ROYAL CENTER FOR DISEASES CONTROL

QUARTERLY BULLETIN: Second Quarter 2025

Contents

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

Immediate reports:

- Total of 60 samples of suspected Measles/Rubella cases were tested, none of the sample tested positive for Measles and Rubella IgM.
- Three suspected cases of Pertussis was reported from JDWNRH, all tested negative for *Bordetella pertussis*.
- Two suspected cases of acute hemorrhagic fever were reported from JDWNRH, both were negative for JE.
- Seven cases of malaria, was reported from Sarpang dzongkhag.

Events reports:

- During the quarter, twenty-four disease outbreaks were reported. Among them six were of Influenza-like Illness, five acute-gastroenteritis, three acute undifferentiated febrile illness (AUFI) caused by leptospirosis, seven chicken-pox, two conjunctivitis and one was of mushroom poisoning. An outbreaks of AUFI was responded by DHRRT with supports from NHRRT while rest were responded to by the DHRRT and 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 second quarter 2025, a total of 3640 weekly reports were expected from 280 health centers across the country. The reporting was in consistence with the first quarter of 2025. Overall 85.0% 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**).

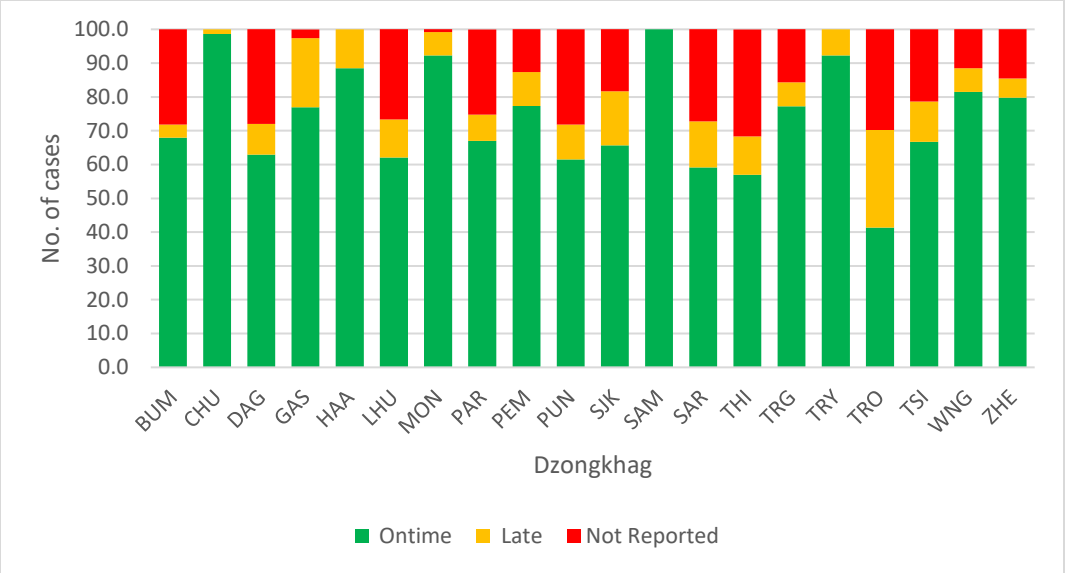


Figure 1: Dzongkhag-wise weekly reporting status for 2nd Quarter 2025

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, 25605 (81.0%) cases of respiratory illnesses and 5138 (15.0%) diarrheal cases were reported **(Table 1).**

Table 1: Notifiable diseases/syndromes reported by Dzonkhag

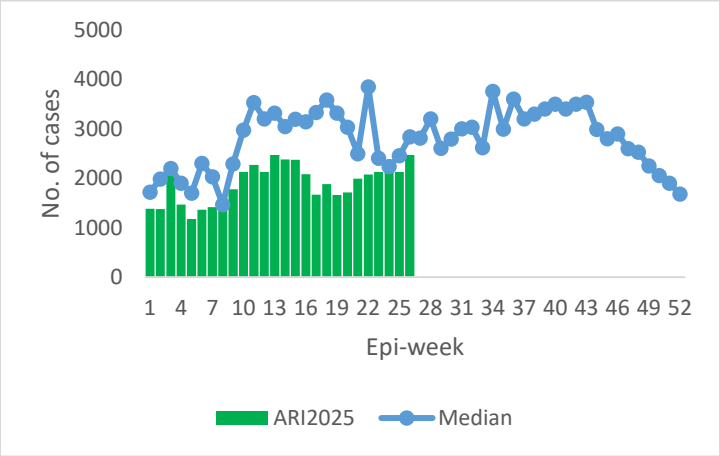
Dzongkhag	ABD	AWD	AJS	ARI	DGF	MUM	FWR	FDP	TPF	SAR	RKS
BUM	0	26	0	183	0	0	0	0	0	57	0
CHU	51	544	20	3524	42	5	20	18	36	48	5
DAG	4	113	0	936	0	1	1	5	1	64	2
GAS	0	32	0	178	0	0	7	0	0	0	0
HAA	0	69	26	303	0	0	0	10	0	0	0
LHU	5	45	1	415	0	0	1	2	0	0	0
MON	19	471	15	1596	0	0	0	38	0	9	5
PAR	8	126	2	373	0	0	0	0	0	1	0
PEM	9	273	17	1248	4	0	86	1	5	5	3
PUN	14	193	1	616	3	0	103	1	0	2	0
SJK	20	198	1	1061	7	0	0	1	8	26	2
SAM	19	641	6	3496	2	0	6	27	1	30	4
SAR	11	354	13	2142	15	7	11	68	32	7	15
THI	52	535	37	3336	10	0	6	0	1	0	0
TRG	18	207	11	1003	0	2	20	2	13	20	11
TRY	35	381	1	816	0	0	27	30	59	1	4
TRO	8	44	0	461	0	0	0	0	0	0	0
TSI	25	150	0	863	3	1	1	4	6	69	2
WNG	20	284	6	1855	4	1	3	1	0	57	0
ZHE	4	130	5	803	7	0	0	0	3	1	3
Total	**322**	**4816**	**162**	**25208**	**97**	**17**	**292**	**208**	**165**	**397**	**56**

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).

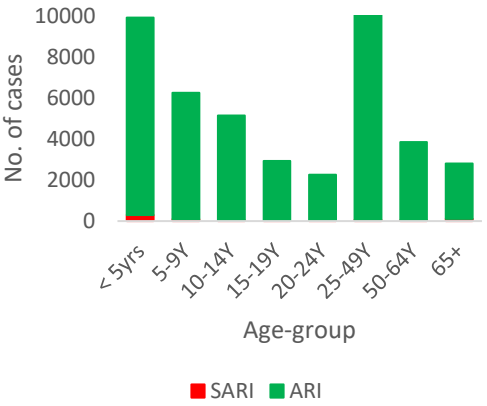
1.3. Descriptive analysis of most common notifiable diseases

1.3.1. Respiratory Illness (ARI and SARI) syndrome

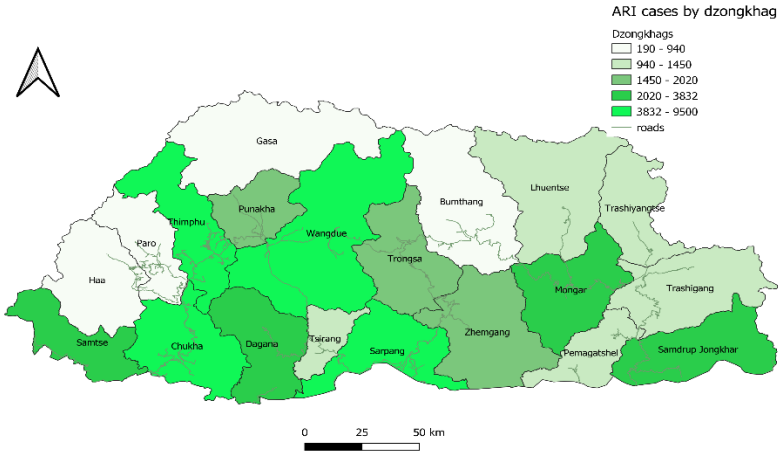
A total of 25605 cases of respiratory illness were reported, of which majority of cases were ARI (99.0%) and rest was SARI cases. The trend of ARI cases was found lower compared with the median of the last three years of the same quarter **(Figure 2A).** The most commonly affected age group by respiratory 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

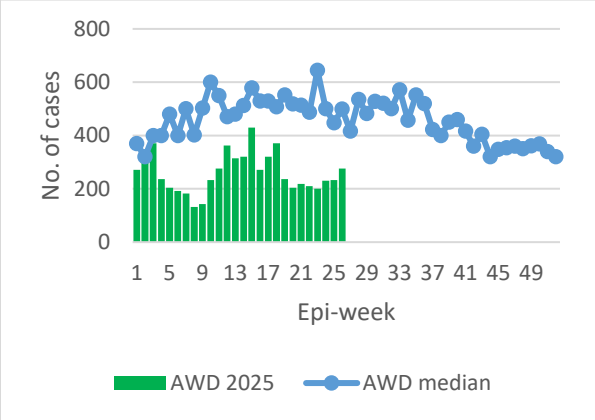


C: Respiratory illness cases by dzongkhag

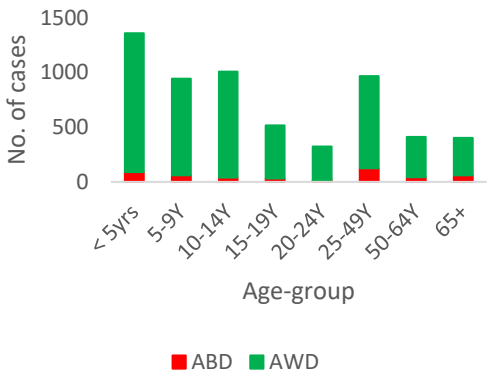
Figure 2: Respiratory illness incidence by epidemiological weeks, age groups and place.

