



**ROYAL CENTERS FOR DISEASE CONTROL**

**QUARTERLY BULLETIN: 3<sup>RD</sup> QUARTER 2023**

**WEEK 27 TO 39**

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

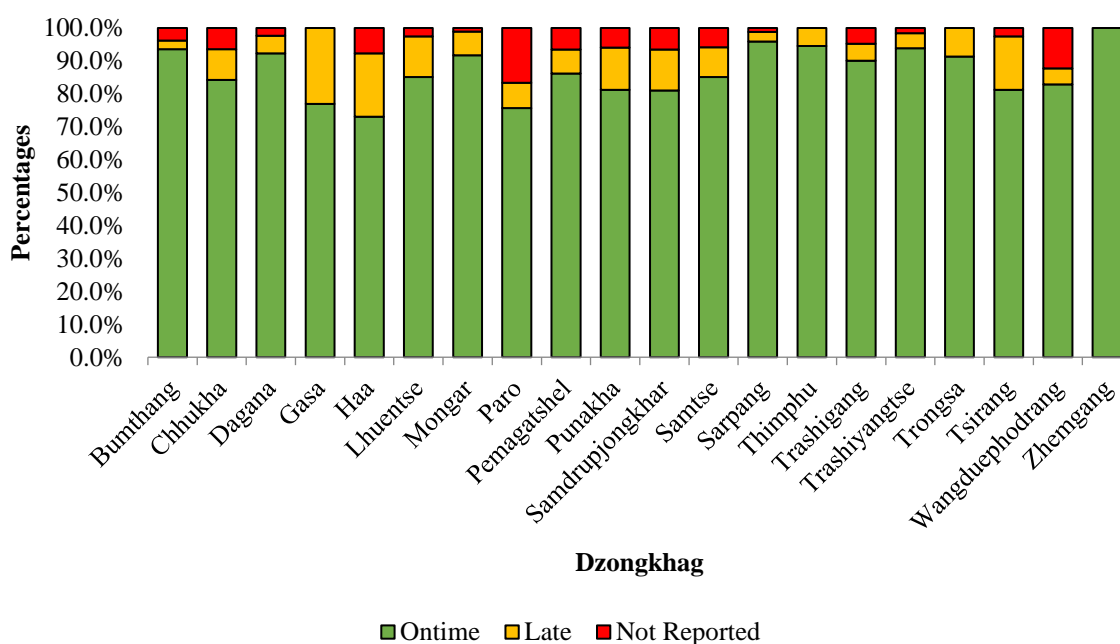
- Overall reporting rate for notifiable diseases had increased compared to the previous quarter
- A majority of the immediately notifiable diseases/syndromes reported were suspected measles/rubella cases
- Two patient samples tested positive for measles IgM. One of the two positive samples also tested positive for PCR. Three samples tested positive for rubella IgM.
- Twelve dengue positive cases, nine suspected bacterial meningitis, seven malaria positive for RDT, seven suspected pertussis cases, five malaria and one suspected rabies case and one AES were reported.

In the third quarter, 91 diseases events were reported, of which 57 were of outbreaks of conjunctivitis cases, 17 outbreaks of flu like illness, four events each of chickenpox and dengue, three events of rabid animal bite in humans, two events of scabies and one event each of food poisoning, HFMD & scrub typhus were reported

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

### 1.1 Reporting status of health centers under 20 Dzongkhags

In the third quarter, a total of 3445 weekly reports were expected from 265 health centers across the country from week 27 to 39. Compared to the second quarter of 2023, there was an overall improvement of 1.0% in total reports submitted. Overall, 95.6% of reports were received in the NEWARS information system of which 88.0% were reported on time, 7.6% 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 centers

Among 11 weekly reportable diseases/syndromes, the highest number of cases reported were respiratory illnesses (ARI & SARI). In total, 53014 (85.8%) cases of respiratory illnesses and 7460 (12.1%) diarrheal cases were reported (**Table 1**). The total cases reported were higher than that of second quarter.

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**Table 1:** Notifiable diseases/syndromes reported by Dzongkhags

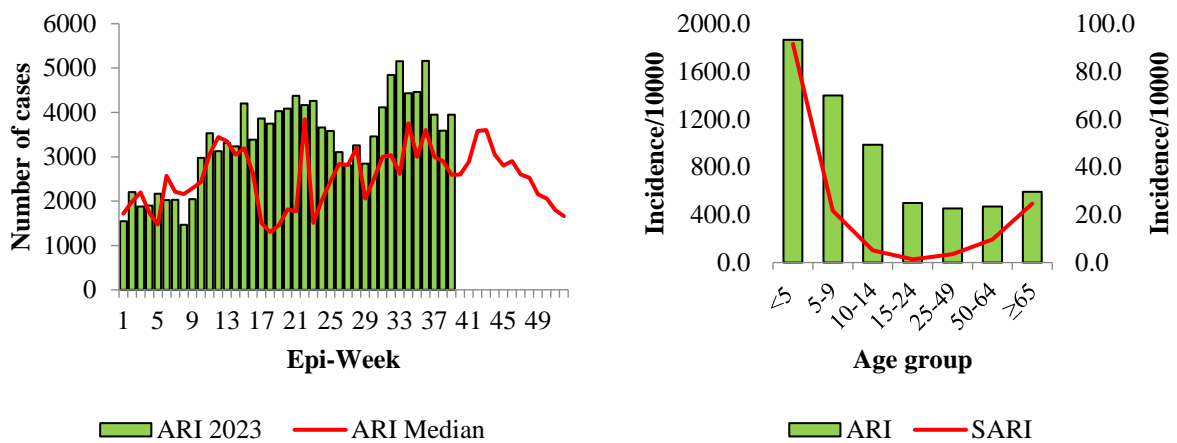
Dzongkhag	ABD	AWD	AJS	ARI	DGF	MUM	FWR	FDP	TPF	SAR	RKS
<b>Bumthang</b>	1	83	1	804	0	0	1	0	0	2	0
<b>Chhukha</b>	107	523	4	5817	73	6	123	19	32	93	32
<b>Dagana</b>	33	368	1	2974	88	4	4	5	4	41	39
<b>Gasa</b>	2	33	0	95	0	0	0	0	0	0	0
<b>Haa</b>	5	100	0	566	0	0	0	13	0	4	0
<b>Lhuentse</b>	10	80	0	1208	0	0	0	0	0	0	0
<b>Mongar</b>	40	353	3	3026	5	0	5	11	1	41	13
<b>Paro</b>	141	713	0	2441	0	0	2	0	0	0	0
<b>Pemagatshel</b>	16	260	0	2444	2	2	0	0	10	7	30
<b>Punakha</b>	54	293	9	1130	0	1	9	1	0	33	0
<b>Samdrupjongkhar</b>	31	379	12	4257	3	1	9	9	1	79	1
<b>Samtse</b>	39	557	4	4321	1	2	32	0	0	49	3
<b>Sarpang</b>	41	396	0	5453	26	2	35	1	2	207	35
<b>Thimphu</b>	92	1139	2	5645	71	79	16	1	0	184	0
<b>Trashigang</b>	22	356	23	2052	0	0	4	9	210	159	17
<b>Trashiyangtse</b>	25	184	0	2004	0	0	3	0	0	16	3
<b>Trongsa</b>	6	198	3	1599	0	2	14	1	0	12	1
<b>Tsirang</b>	16	127	6	1177	5	0	1	1	0	16	0
<b>Wangduephodrang</b>	77	421	6	3678	15	4	59	1	1	41	3
<b>Zhemgang</b>	48	91	0	1334	3	3	0	1	18	5	20
<b>Total</b>	<b>806</b>	<b>6654</b>	<b>74</b>	<b>52025</b>	<b>292</b>	<b>106</b>	<b>317</b>	<b>73</b>	<b>279</b>	<b>989</b>	<b>197</b>

