**250 ppb**	**500 ppb**	**1000 ppb**
Aldrin	93.0	105.3	99.1	93.0	105.3	99.1
Malathion	107.6	95.0	100.9	100.1	100.0	100.0
Chlorphyrifos	99.2	100.6	99.9	99.2	100.6	99.9
Terbufos Sulfone	97.2	102.1	99.7	96.5	102.7	99.6
Bioallethrin	107.5	94.7	100.9	103.7	97.2	100.5
Bifenthrin	99.8	100.1	100.0	103.5	97.3	100.4
Amitraz	98.5	101.2	99.8	101.9	98.5	100.2
Permethrin	102.6	98.0	100.3	104.6	96.6	100.6
Cyfluthrin	105.1	95.8	100.7	109.9	92.5	101.2
Cypermethrin	91.1	107.9	98.8	100.2	99.9	100.0
Etofenprox	103.1	97.7	100.4	103.3	97.5	100.4
Deltamethrin	127.7	7810.9	103.5	131.8	76.2	104.0

NTESIS (National Toxic Exposure Surveillance Information System) Report

A total of three cases were reported in NTESIS. The decrease in this figure may be attributed to the recent modifications and upgrades made to the NTESIS system. Initially, all poison-related cases were logged as event reports. However, due to certain inconveniences encountered with the NEWARIS system, these event-based cases were converted to non-event status with corresponding alterations in the NTESIS system. The reduction in the number of poison cases may be a result of these changes, compounded by a lack of awareness among hospital staff regarding these modifications. Therefore, there is a need to sensitize and train the healthcare professionals on the NTESIS.

Regarding the cases, two cases was due to intentional ingestion of amitriptyline and cypermethrin (pesticides) suspecting attempted suicide case. Other was the accidental ingestion of diesel case. An 18 year old woman attempted suicide by ingesting 50 tablets of 25 mg amitriptyline. She was promptly admitted to the hospital, where she underwent thorough blood tests, CT scans, and x-rays. Based on the results, she received treatment including a stat dose of 2gm ceftriaxone and 1gm of ceftriaxone twice daily, intravenous thiamine at 100mg, IV ranitidine at 50mg, and IV ondansetron at 4mg. Subsequently, she was referred to JDWNRH.

2.10 Blinded rechecking of malaria slides

2.10.1 Reporting status of health centers

A total of 132 health centers have participated in malaria blinded rechecking. The overall average reporting rate with 21.3% were reported on time, 28.4% were zero reported,19.1% were reported late and rest were not reported (**Figure 22)**

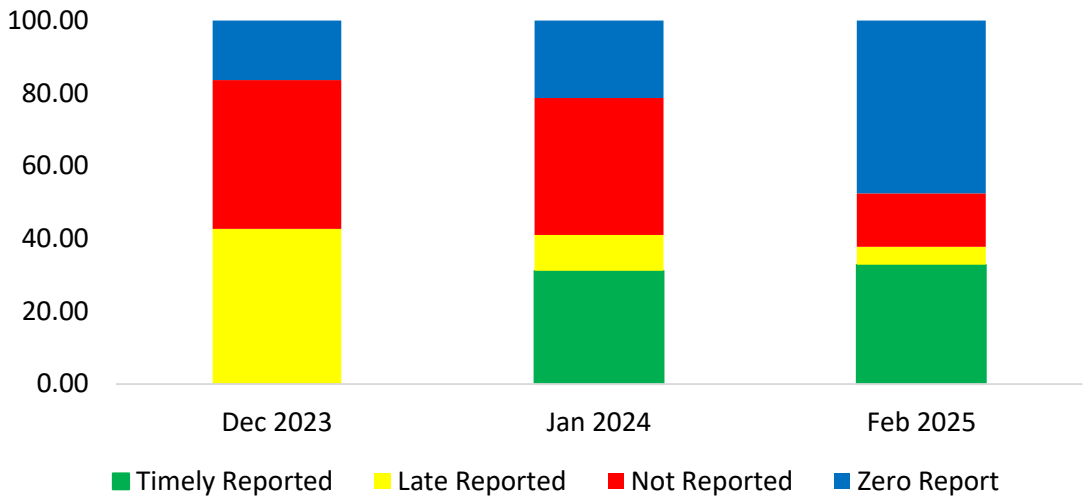

Figure 22: Monthly reporting status for 1st quarter 2024

2.11. Blinded rechecking status.

A total of 562 malaria slides were received at the National Malaria Reference Laboratory for blinded rechecking. From the total slides examined, five malaria-positive slides are detected (0.89%)

From 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 83.3% ,parasite density determination was 66.7%,quality of blood film was 92.1% and quality of stain was 79.7% **(Table 12 and 13)**

Table 13. Quarterly report on Malaria Blinded Rechecking

Month	Dec-23	Jan-24	Feb-24	Total
Health center participated in blinded rechecking	37	40	55	132
Total slides received for blinded rechecking	203	183	176	562
Total positive detected	5	0	0	5
Total Nmps detected	198	183	176	557
	Total slide Examine			1256

Table 14: Performance score on blinded rechecking

Month	Dec-23	Jan-24	Feb-24	Quarterly Score
Sensitivity (True positive detection)	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
Mp Stages Identification	83.3			83.3
Mp Parasite density	66.7			66.7
Stain Quality	81.9	77.0	80.3	79.7
Blood film Quality	93.5	92.0	91.1	92.1

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