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

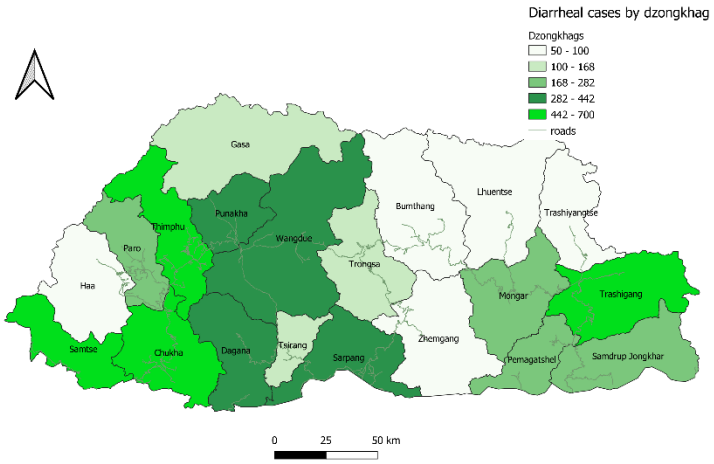
Of the total 5138 diarrheal cases reported, 90.0% were AWD and rest were ABD. The trend for diarrheal cases found lower compared with the median for the last three years, **(Figure 3A).** A high incidence of diarrheal diseases was observed in children 0-4 years **(Figure 3B)**. By dzongkhag, Thimphu, Chhukha, Sarpang and Trashigang reported the maximum number of cases **(Figure 3C).**



A: Cases by Epi-week



B: Incidence by age group

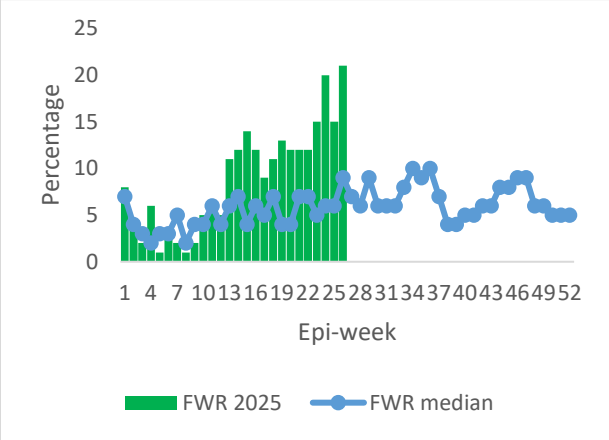


C: Diarrheal Cases by dzongkhag

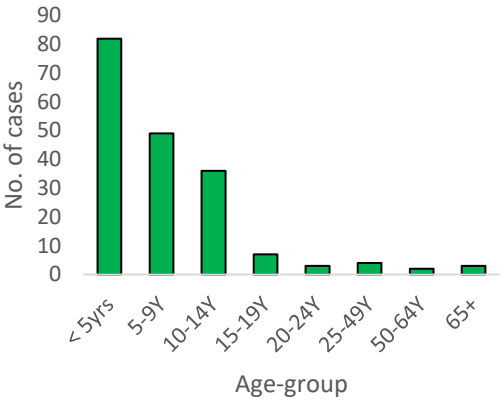
Figure 3: Diarrheal disease incidence by epidemiological weeks, age groups and place.

1.3.3. Fever with Rashes syndrome

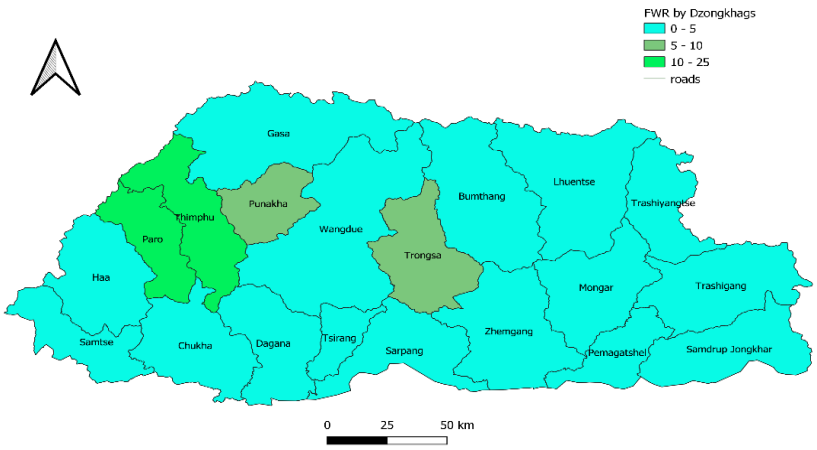
A total of 292 cases of fever with rash (FWR) syndrome were reported in the quarter **(Figure 4A)**.The trend of FWR was found consistently compared to the median of last three years. A majority of FWR were reported in the age group < 14 years **(Figure 4B).** As usual Thimphu and Paro reported maximum 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=60). Of the total samples tested, none of the samples tested positive for measles IgM and rubella IgM. Additionally, three suspected pertussis, and three suspected acute hemorrhagic fever were reported from JDWNRH. Seven confirmed malaria cases were reported during the quarter from Sarpang. Two cases of severe dengue was reported during the quarter.

1.5. Description of Key events

1.5.1 Influenza-like Illness:

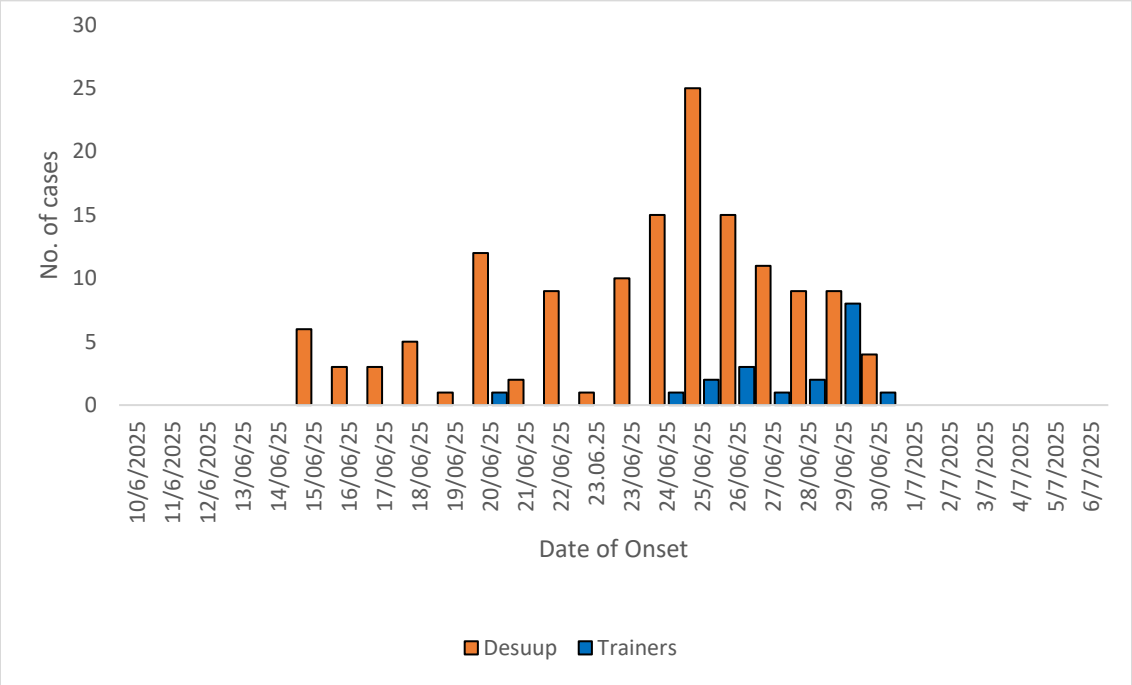
Between 1st April and 30 June 2025, six outbreaks of ILI events/outbreaks were reported in the event-based surveillance NEWARS. The cumulative number of cases affected was a total of --- cases, with no ILI-related mortality. The National Influenza Center received a total of 43 samples for confirmation. The samples were tested and detected Influenza subtype A/H1pdm09 as well as a few with COVID-19**.** A significant increase in the number of ILI outbreaks was observed, as compared to those reported in the first quarter of 2025. An outbreaks were responded to by the respective health centers upon the recommendations of RCDC.

Influenza like-illness is a seasonal illness and the district health authority in collaboration with district education authority should conduct awareness activities on the prevention of illness before influenza season in all schools and institutes. The concerned programe may create mass awareness on the prevention of /acute respiratory illness/influenza illness before the season through TV, radio, and social media platforms.

1.5.2. Outbreak Acute Undifferentiated Febrile Illness (AUFI):

During April – June 2025, a total of three events of AUFI was reported on event-based surveillance in NEWARS. Among them one was reported from Nganglam, one from CRRH Gelephu and Dewathang. On verification by RCDC, it was learned that all affected individuals were from the same cohort who completed 69th batch of Desuung training on June 24, 2025, from Dewathang

RBA. The CMO Samdrupjongkhar led the field investigation at Dewathang. A member from DoPH and RCDC joined DHRRT on June 30, 2025 to support the DHRRT. A total of 140 Desuup and 19 trainers complained of at least one symptom giving an overall attack rate of 55.0%. The age ranges from as young as 19 years old to as old as 50 years old. The onset of first cases among Desuup was on 15/06/2025 and first case among trainers was reported on 20/06/2025 as shown in figure 1. The index case was an individual who succumbed from acute hemorrhagic disorder on 28/06/2025 at JDWNRH.



The most common symptoms were Cough 78.0% followed by sore-throat 40.0% and Fever 31.0% (Figure 2). Other symptoms included light headedness, headache, epigastric pain, abdominal pain, myalgia, generalized weakness, runny nose, etc. The laboratory testing results at RCDC confirmed leptospirosis infection and Covid-19 (**Table 1).** There were three case fatality following this outbreak.

Table 1: Laboratory investigation of AUFI cases as June 30, 2025

Type of test	Total Sample	Result (Positive)	Percentage
Leptospirosis	43	19	44%
Scrub Typhus	34	7	16%
Brucellosis	32	2	2%
Dengue	34	0	0%
Chikungunya	34	1	2%
JE (Japanese Encephalitis)	34	0	0%
COVID 19	34	14	41%

The clinical, epidemiological and laboratory findings are suggestive of outbreak with mixed infection probably leptospirosis and Covid-19 among Desuup trainees in Dewathang. There is a strong epidemiological link to a mass water immersion activity on 14th June 2025. The clustering of symptom onset within the expected incubation period (5–14 days), combined with conducive environmental are clear evidence of pond being contaminated.

1.5.3. Acute Gastro-enteritis:

Between 1st April and 30 June 2025, RCDC was notified of five outbreak of acute gastroenteritis affecting general public, School students' three schools under Trashiyangtshe Punakha and attendees of the gathering held at paro. A team from DHRRT promptly initiated an epidemiological investigation to determine the cause, and mode of transmission of the outbreak. Active surveillance was conducted for case finding. Clinical and Environmental samples were collected and tested. Laboratory testing of clinical specimens revealed shigella sonnai in one outbreak while all

environmental samples revealed consistent with **e.Coli.**

Timely response and collaborative efforts between local hospitals RCDC, and BAFDA were crucial in mitigating further spread. Continue surveillance for early detection, underscore the need for vigilance and coordinated action to prevent future occurrences of such outbreaks particularly linked to social gatherings

1.5.4. Mushroom poisoning:

An outbreak of mushroom poisoning was reported on event-based surveillance, NEWARS from Sarpang Hospital. Altogether five persons who consumed mushrooms reported mild to severe gastrointestinal illness within 24 hrs. There was no any complication or mortality following the outbreaks.

The incidents reported may only represent a portion of actual mushroom poisonings. The low level of awareness of mushroom poisoning, in contrast to the high species diversity in this seasons is a huge challenge for mushroom poisoning control and prevention. Mushroom poisoning is a seasonal illness and the district health authority in collaboration with relevant stakeholders should conduct awareness activities on prevention of such incidents prior to mushroom season in the communities.

1.5.5. Conjunctivitis:

Three incidents of conjunctivitis's outbreak was reported from different schools under Lhuentse and Bumthang dzongkhag during the quarter. There was no complication following the outbreaks, all improved with symptomatic management. Health education on prevention of conjunctivitis and hygiene was given by concerned health centers to the schools. 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 466 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 243 (52.1%) were pulmonary tuberculosis (PTB) cases, 82 (17.6%) were culture follow-up samples from MDR-TB patients under treatment, 68 (14.6%) were pulmonary samples received for TB screening for VISA purposes and 73 (15.7%) were extra-pulmonary tuberculosis cases.