**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), SAR (Severe Acute Respiratory Infection), RKS (Rickettsioses).

1.3 Descriptive analysis of most common notifiable diseases

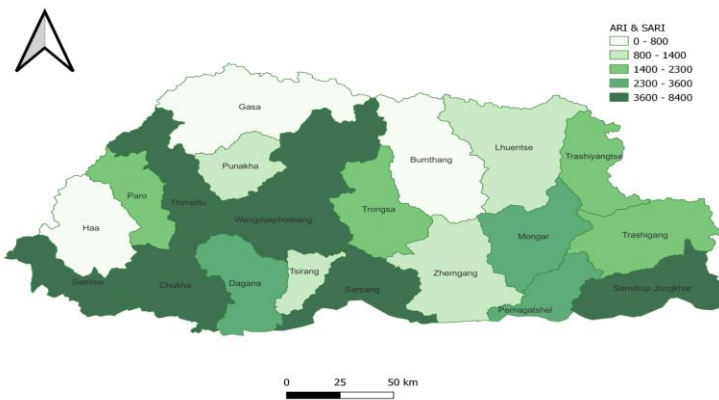
1.3.1 Respiratory Illness (ARI and SARI) syndrome

A total of 53014 cases of respiratory illness were reported, of which majority of cases were ARI (98.1%) 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, Chhukha reported the maximum number of ARI cases (**Figure 2C**).



A: Cases by Epi-week

B: Incidence by age group

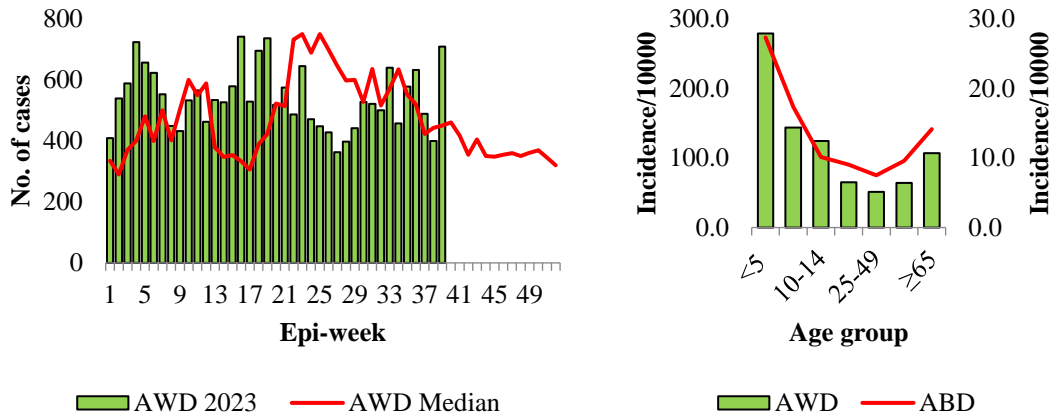


C: Respiratory illness cases by district

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

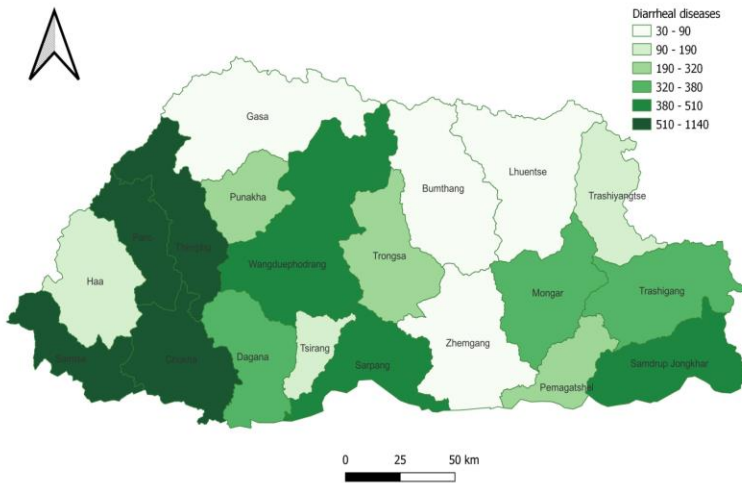
**1.3.2 Diarrheal syndrome: (Acute Watery Diarrhea [AWD] and Acute Bloody Diarrhea [ABD])**

Of the total 7460 diarrheal cases reported, 89.2.0% (n=6654) 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 district, Thimphu reported the maximum number of cases (Figure 3C).