Among the PTB cases, new smear-negative (NSN) constituted 50.6% (n=123) of the cases, followed by 25.1% (n=61) of new smear-positive (NSP) cases, 20.2% (n=49) were cases with uncertain treatment history, 1.6% (n=4) with history of previous TB treatment and 2.5% (n=6) was received for re-culture (**Figure 1**).

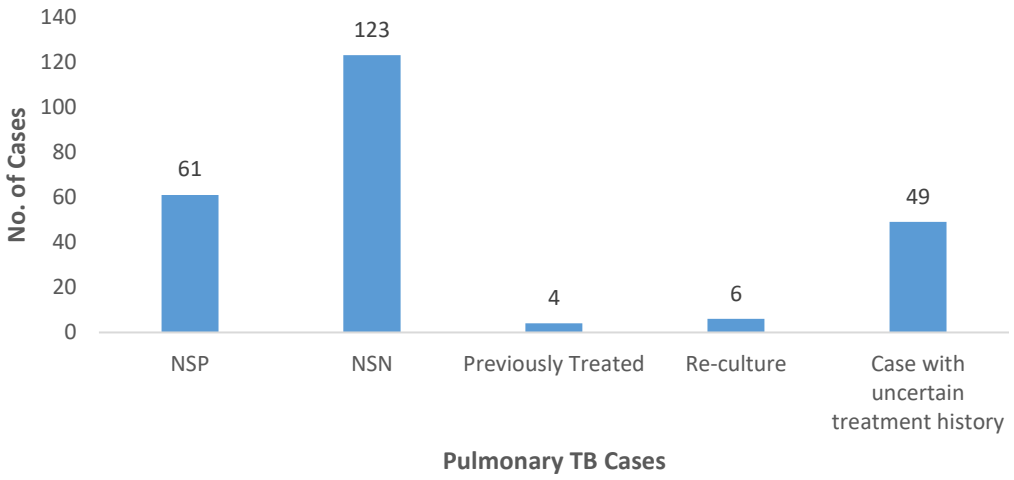


Figure 1: Classification of Pulmonary TB samples

2.1.2. Drug Sensitivity Test:

A total of 5 out of 466 (1.1%) patients had complete drug susceptibility testing (DST) reports using rapid molecular line probe assay, while 119 (25.5%) had DST reports using GeneXpert assay. A total of 6 multi-drug resistant tuberculosis (MDR-TB) cases were detected among which, one was detected using molecular line probe assay, and the other five through molecular WHO-recommended rapid diagnostic test (GeneXpert assay). Among the MDR-TB cases, all 6 were new cases. MDR-TB cases were highest in the age groups of 10-19 years (**Figure 2**).

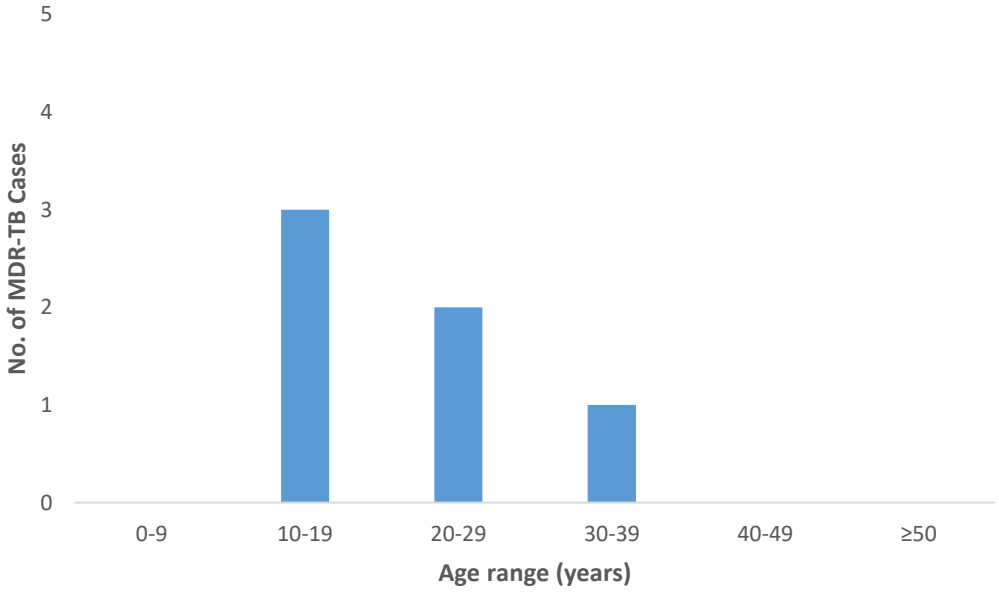


Figure 2: Distribution of MDR-TB cases by age group

2.2. National Respiratory Virus Sentinel Surveillance Report

2.2.1 Epidemiological Surveillance ILI and SARI

Seven ILI and eleven SARI sentinel hospitals report weekly influenza-like illness (ILI) and severe acute respiratory infection (SARI) aggregate cases to the RCDC. ILI cases increased by 57.6% in the second quarter (April to June 2025), with 2133 ILI cases. Similarly, SARI cases increased by 18.6% (332) compared to the previous quarter (**Figure 1 & Table 1**).

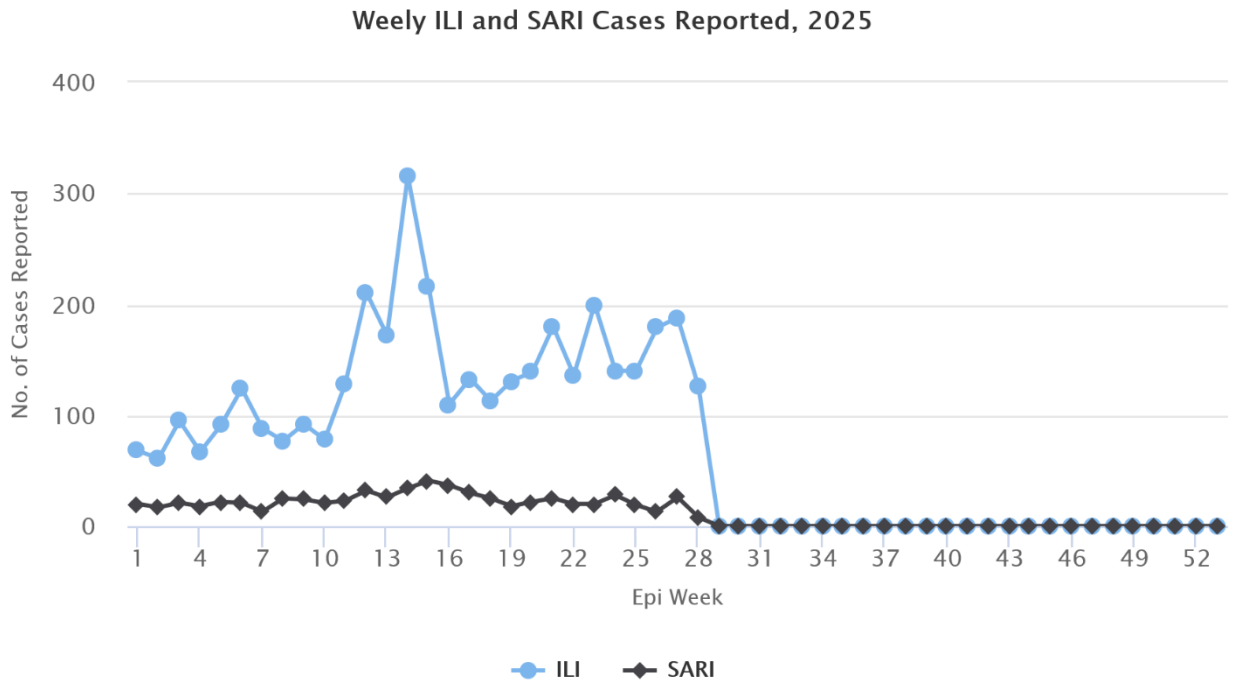


Figure 1: Weekly ILI and SARI aggregated cases reported (Note: week 14 – 26 is for the 2nd quarter of 2025)

All the ILI and SARI sentinel hospitals have reported, however few departments have missed SARI reporting. Paro (765) and Punakha Hospitals (516) have reported more ILI cases compared to other sentinel hospitals. Similarly, JDWNRH (101) and Gelephu (59) have reported more SARI cases compared to other sentinel hospitals during the quarter (**Table 1**).

Table 1: ILI and SARI cases reported by sentinel Hospitals

Sentinel Sites	ILI cases	SARI cases
Gelephu CRRH	NA	59
JDWNRH	NA	101
Monggar ERRH	NA	36
Phuentsholing Hospital	NA	38
Paro Hospital	765	12
Punakha Hospital	516	11
Samdrup Jongkhar Hospital	149	2
Samtse Hospital	202	0
Trashigang Hospital	134	15
Trongsa Hospital	183	8
Tsirang Hospital	184	50
Grand Total	**2133**	**332**

2.2.1. Influenza and SARS-CoV-2 (COVID-19)

A total of 1322 samples (ILI- 934, SARI- 220, eSARI- 93, and ARI outbreak- 75) were received from sentinel hospitals and non-sentinel hospitals (**Table 2**). The samples were tested for Influenza and SARS-CoV-2 by multiplex RT-PCR (Flu SC2) assay, and selective samples for RSV. Overall influenza positivity was 29.0% (384), increased by 11.1% compared to the previous first quarter, which was 17.9% (151). SARS-CoV-2 positivity was 2.2% (29), increased by 1.8% compared to the first quarter, which was 0.4% (3). Influenza B/Victoria (69.5%) was the most predominant influenza strain, followed by Influenza subtype A/H3 (26.6%) and Influenza A/H1pdm09 (3.9%) (**Figure 2)**.

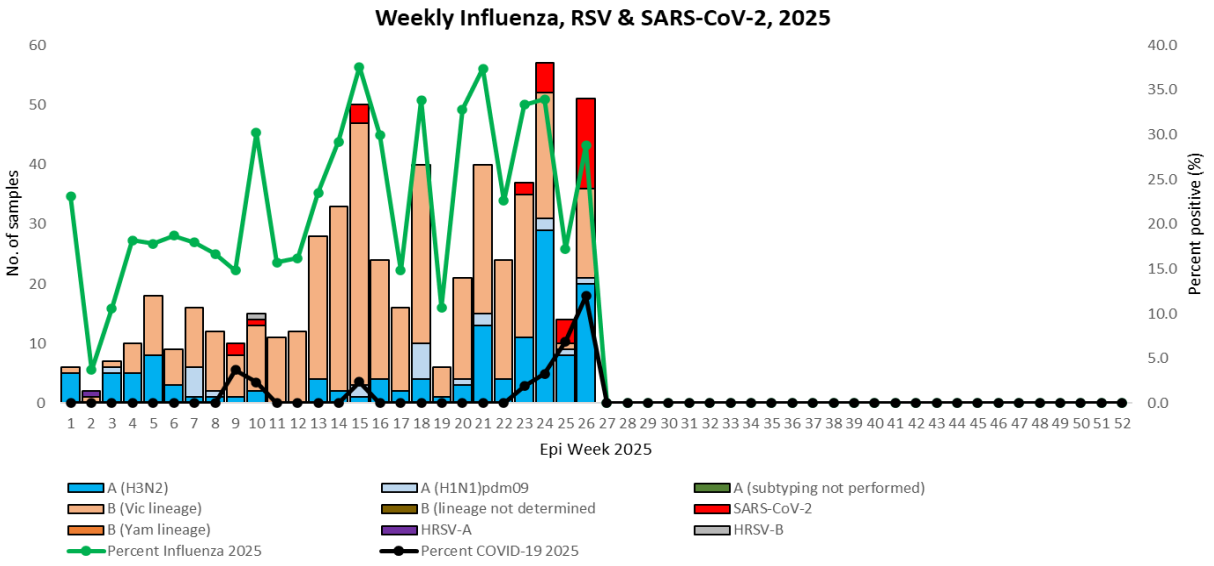


Figure 2: Weekly Influenza subtypes, SARS-CoV-2 and RSV in the first quarter of 2025

The median age group for Influenza was 14 years (IQR: 8 – 32 years, range: 5 months – 93 years), and the median age group for SARS-CoV-2 was 30.5 years (IQR: 4.4 – 45.5 years, range: 1 month – 89 years). The most affected age group for influenza was 5-14 years (41.3%), followed by 30 – 64 years (24.9%), while the sex distribution was not significant. Similarly, the most affected age group for SARS-CoV-2 was 30 – 64 years (46.4%), followed by 0 – 1 years (25.0%) (**Figure 3**).

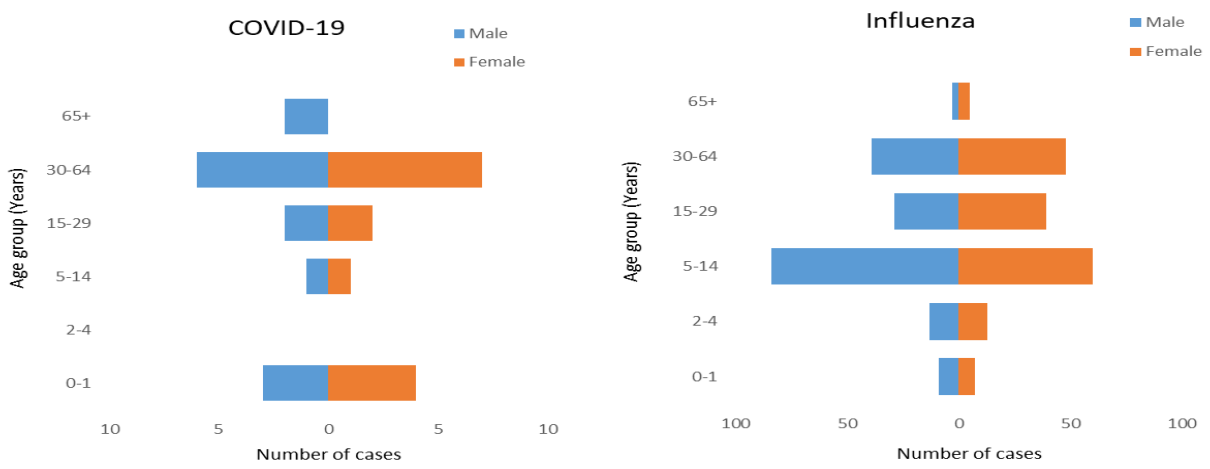


Figure 3: Influenza and SARS-CoV-2 age groups distribution by sex

Table 2: Sample receiving for ILI, SARI, eSARI**,** and ARI outbreak

Sentinel Sites/ Hospitals	ILI	SARI	eSARI	ARI Outbreak	Grand Total
Gelephu	10	12	18		40
JDWNRH	5	64	59	4	132
Mongar	86	21	9		116
Paro	67	10			77
Phuntsholing	73	35	7		115
Punakha	82	14			96
Samdrup Jongkhar	61	2			63
Samtse	133	3			136
Trashigang	118	14			132
Trongsa	118	8			126
Tsirang	154	37			191
Lungtenphu	10				10
Dewathang	5				5
Trashiyangtse	3				3
Balam PHC, Mongar				11	11
Bjoka,Zhemgang				13	13
Dagana	1				1
Gaselo HSS, Wangdue				15	15
Gidakom	3				3
Narang BHU, Mongar				10	10
Pemagatshel	5				5
Shemagangkha Pry School				15	15
Tenzin Academy, Paro				7	7
Grand Total	**934**	**220**	**93**	**75**	**1322**

2.2.2. Respiratory Syncytial Virus (RSV)

A total of 93 RSV samples were received from four RSV sentinel sites (**Table 2**). As per the guideline, 5 RSV samples were expected to be enrolled per week by each sentinel site. Therefore, 20 samples are expected to be tested (4*5 = 20 samples per week.

Samples were tested for RSV. If detected 'Negative' for RSV, they were tested for Influenza and SARS-CoV-2 as per the testing algorithm of the 'National Respiratory Virus Sentinel Surveillance Guidelines'. No RSV positive was detected; however, two samples tested positive for Influenza subtype A/H3, and 12 for Influenza lineage B/Victoria, while five were tested positive for SARS-CoV-2 (**Figure 4**).

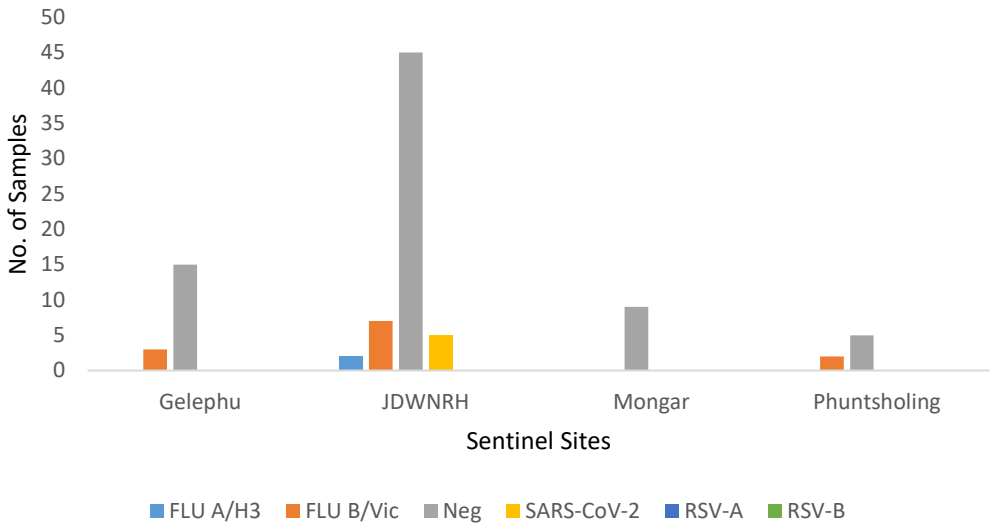


Figure 4: RSV Subtypes, Influenza subtypes and SARS-CoV-2 from eSARI samples

2.2.3. SARS-CoV-2 Sequencing Report

This report presents the findings of a SARS-CoV-2 sequencing analysis conducted from samples collected in between 1st May to 23rd June 2025.

Sample Collection and Testing

From 1st May to 23rd June 2025, RCDC received 709 throat swab specimens from 11 National Respiratory Virus Sentinel Surveillance Sites monitoring influenza-like illness (ILI) and severe acute respiratory illness (SARI). Among these, 26 samples (≈ 3.7%) tested positive for SARS-CoV-2 using the CDC's Influenza and SARS-CoV-2 multiplex RT-PCR assay. To ensure high-quality genomic data, samples with strong viral loads (Ct < 28) were selected for Next Generation Sequencing.

2.2.3.1. Genomic Classification

Sequenced samples were classified as belonging to the 24A, 25B, 24D, and 24H Nextstrain clades. The Lineage (Pangolin) classification revealed three subtypes: JN.1.16.5, PY.1, NB.1 and NB.1.8.1. According to the World Health Organization's classification, all samples were identified as the Omicron variant (**Table 3**).

Table 3: Circulating SARS-CoV-2 strains in Bhutan

Health Centre	Date of Collection	Clade	WHO Clade	Pango Lineage	WHO Classification
JDWNRH	14-Feb-25	24D	Omicron	NB.1	-
Trongsa	28-May-25	25B	Omicron	NB.1.8.1	**Variants Under Monitoring (VUM)**
JDWNRH	30-May-25	25B	Omicron	NB.1.8.1	**VUM**
Phuntsholing	31-May-25	24A	Omicron	JN.1.16.5	-
Phuntsholing	04-Jun-25	24H	Omicron	PY.1	-
Samtse	09-Jun-25	25B	Omicron	NB.1.8.1	**VUM**
Paro	09-Jun-25	25B	Omicron	NB.1.8.1	**VUM**
Gidakom	10-Jun-25	25B	Omicron	NB.1.8.1	**VUM**
JDWNRH	10-Jun-25	25B	Omicron	NB.1.8.1	**VUM**
JDWNRH	**14-Jun-25**	**25B**	**Omicron**	**NB.1.8.1**	**VUM**

2.3. Surveillance for Measles and rubella (MR) and Pertussis:

In the 2nd quarter of the year, VPVDL saw an increase of almost two folds in the number of suspected measles and *rubella* (MR) cases as compared to the previous quarter. VPVDL tested a total of 60samples as compared to 34 in the previous quarter.

It is a general recommendation to collect both blood and throat swab from every case. However, we received blood samples from 56 cases for IgM assay and 56 throat swabs for PCR. All samples tested negative for both MR IgM as well as RNA detection. All cases have been classified as "discarded" for MR. VPVDL received one sample for *pertussis* IgM testing from JDWNRH. The sample tested negative.

Stool samples from three Acute Flaccid Paralysis (AFP) were received for investigation of *poliomyelitis*. The samples will be sent to the Regional Reference Laboratory in Thailand for polio virus culture and isolation. So far, all samples sent by VPVDL to RRL, Thailand, have tested negative for Polio Virus.