A: Cases by Epi-week

B: Incidence by age group



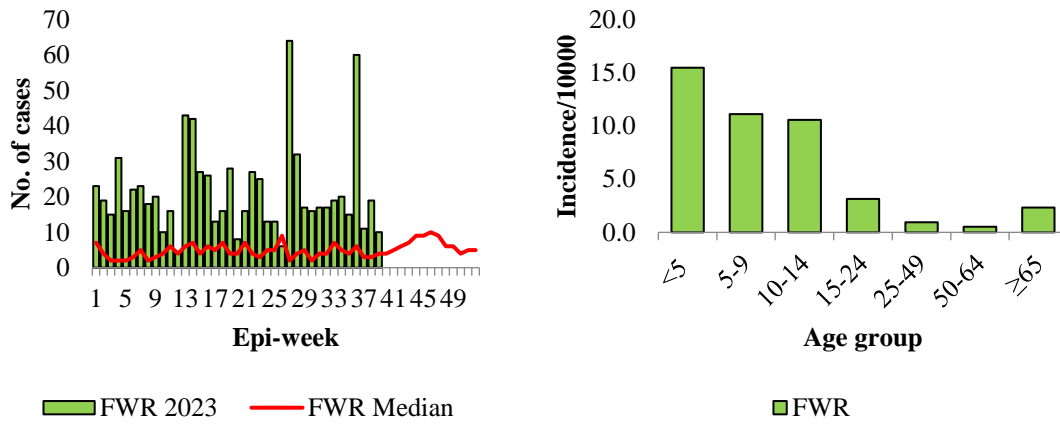
C: Diarrheal Cases by districts

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



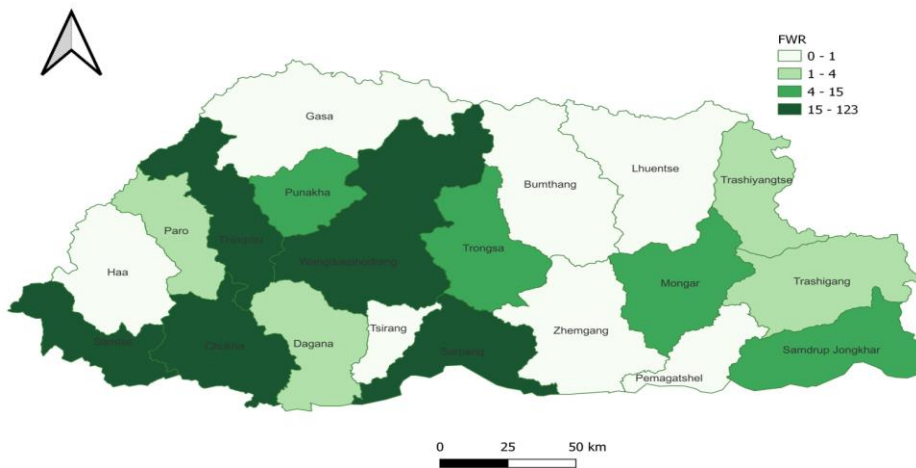
1.3.3 Fever with Rashes syndrome

A total of 317 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 Trashigang and Chhukha reported maximum number of the cases (**Figure 4C**).



A: Cases by Epi-week

B: Incidence by age group

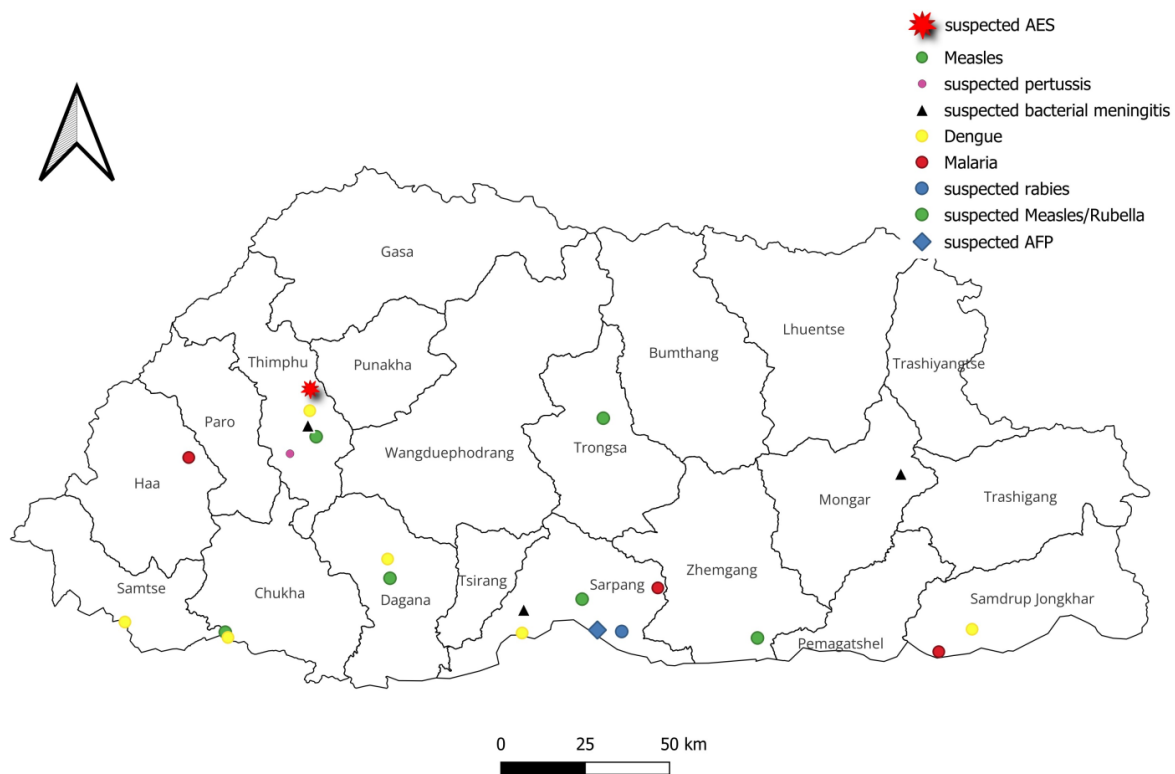


C: Fever with rash cases by districts

**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=21). Of the total, two samples were positive for measles IgM and one sample was positive for measles PCR. Three samples were positive for Rubella IgM. Additionally, 12 dengue positive cases, nine suspected bacterial meningitis, seven malaria positive for RDT, seven suspected pertussis cases, five malaria and one suspected rabies case and one AES were reported.