Table 1: Total number of samples received from different parts of Bhutan for investigation of Measles, Rubella, Pertussis and Poliomyelitis

Place	Measles/ rubella	Pertussis	AFP
Gedu	1		
Gelephu	1		
Gidakom	2		
Gomtu	2		
Thimphu	11	1	1
Mongar	2		
Paro	7		1
Pemagatshel	2		
Phuntsholing	1		
Punakha	7		
Samdrup Jongkhar	2		
Sarpang	5		
Trashiyangtse	3		
Trashigang	2		1
Trongsa	1		
Tsirang	6		
Bumthang	2		
Wangdiphodrang	1		
Yebilaptsha	2		

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

A total of 122 samples were received from the sentinel site for AUFI testing. The AUFI parameters include Dengue, Chikungunya, Scrub typhus, Japanese encephalitis, leptospirosis, and Brucellosis. All tests were conducted using the ELISA method and Microscopic Agglutination Test (MAT). Out of the 122 samples, one sample tested positive for Dengue, Japanese Encephalitis and Leptospirosis. 33 samples tested positive for Scrub Typhus and 11 sample tested positive for Brucellosis. All samples for Chikungunya were tested negative. These samples were obtained for the purpose of surveillance through independent testing. **(Table 1)**

Table 1: Total Number of AUFI samples test during 2nd Quarter

Surveillance site	Total samples	DEN (-)	DEN (+)	CKG (-)	CKG (+)	ST (-)	ST (+)	JE (-)	JE (+)	LPS (-)	LPS (+)	BRU (-)	BRU (+)
Phuntshoing Hospital	21	21	0	21	0	12	9	21	0	21	0	19	2
Samdrup Jongkhar Hospital	19	18	1	19	0	15	4	19	0	19	0	18	1
Tsirang Hospital	70	70	0	70	0	53	17	70	0	69	1	63	7
Gelephu CRRH	8	8	0	8	0	6	2	7	1	8	0	7	1
Wangdue Hospital	1	1	0	1	0	0	1	1	0	1	0	1	0
JDWNRH	1	1	0	1	0	1	0	1	0	1	0	1	0
Trongsa Hospital	1	1	0	1	0	1	0	1	0	1	0	1	0
Mongar ERRH	1	1	0	1	0	1	0	1	0	1	0	1	0
Total	**122**	**121**	**1**	**122**	**0**	**89**	**33**	**121**	**1**	**122**	**1**	**111**	**11**

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

In addition, during the second quarter, 57 serum samples were received from district hospital for confirmation. From the tested samples, five samples tested positive for Brucellosis and tested negative for Dengue, Leptospirosis, Scrub Typhus and Japanese encephalitis. During the Second quarter, one samples received for AES testing. The samples tested negative. **(Table 2)**

Table 2: Total number of samples tested for confirmation

Test	Total samples	DEN (-)	DEN (+)	CKG (-)	CKG (+)	ST (-)	ST (+)	JE (-)	JE (+)	LPS (-)	LPS (+)	BRU (-)	BRU (+)
Dengue	41	41	0										
Brucellosis	7											2	5
lepto	6									6	0		
Scrub Typhus	3					3	0						
Japanese encephalitis	1							1	0				
Total	57	41		0	0	3	0	1	0	6	0	2	5

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

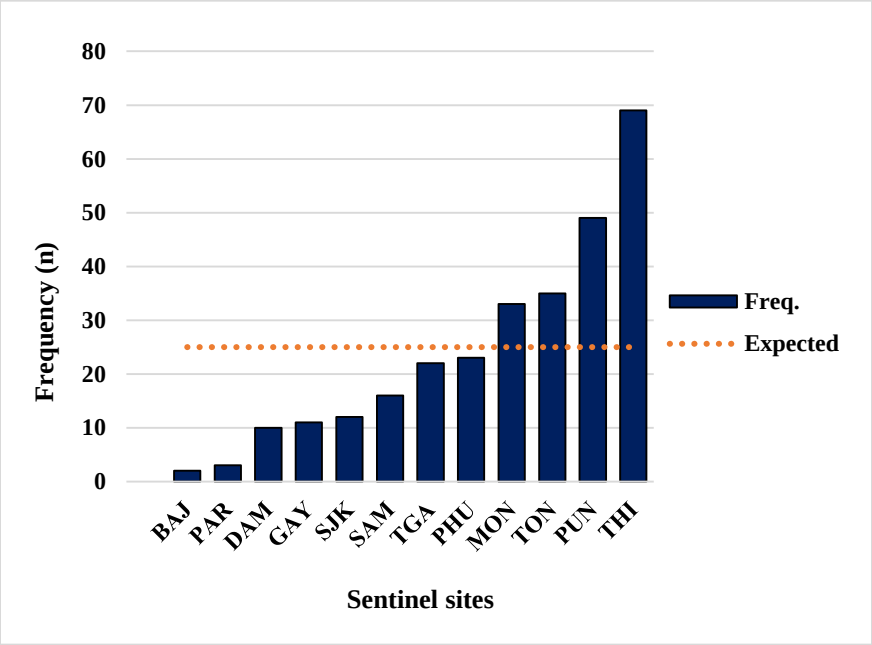
At the end of second quarter, total of 43 samples were received linked to outbreak of AUFI among same cohort who completed 69th batch of Desuung training on June 24, 2025, from Dewathang RBA and the samples were tested for AUFI test panel. Total of 19 (44.0%) sample tested positive for Leptospirosis, seven sample (16.0) tested positive scrub typhus, two tested positives for Brucellosis and one tested positive for Chikungunya.

Date	Site (Hospital)	Total samples received	AUFI Panel	Test status	Total Positive	Total Negative
			Leptospirosis	Complete	**13**	3
			Scrub typhus	Complete	**6**	10
			Brucella	Complete	**1**	15
			Leptospirosis	Complete	**4**	9
			Scrub typhus	Complete	0	4
			Brucella	Complete	0	4
			Chikungunya	Complete	0	4
			JE	Complete	0	4
	Wangdue	14	Dengue	Complete	0	14
			Leptospirosis	Complete	**2**	12
			Scrub typhus	Complete	**1**	13
			Brucella	Complete	**1**	13
			Chikungunya	Complete	**1**	13
			JE	Complete	0	14

2.5. Sentinel Surveillance for Diarrheal Etiologic Agents

In the second quarter, surveillance activities resulted in the collection of a total of 285 stool samples from 12 designated sentinel sites. Among these, JDWNRH, Punakha, Trongsa, and ERRH were the highest contributors, each meeting their quarterly sample submission targets. An additional 24 samples were obtained from enteric disease outbreaks that underwent laboratory investigation during the reporting period.

Of the samples collected, 52% (n = 66) were from male patients, while 48% (n = 50) were from females, indicating a slight male predominance. The mean age of patients was 20.1 years. The geographic distribution of the collected samples is presented in Figure 1.



MON	ERRH
PUN	Punakha Hospital
BAJ	Wangdue Hospital
TON	Trongsa Hospital
THI	JDWNRH
PAR	Paro Hospital
PHU	P/ling Hospital
GAY	CRRH
SAM	Samtse Hospital
TGA	T/gang Hospital
DAM	Tsirang Hospital
SJONG	S/jong Hospital

Figure 1: Specimens received by site

Clinically, the majority of patients presented with loose stools (70.0%, n = 199), followed by watery diarrhea in 29.0% (n = 83) of cases. A small proportion (1.0%, n = 3) exhibited bloody stools, suggesting a less common presentation of invasive diarrheal disease. The average duration of diarrheal illness was 36.5 hours, with most cases resolving within a few days.

Regarding healthcare utilization, 89.0% (n = 103) of patients were managed in outpatient settings, while 9.1% (n = 26) required hospitalization, reflecting a generally mild to moderate disease severity profile.

Epidemiological investigations identified five cases with potential links to the consumption of specific food items, including pizza, pork, rice, and mixed vegetables, indicating possible foodborne transmission. An additional five cases were associated with recent travel to neighboring border areas or international travel (notably from Australia) before symptom onset, suggesting potential exposure to external sources of infection.

In terms of occupational distribution, the highest proportion of cases was reported among students (n = 91; 32.0%), followed by dependents (n = 62; 22.0%) and farmers (n = 41; 14.3%). This distribution may reflect differences in exposure risk, mobility, and healthcare-seeking behavior across occupational groups. The average time interval between the onset of illness and the first hospital visit was 2.3 days, indicating a moderate delay in health-seeking behavior, which could have implications for early diagnosis and the containment of outbreaks.

Laboratory investigations identified Diarrheagenic *Escherichia coli* (DEC) as the predominant pathogen, detected in 28 out of 193 E. coli isolates tested. This highlights *E. coli* as a major contributor to diarrheal illnesses during the reporting period. Other important pathogens isolated included *rotavirus*, *norovirus*, *Shigella sonnei*, and *Giardia lamblia*. These findings reflect the mixed etiology of diarrheal diseases, involving both viral and bacterial agents, as well as protozoan parasites. The distribution of confirmed pathogens is illustrated in Figure 2.

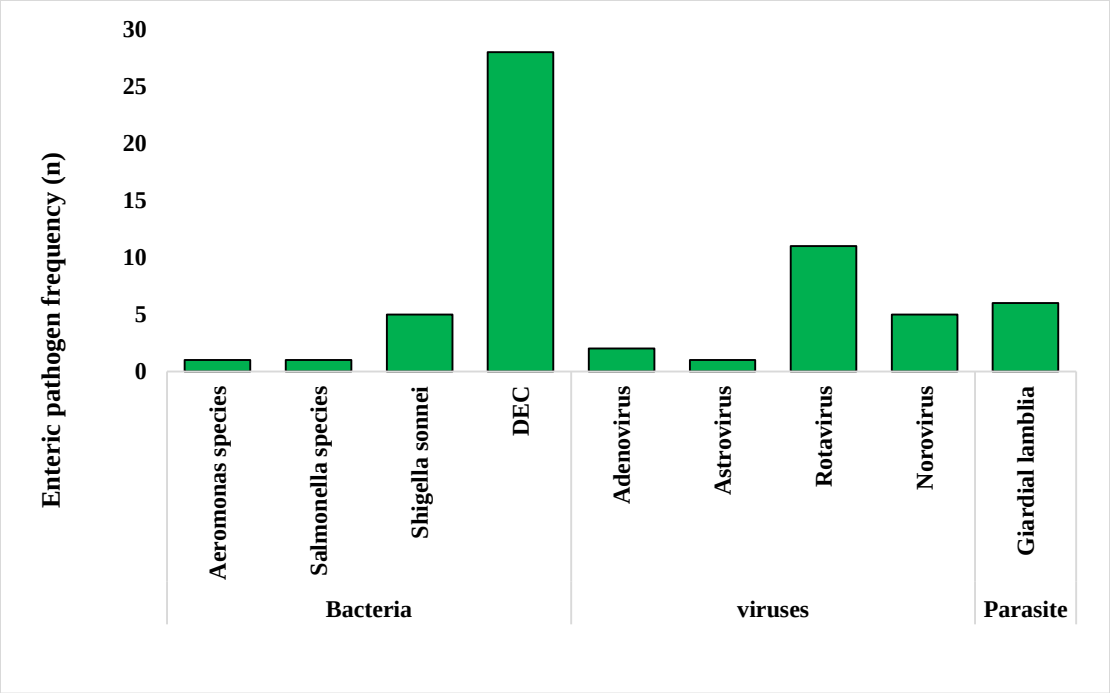


Figure 2: Proportion of isolated pathogens; DEC: Diarrheagenic *Escherichia coli*

A total of 35 bacterial isolates demonstrated resistance to first, second, and third-generation cephalosporins among the antibiotics tested, indicating a concerning level of multidrug resistance. These resistant isolates were further subjected to phenotypic screening for the presence of Extended-Spectrum Beta-Lactamase (ESBL) and AmpC beta-lactamase enzymes, two key mechanisms responsible for beta-lactam antibiotic resistance. The distribution of ESBL and AmpC producers among cephalosporin-resistant isolates is presented in Figure 3.

Figure 3. Phenotypic detection of ESBL and AmpC among cephalosporin-resistant isolates

Figure 4 presents the susceptibility patterns of bacterial isolates against a panel of commonly tested antibiotics. The findings highlight a notable level of resistance across multiple antibiotic classes. High resistance rates were observed to beta-lactam antibiotics, with ampicillin showing 75.0% resistance, cefazolin 70.0%, ceftriaxone 65.0%, and cephalexin 72.0%. Among other antibiotics, gentamicin and nalidixic acid exhibited moderate to high resistance at 40.0% and 45.0%, respectively. Chloramphenicol and ciprofloxacin both had resistance rates of 25.0%, while tetracycline and trimethoprim-sulfamethoxazole showed lower resistance levels of approximately 20.0%.

These findings emphasize the emergence of multidrug resistance and underscore the importance of routine susceptibility testing to guide effective antibiotic use and inform stewardship policies.

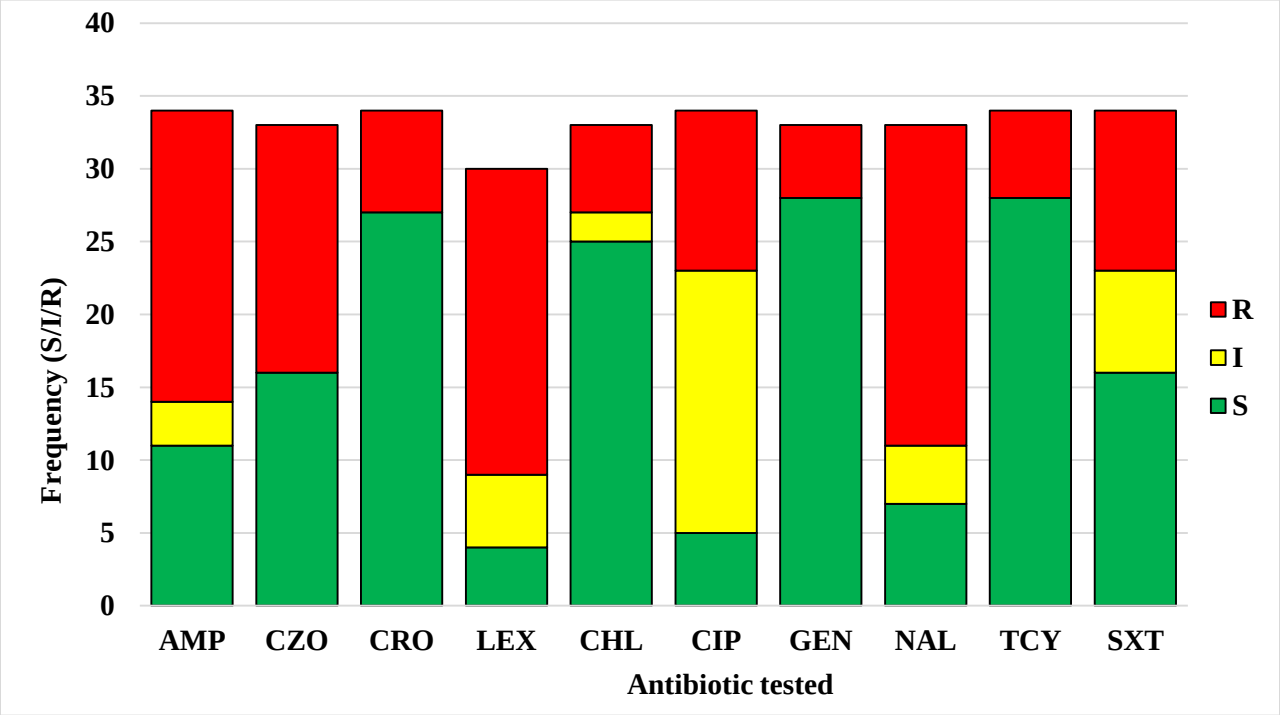


Figure 4: Antibiotic susceptibility pattern for bacterial pathogens (n=16)

Note: AMP (Ampicillin), CZO (Cefazolin), CRO (Ceftriaxone), LEX (Cefalexin), CHL (Chloramphenicol), CIP (Ciprofloxacin), GEN (Gentamycin), NAL Nalidixic Acid, TCY (Tetracycline), SXT (Trimethoprim and sulfamethoxazole

S – Susceptible, I – Intermediate, R – Resistant DEC: Diarrheagenic *E. coli*

2.6 Food safety surveillance:

Total of 49 ready to eat food samples were received from April to June 2025 from five food safety surveillance sites (Figure 1).

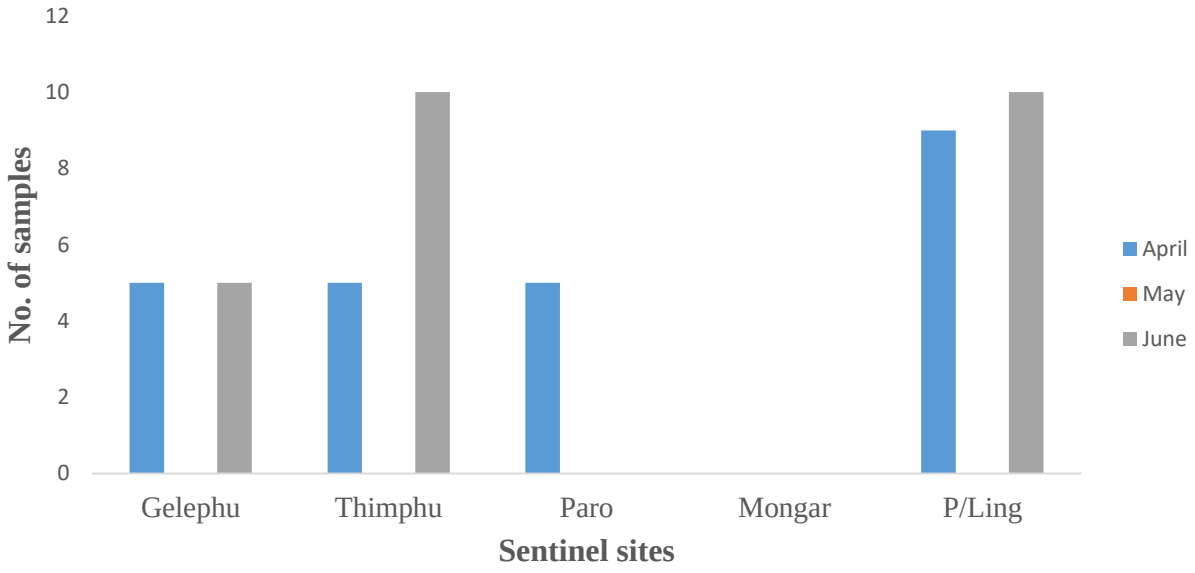


Figure 1. Number of food safety surveillance samples received from surveillance sites

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 assessing food safety and surrogacy for hygiene practice. During the current period 20.4% (n=10) of food samples had been contaminated with pathogenic organisms and 16.3% (n=8) with indicator organisms. The common pathogen isolated is *Staphylococcus aureus* (16.3%) followed by *Bacillus cereus* (4.1%). There was no event of foodborne disease outbreak reported during the sampling period.

2.7 Urban Drinking Water Quality Monitoring (UDWQM)

2.7.1 Bacteriology test (Thermotolerant coliform) Report

A total of 591 samples were collected and tested for *E. coli* from urban health centers for the 2nd quarter 2025. The result showed that the mean compliance rate across the health centers was 36.2%, quite lower than previous quarter of 49.2% (quarter 1, 2025) and 53.6% (quarter 4, 2024) respectively (**Figure 1**). Figure 2 shows the *E. coli* compliance for 20 districts. Figure 3 presents a breakdown of compliance data from all the reporting centers.

As shown in the figure 3, some urban water surveillance sites including Deothang, Gedu, Gomtu, Khamdang, Lhamoizingkha, Mongar, Riserboo, Samtse, Trashigang and Trirang Hospitals failed to report for this quarter.