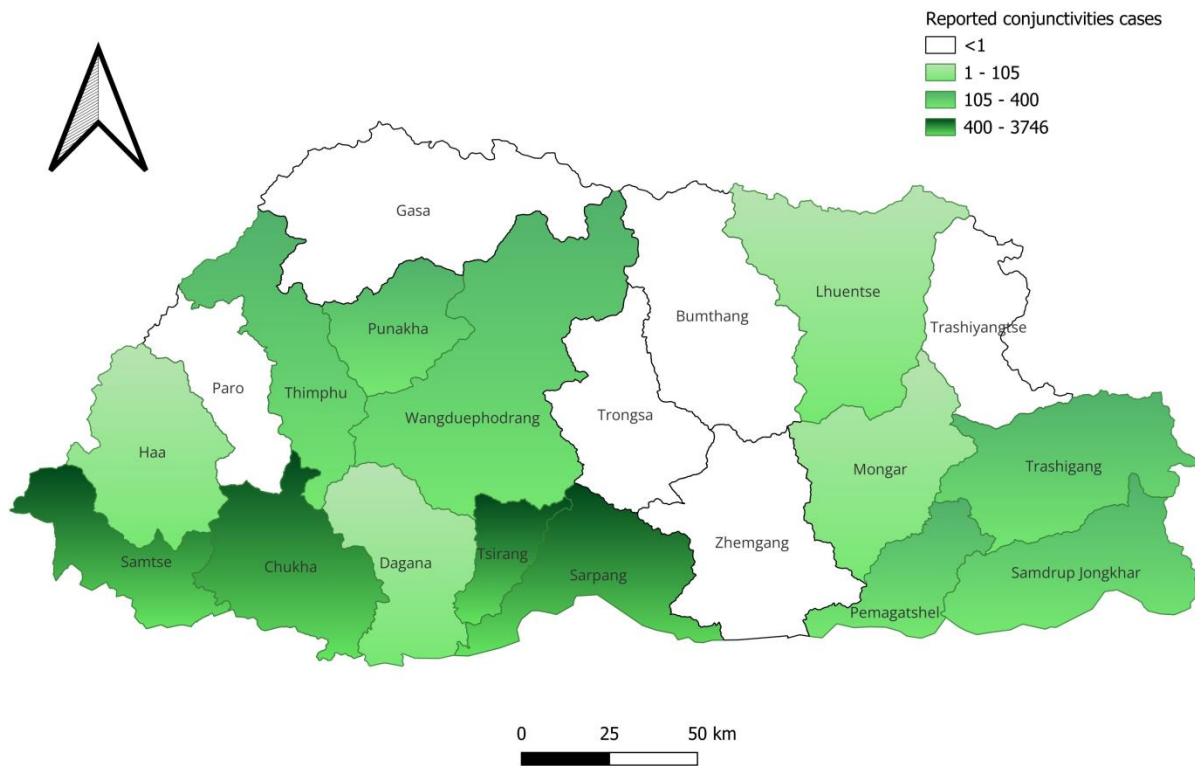


**Figure 5:** Distribution of immediately notifiable diseases/syndrome by dzongkhag

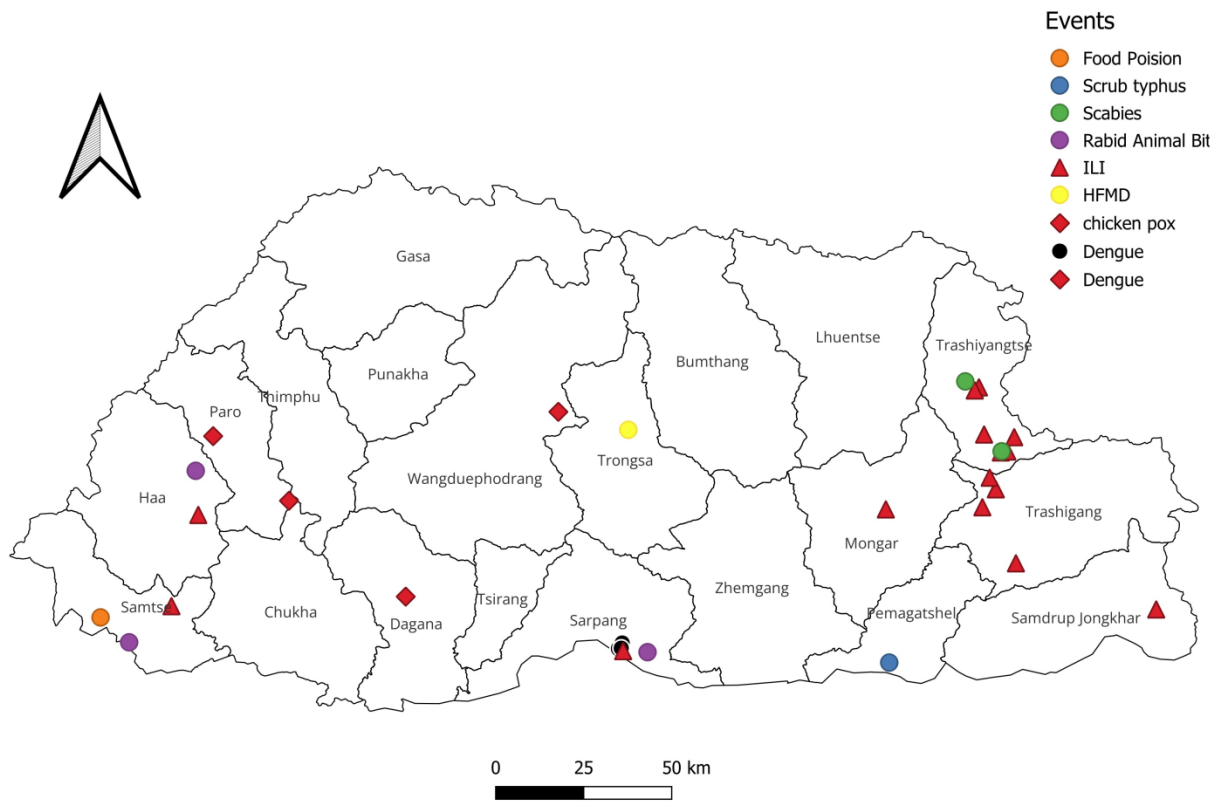
### 1.5 Event

In the third quarter, 91 diseases events were reported, of which 57 were of outbreaks of conjunctivitis cases (**Figure 6A**). In addition, 17 outbreaks of flu like illness, four events each of chickenpox and dengue, three events of rabid animal bite in humans, two events of scabies and

one event each of food poisoning, HFMD & scrub typhus were reported (**Figure 6B**). Eye and throat swab samples from the conjunctivitis outbreaks were sent to reference Lab in Thailand for testing and adenovirus, enterovirus, Rhinovirus, Bocavirus and influenza virus were detected in the samples. Similarly, ARI outbreak samples detected Flu A and B as well as COVID-19. 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 6A:** Distribution of conjunctivitis cases by Dzongkhag



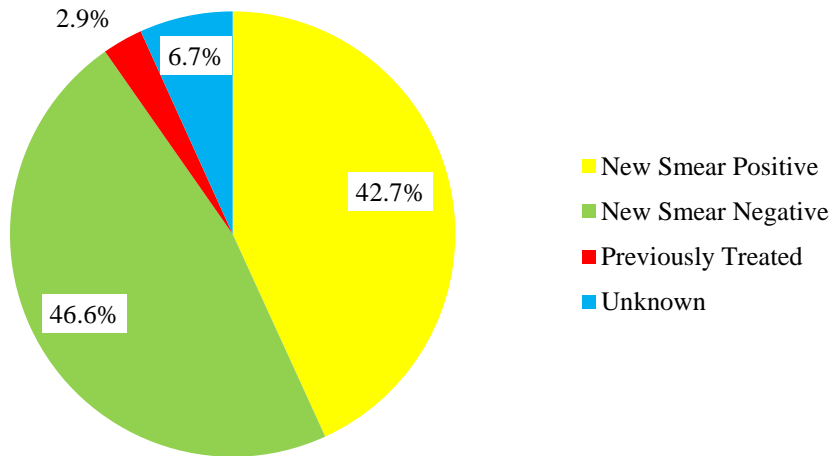
**Figure 6B:** Distribution of events by Dzongkhag (excluding conjunctivitis)

**2. Laboratory Based Surveillance**

**2.1 Drug Resistant Surveillance for Tuberculosis**

A total of 464 patient samples were received at National Tuberculosis Reference Laboratory (NTRL) for culture and drug susceptibility testing (DST) for anti-tuberculosis drugs. Of the total, 208(44.8%) were pulmonary samples, 27(5.8%) were extra-pulmonary samples and 106(22.8%) were pulmonary samples received for TB screening for VISA. In addition, 123(26.5%) follow-up samples were received for culture for MDR-TB patients under treatment.

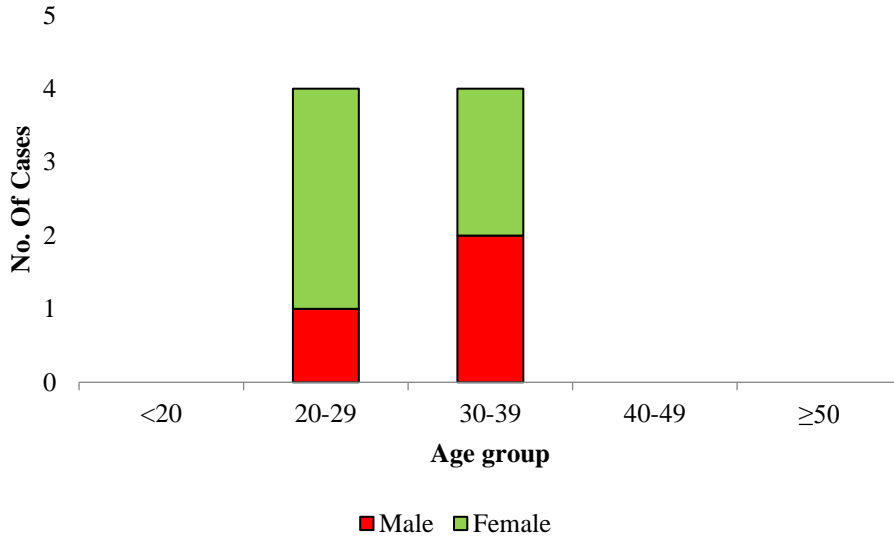
Among the pulmonary samples, new smear positive (NSP) constituted 42.7% (n=89) of the total samples, and 2.9% (n=6) were previously treated cases (**Figure 7**).



**Figure 7:** Classification of Pulmonary TB samples

**2.1.1 Drug Sensitivity Test**

Drug sensitivity test report was available for 90 samples using Line Probe Assay and 39 samples had reports for Liquid DST for first line drugs. A total of eight multi-drug resistant tuberculosis (MDR-TB) cases were detected among patients with complete DST report. All the eight MDR-TB cases were new smear positive samples. All MDR-TB cases were in the age group of 20-39 years (**Figure 8**). Of the eight MDR-TB samples, second line DST was available for six cases. Five samples were sensitive to both fluoroquinolones and aminoglycoside. One sample was resistant to fluoroquinolone.