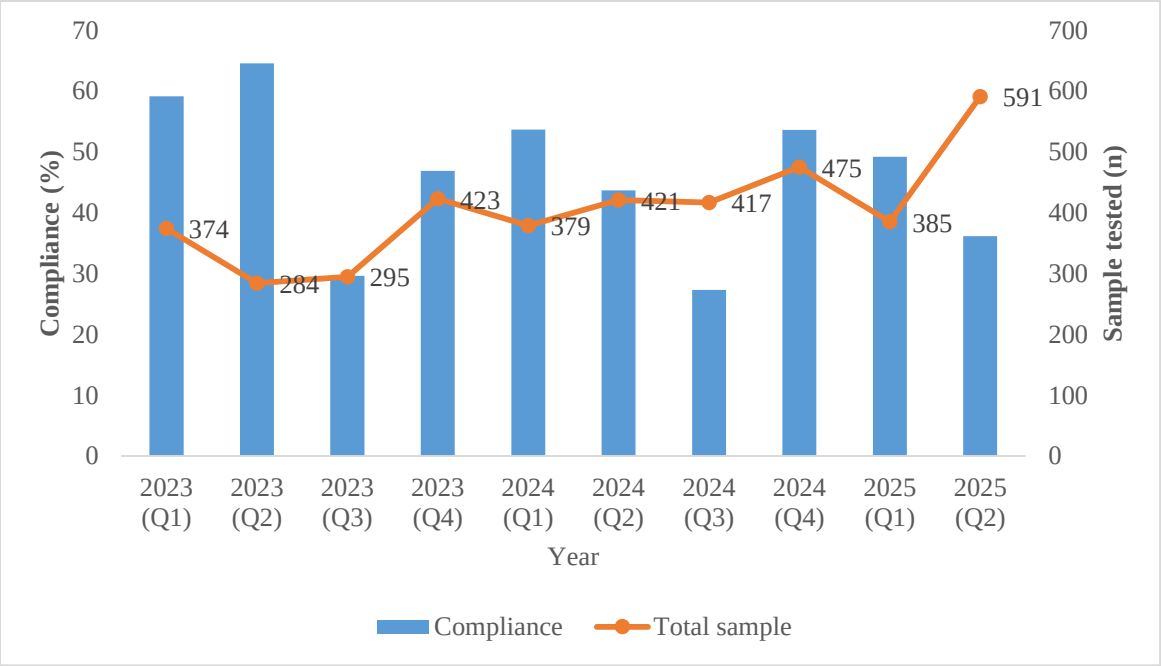


Figure 1. Microbial test result compliance in urban areas for past 10 quarters

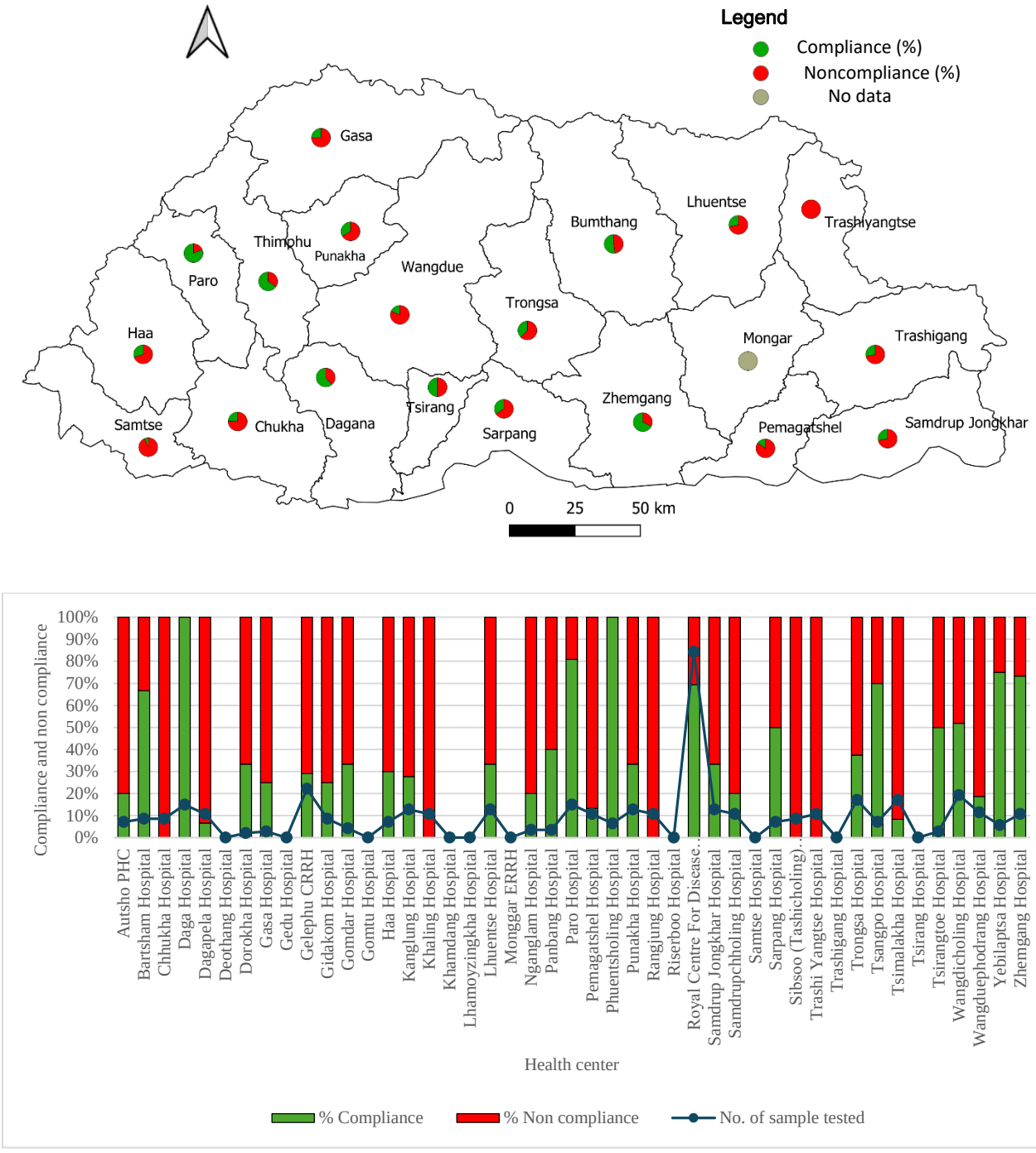


Figure 3. Microbial test result compliance for urban health centers

Physical test

The compliance rate for physical parameter shows a comparatively better when compared with the microbial parameters, with the mean compliance rate of 99.4% and 92.9% for pH (recommended value; 6.5-8.5) and turbidity (recommended value; <5NTU), respectively.

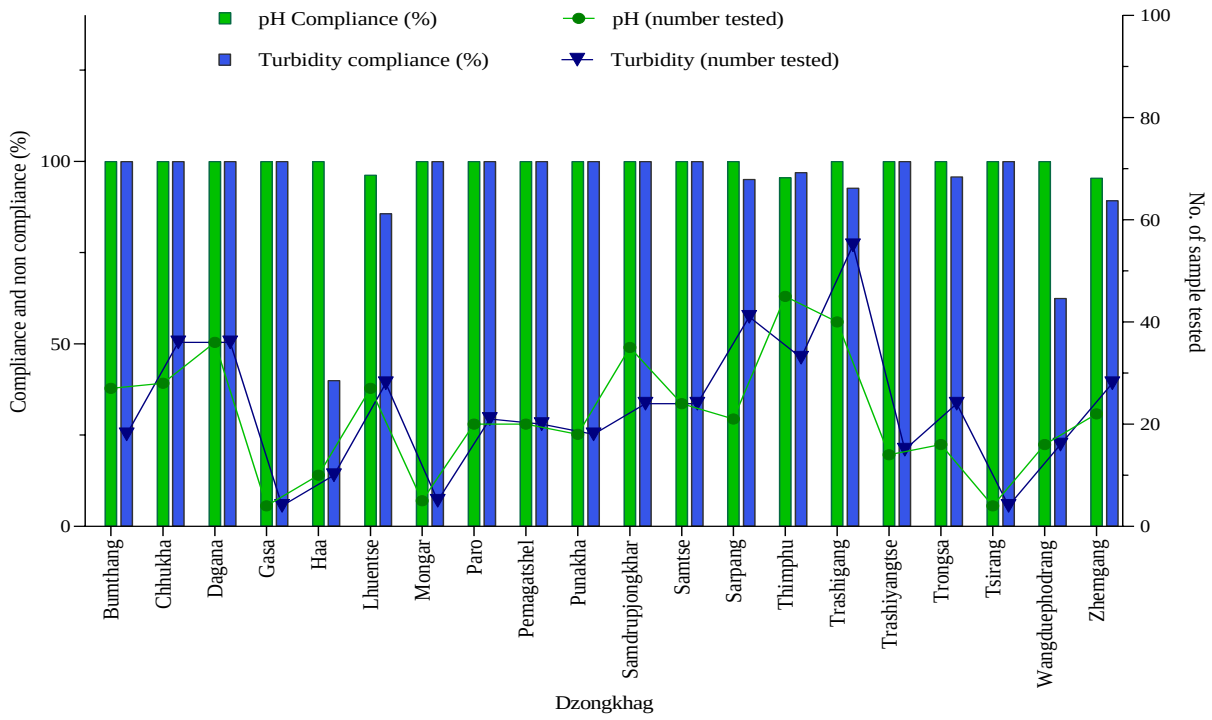


Figure 4. Physical test compliance rate from 20 dzongkhags

2.7.2. Drinking water quality surveillance (Rural)

This bulletin covers the rural drinking water quality surveillance report made for February-March months, 2025 (Dry season). A total of 850 water samples were analyzed, with 74.6% meeting safety standards (0 CFU/100mL) showing improvement in the proportion of safe drinking water compared to the previous half-yearly report for the wet season of 2024 (67.40%).

Figure 5 shows the *E. coli* compliance rates across 20 districts in rural areas, while Figure 6 compares compliance rates between the dry and wet seasons over the past three years. No significant differences were observed in compliance rates between the dry and wet seasons.

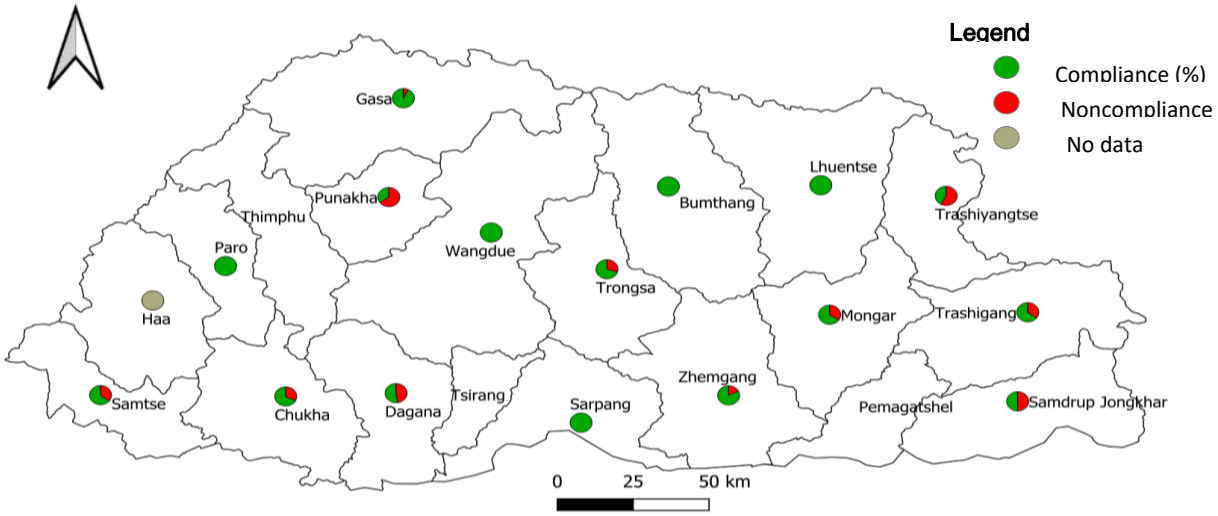


Figure 5. *E. coli* compliance for 20 districts in rural areas.

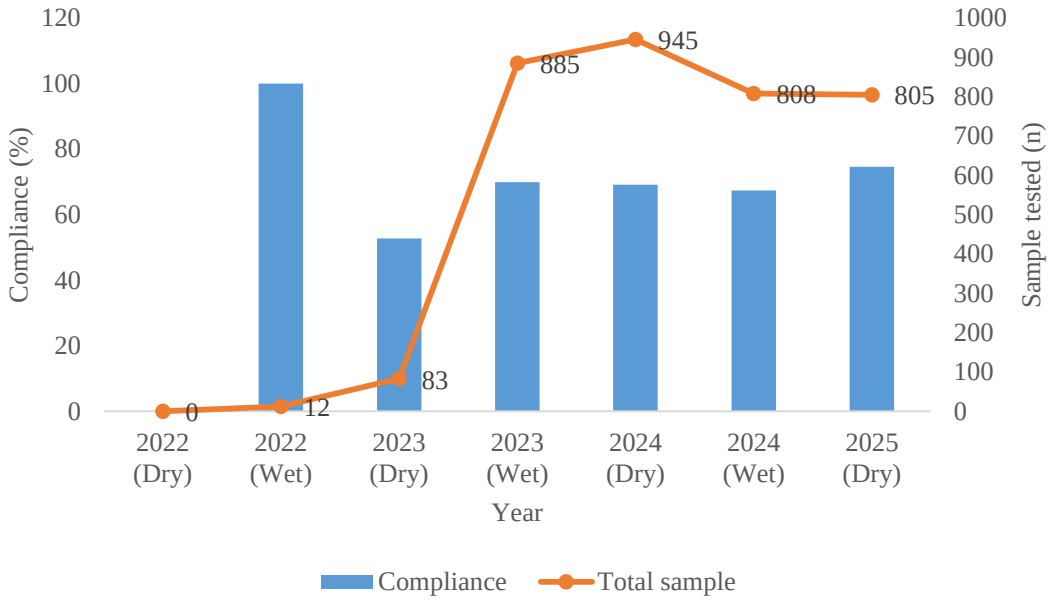


Figure 6. Compliance rate for various seasons over the past 3 years.

2.8. Drug Quality Monitoring:

The National Medical Product Testing Laboratory (NMPTL), Royal Centre for Disease Control, Ministry of Health in the 2nd quarter of 2025, conducted quality assessments on pharmaceutical products to monitor the prevalence of substandard and non-compliant medicines in the healthcare system. The objective was to ensure medicines available in both public and private healthcare sectors meet the required pharmacopoeial standards.