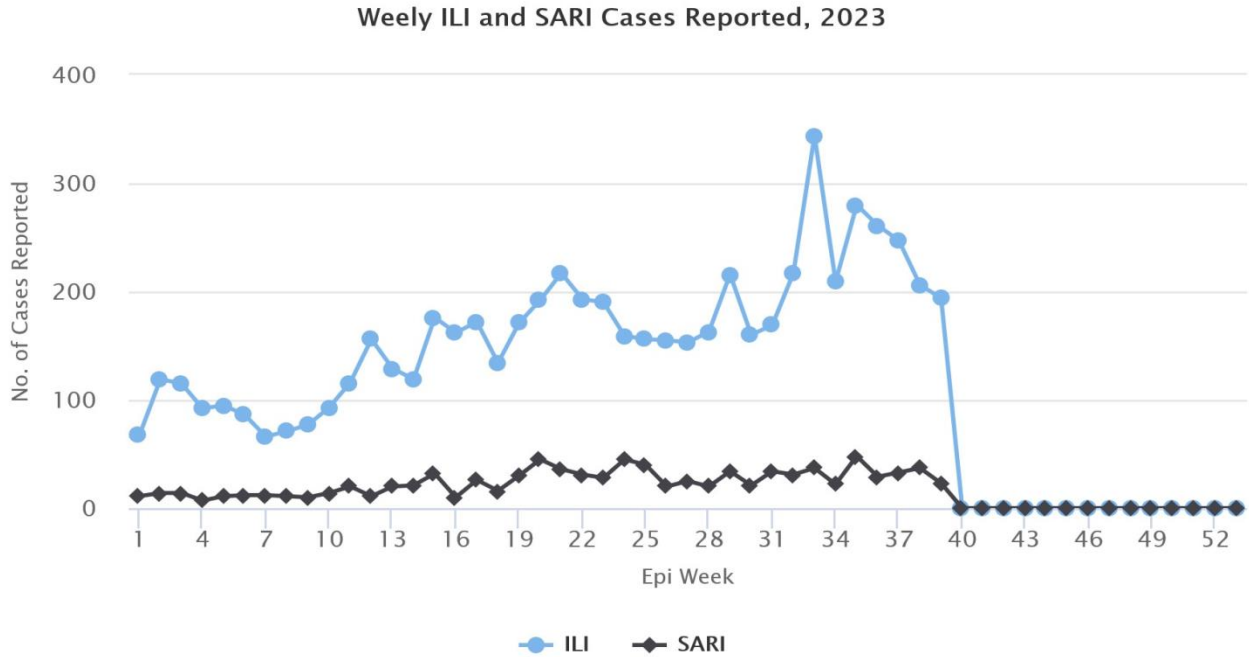


**Figure 8:** Distribution of MDR-TB cases by age group and gender

## 2.2 COVID-19 Integrated Influenza surveillance

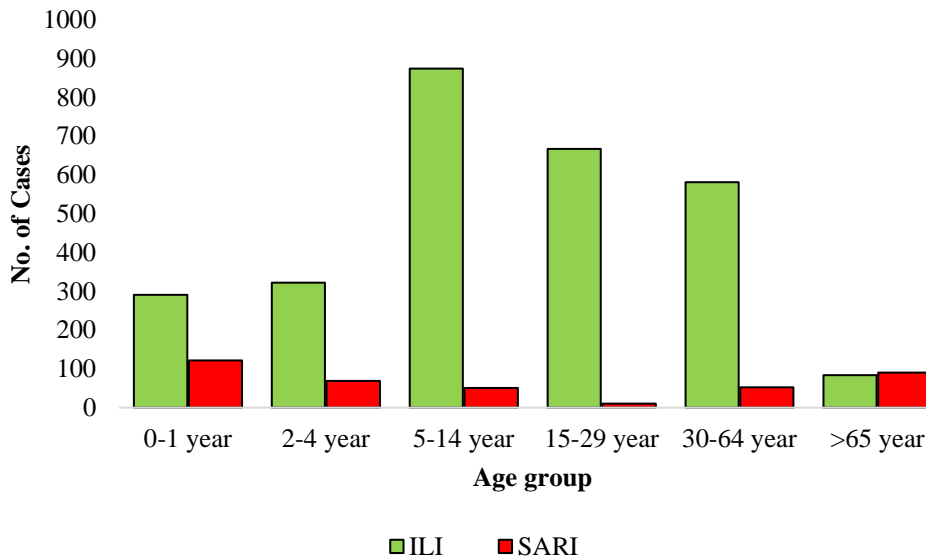
### 2.2.1 Epidemiological Surveillance

Influenza-like illness (ILI) and severe acute respiratory infection (SARI) activity increased during the third quarter (Jul – Sep 2023). A total of 2819 ILI cases and 394 SARI cases were reported by respective sentinel hospitals (**Figure 9**). The ILI positivity rate was 2.3% compared to the 2<sup>nd</sup> quarter (2.0%), while the SARI positivity rate was 8.4% compared to the previous quarter (8.3%). The positivity rate increased by 0.3% for ILI and 0.1% for SARI respectively.



**Figure 9:** Weekly ILI and SARI cases reported from Sentinel sites

The most affected age group for ILI was 5 – 14 years (31.0 %) followed by 15 – 29 years (23.7 %), while for SARI the most affected age group was from 0 – 1 years (31.0 %) followed by 2 – 4 years (18.0 %) (**Figure 10**).



**Figure 10:** ILI and SARI cases by age group

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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=1,158) and Samdrup Jongkhar Hospital (n=691) has reported the highest ILI cases, while, Paro Hospital (n=88) and Gelephu (n=83) reported the highest SARI cases during the third quarter of 2023 (**Table 2 & 3**).

**Table 2:** Weekly ILI cases reporting Status from week 27 – 39, 2023 (Source RCDC)

Sentinel Hospitals	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32	Wk 33	Wk 34	Wk 35	Wk 36	Wk 37	Wk 38	Wk 39	Grand Total
Paro Hospital	71	70	91	60	66	77	153	89	101	83	113	93	91	1158
Punakha Hospital	9	5	3	0	3	9	12	9	41	30	16	4	16	157
Samdrup Jongkhar Hospital	30	27	57	41	49	65	89	46	85	65	36	48	53	691
Samtse Hospital	14	13	18	16	19	22	26	29	10	25	18	15	10	235
Trashigang Hospital	13	13	14	15	9	8	16	11	10	14	27	10	6	166
Trongsa Hospital	10	22	13	14	17	20	39	17	27	33	27	36	15	290
Tsirang Hospital	6	13	20	14	6	16	9	8	6	11	10	0	3	122
<b>Total</b>	<b>153</b>	<b>163</b>	<b>216</b>	<b>160</b>	<b>169</b>	<b>217</b>	<b>344</b>	<b>209</b>	<b>280</b>	<b>261</b>	<b>247</b>	<b>206</b>	<b>194</b>	<b>2819</b>

**Table 3:** Weekly SARI cases reporting Status from week 27 – 39, 2023 (Source RCDC)

Sentinel Hospitals	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32	Wk 33	Wk 34	Wk 35	Wk 36	Wk 37	Wk 38	Wk 39	Total
Gelephu CRRH	8	5	11	12	3	5	9	3	6	6	7	7	1	83
JDWNRH	1	1	5	1	0	6	3	2	8	1	2	4	1	35
Monggar ERRH	0	0	0	0	1	0	0	0	0	0	2	1	1	5
Paro Hospital	4	4	6	4	5	9	11	8	7	4	6	7	13	88
Phuentsholing Hospital	5	6	8	1	6	5	6	6	7	6	5	10	4	75
Punakha Hospital	5	2	1	2	7	4	3	2	5	7	7	2	0	47
Samdrup Jongkhar Hospital	2	0	2	1	2	0	1	0	3	3	0	2	0	16
Samtse Hospital	0	0	0	0	0	1	0	0	2	0	0	0	0	3
Trashigang Hospital	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Trongsa Hospital	0	0	0	0	0	0	2	0	3	0	1	0	0	6
Tsirang Hospital	0	2	1	0	10	0	2	1	7	2	3	4	3	35
<b>Total</b>	<b>25</b>	<b>20</b>	<b>34</b>	<b>21</b>	<b>34</b>	<b>31</b>	<b>37</b>	<b>22</b>	<b>48</b>	<b>29</b>	<b>33</b>	<b>37</b>	<b>23</b>	<b>394</b>

Note: NR- Note Reported



2.2.2 Virological Surveillance

2.2.2.1. Laboratory-Confirmed Influenza and COVID-19 Surveillance

Influenza positivity increased by 8.8% during the third quarter of 2023 (epi week 27 – 39) compared to the previous quarter. The overall Influenza positivity rate was 29.3%, more than the previous quarter (20.7 %). A total of 1,276 flu specimens (ILI- 951, SARI- 325) were received and tested for Influenza and SARS-CoV-2 through multiplex RT-PCR (Flu SC2) and detected 29.3% (21/1276) Influenza positives, 8.0% SARS-CoV-2 positives, and detected one co-infection (0.3%). Influenza subtype A(H3N2) (45.7%) was the most predominating circulating strain, followed by Flu B/Victoria (19.5%) (**Figure 11 & Table 4**).

The mean age for Influenza positive was 22.9 (0.08 – 80) years while the mean age for COVID-19 was 32 (0.2 – 90) years. The most affected age group for Influenza was 30 – 64 years (31.3%), followed by 5 – 14 years (29.1%) and 15 – 29 years (24.9%). While the most affected age group for COVID-19 was 30 – 64 years (52.9%) followed by 5 – 14 years (16.7%). Almost all the Influenza positive cases had cough (95.7%) and fever (92.8%), followed by sore throat (64.7%) and headache (52.1%) (**Table 4**).

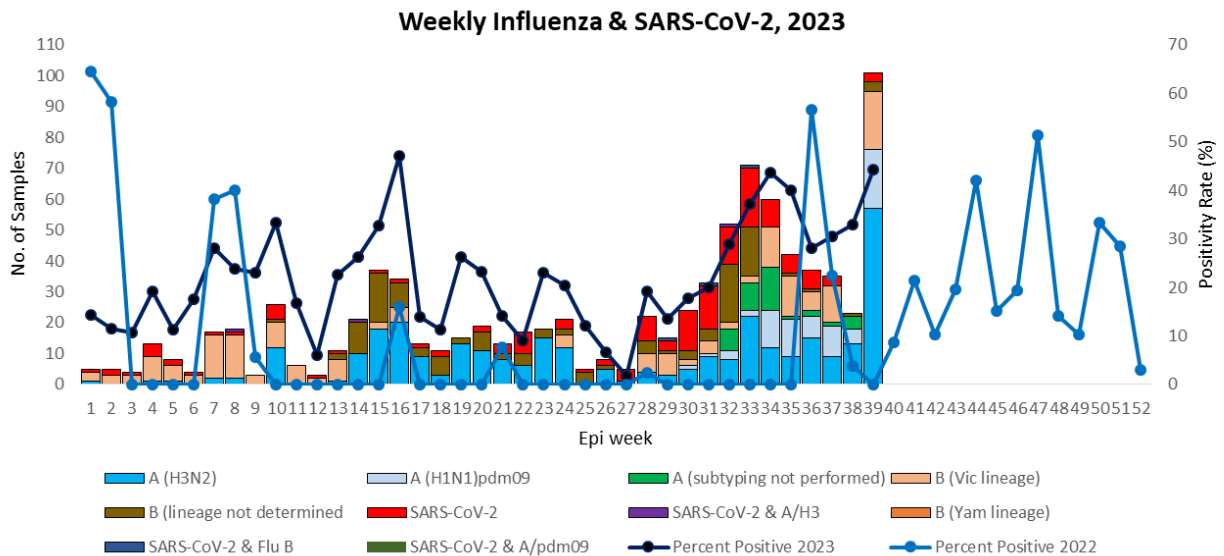


Figure 11: Weekly Influenza subtypes and SARS-CoV-2 (Source: RCDC)