A total of 275 medicine samples were tested in accordance with their respective pharmacopeial monographs. The samples were collected from various hospitals and private pharmacies, and were subjected to standard quality control parameters, including Uniformity of weight, Average weight, Assay, Disintegration, Friability. In addition, oral liquid formulations sampled from private pharmacies were screened for Diethylene Glycol (DEG) and Ethylene Glycol (EG) contamination.

Out of the 275 medicine samples tested during the reporting period, 21 samples (7.64%) were found to be non-compliant with established quality specifications. The highest number of non-compliant samples were reported from Mongar and Wangdue Phodrang, followed by other districts.

Table 1: Distribution of samples collected from different sites

Site	Complies	Does not complies	Total
Thimphu	70	2	72
Chhukha	27	2	28
Sarpang	47	2	49
Samtse	25	1	26
Punakha	12	0	12
Mongar	23	4	27
SamdrupJongkhar	12	2	14
Bumthang	11	1	12
Trongsa	7	1	8
Trashigang	15	1	16
Tsirang	7	2	9
WangduePhodrang	9	3	12
Paro	10	0	10
Total	**275**	**21**	**296**

The findings from Q2 2025 highlight the continued need for robust post-marketing surveillance and enforcement of quality assurance practices. The detection of non-compliant samples emphasizes the importance of maintaining stringent oversight across the health facilities and private pharmacies. The forthcoming results from the baseline study will further support policy and regulatory decisions to combat the circulation of SF medicines in the country.

2.9. National Toxic Exposure Surveillance Information System (NTESIS) Report

The PITL operates a real-time online web-based poisoning surveillance information system called the National Toxic Exposure Surveillance Information System (NTESIS). It collects toxic exposure cases from all the health centers across Bhutan with the support of focal health professionals appointed in each health center. Some reports, especially that fall under the events category, are received through the National Early Warning, Alert and Response Surveillance Information System (NEWARS). The report provided here outlines the types of toxic exposures reported from the health centers during the months of April to June, 2025.

A total of 44 poisoning cases were reported in the NTESIS (16 cases) and NEWARS (28 cases) from 8 health centers, table 1. The agents of exposure included wild plants, food, snake bites, pharmaceuticals and mushroom.

Table 1: Types of toxic exposure cases from April to June, 2025

Case date	Agent	Health center	Dzongkhag	Remarks
06/03/2025	Mushroom	Autsho PHC	Lhuentse	
25/05/2025	Wild plants	Khamdang Hospital	Trashiyangtse	
05/08/2025	Wild plants	Gedu Hospital	Chhukha	
15/05/2025	Pharmaceuticals	Khoma PHC	Lhuentse	
05/02/2025	Snake Bite	Jomotsangkha Hospital	Samdrupjongkhar	
27/04/2025	Snake Bite	Jomotsangkha Hospital	Samdrupjongkhar	
22/04/2025	Pharmaceuticals	Khamdang Hospital	Trashiyangtse	
25/04/2025	Wild plants	Autsho PHC	Lhuentse	
04/04/2025	Food	Ugyentse PHC	Samtse	23 cases
27/05/2025	Mushroom	Sarpang Hospital	Sarpang	5 case

Confirmation tests for drugs of abuse from April to June, 2025

The PITL also provide the service as a National Reference Laboratory for the confirmation of drugs of abuse. From April to June, 2025, a total of 64 samples were received in this quarter for confirmation of Benzodiazepines, Delta-9-THC, Heroin, Opoids, Tramadol and other toxins, in both biological and non-biological samples namely capsules, coin, plant parts, powder, tablets and urine, **(Figure 1)**. The main methods used for testing was Gas Chromatography coupled with Mass Spectrometry (GC-MS).

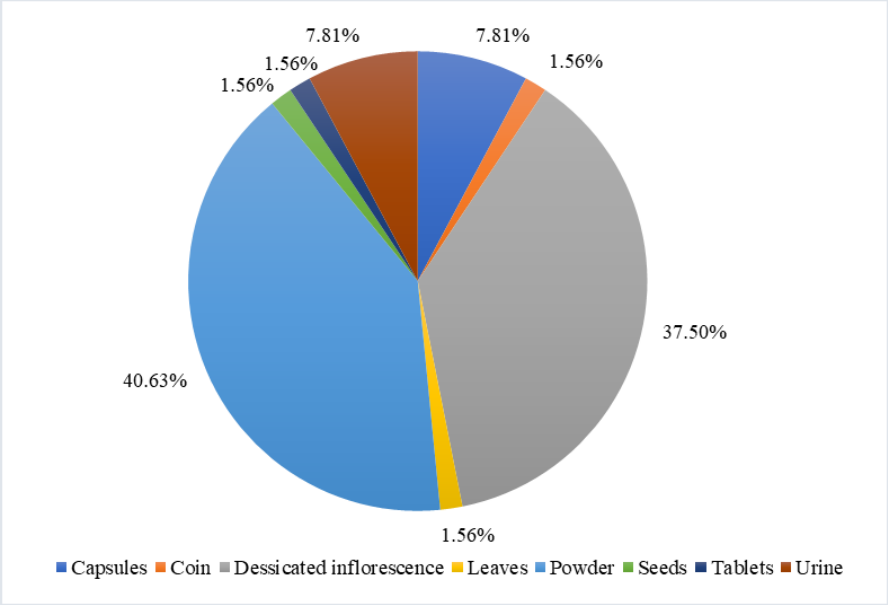


Figure 1: Different types of samples.

The positive detection rate of the samples tested was 92.19% (n=59). The top two agents with the highest frequency of testing were Delta 9 THC (43.75%, n=28), followed by Heroin (40.63%, n=26), (**Figure 2**).

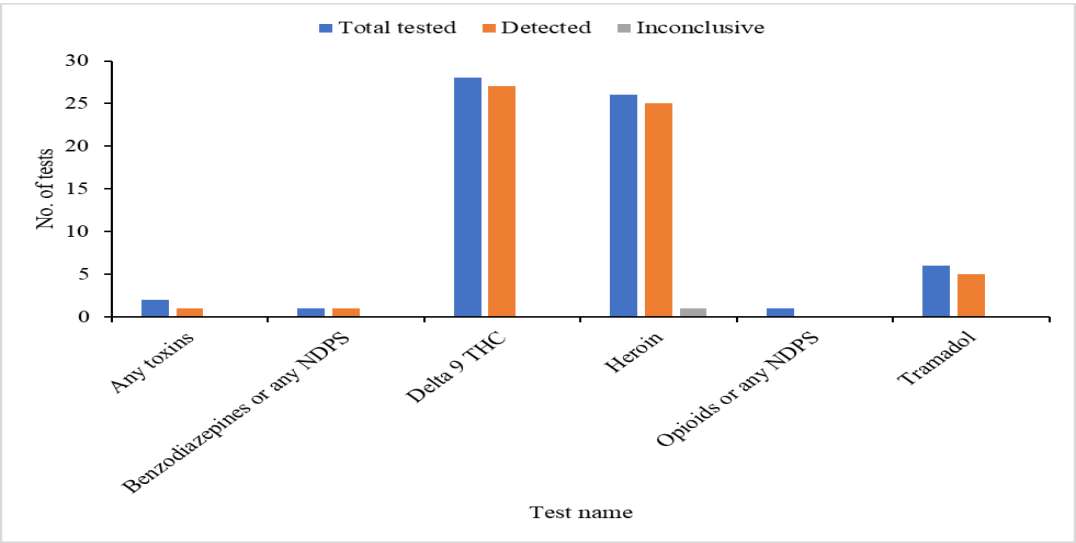


Figure 2. Types of tests and results

2.10. Blinded rechecking of malaria slides

A total of 151 health centers has participated in malaria blinded rechecking. The overall average reporting rate with 34.7% were reported on time, 40.33% were zero reported, 5.3% were reported late and rest 19.7% were not reported **(Figure 1).**

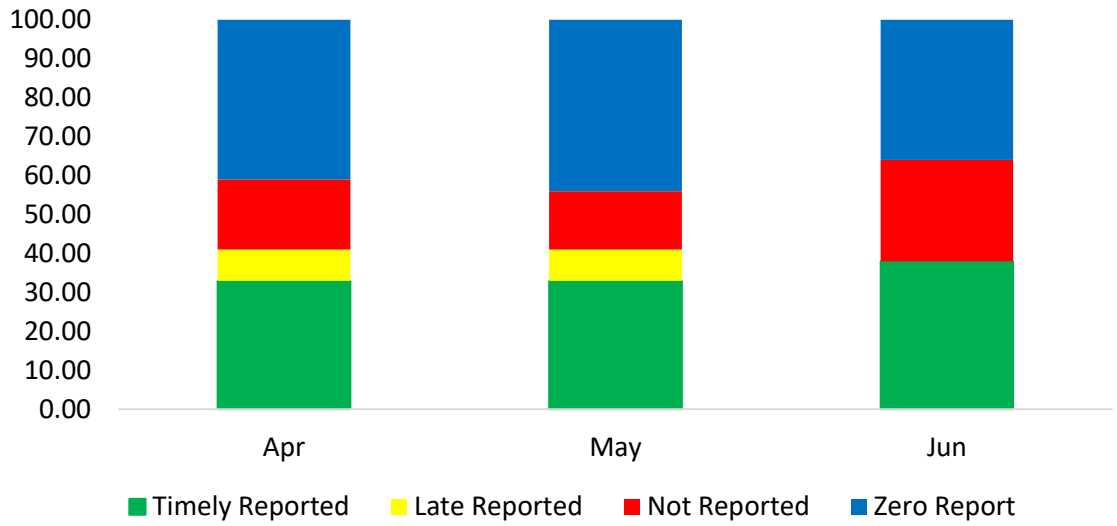


Figure 1: Monthly reporting status for 2nd quarter 2025

2.10.1 Blinded rechecking status.

A total of 833 malaria slides were received at the National Malaria Reference Laboratory for blinded rechecking. From the total slides examined, 1 malaria-positive slide is detected (0.12%)

All the slides 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 50.0%, parasite density determination was 0.00%, quality of blood film was 90.52% and quality of stain was 68.20% **(Table 1 and 2)**

Table 1: Report on Malaria Blinded rechecking for 2nd quarter 2024

Quarterly report on Malaria Blinded Rechecking 2025				
Month	**Apr**	**May**	**Jun**	**Total**
Health center participated in blinded rechecking	50	52	50	152
Total slides received for blinded rechecking	253	289	291	833
Total positive detected	0	1	0	1
Total Nmps detected	253	288	291	832
	Total slide Examine			1818

Table 2: Report on performance score for Blinded rechecking

Performance score on blinded rechecking				
Month	**Apr**	**May**	**Jun**	**Quarterly Score**
Sensitivity (True positive detection)		100		50
Specificity (True negative detection)	100	100	100	100
Malaria parasite detection	100	100	100	100
Mp Species Identification		100		50
Mp Stages Identification		50		25.00
Mp Parasite density		0		0.00
Stain Quality	70.94	64.45	69.21	68.20
Blood film Quality	88.16	91.73	91.68	90.52