**Table 4:** Patient demographic and clinical characteristics

Variables	All ILI n(%)	All SARI n(%)	Influenza n(%)	SARS-CoV-2 n(%)
<b>All Specimens</b>	951 (74.5)	325 (25.5)	374 (29.3)	102 (8.0)
<b>Mean Age (range) years</b>	23.3 (08 – 85)	20.5 (0.08 – 94)	22.9 (0.08 – 80)	32 (0.2 – 90)
<b>Median Age (IQR) years</b>	20 (9 – 35)	3 (1 – 37)	20 (8 – 36)	34 (12 – 45)
<b>Age Group</b>				
<b>0 – 1 year</b>	31 (3.3)	124 (38.2)	19 (5.1)	8 (7.8)
<b>2 – 4 year</b>	66 (6.9)	57 (17.5)	27 (7.2)	2 (1.9)
<b>5 – 14 year</b>	282 (29.6)	40 (12.3)	109 (29.1)	17 (16.7)
<b>15 – 29 year</b>	266 (27.9)	11 (3.4)	93 (24.9)	16 (15.7)
<b>30 – 64 year</b>	284 (29.9)	41 (12.6)	117 (31.3)	54 (52.9)
<b>&gt;65 year</b>	22 (2.3)	52 (16.0)	9 (2.4)	5 (4.9)
<b>Sex</b>				
<b>Female</b>	405 (42.6)	156 (48.0)	176 (47.1)	60 (58.8)
<b>Male</b>	546 (57.4)	169 (52.0)	198 (52.9)	42 (41.2)
<b>Clinical Symptoms</b>				
<b>Fever</b>	868 (91.3)	294 (90.7)	347 (92.8)	96 (94.1)
<b>Cough</b>	916 (96.3)	299 (92.0)	358 (95.7)	101 (99.0)
<b>Headache</b>	497 (52.3)	23 (10.8)	195 (52.1)	54 (52.9)
<b>Sore throat</b>	668 (70.2)	135 (41.5)	242 (64.7)	74 (72.6)
<b>Breathing problem</b>	100 (10.5)	209 (64.3)	55 (14.7)	18 (17.7)
<b>Flu A/H3</b>			171 (45.7)	
<b>Flu A/Pdm09</b>			73 (19.5)	
<b>Flu A/Not Subtyped</b>			6 (1.6)	
<b>Flu B/Victoria</b>			73 (19.5)	
<b>Flu B/Lineage Not Determined</b>			50 (13.4)	
<b>Flu A/Pdm09 &amp;Flu B/Victoria</b>			1 (0.3)	

A total of 1276 samples were received from the Sentinel and non-sentinel sites. RCDC recommends 10 – 15 ILI samples per week from each sentinel site. While all the SARI cases are recommended to collect samples. Samtse Hospital (n=254) and Trongsa Hospital (n=173) followed by Phuentsholing (n=160) and Trashigang Hospital (n=122) have collected more samples compared to the rest of the sentinel hospitals (Figure 12 & Table 5).

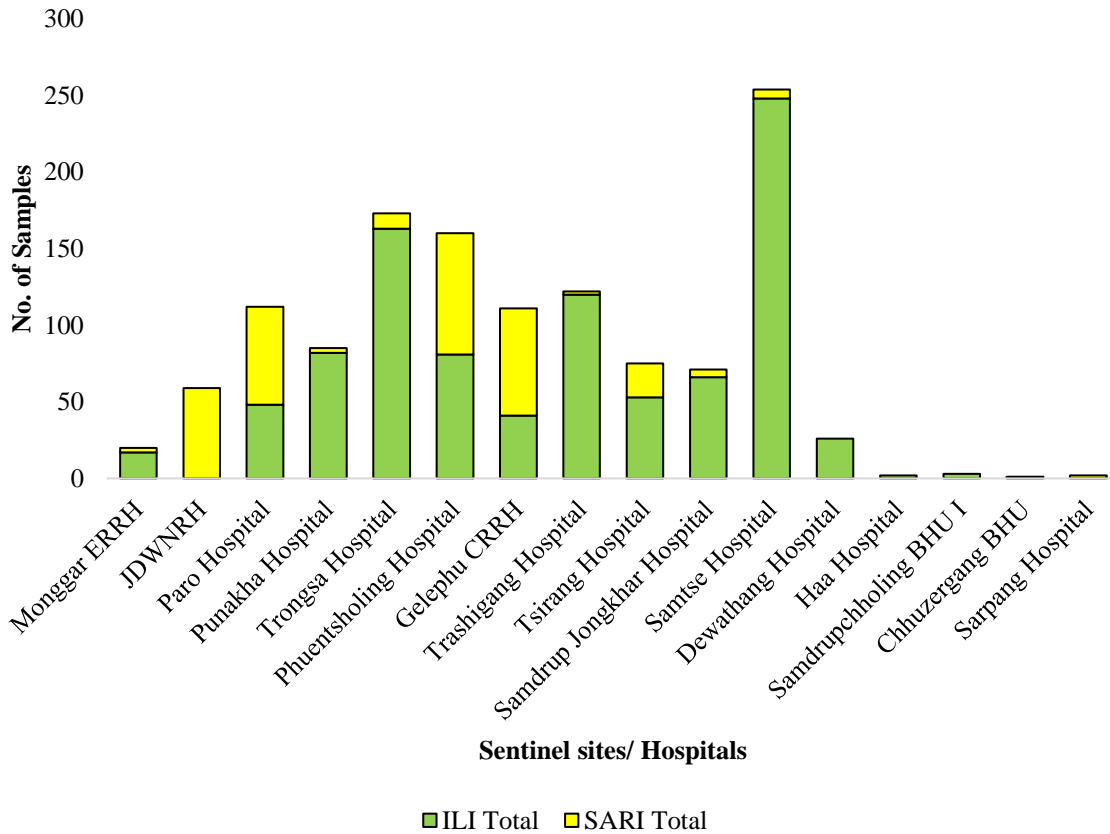


Figure 12: Number of ILI, SARI and Flu Outbreak Specimens received in 2<sup>nd</sup>quarter 2023

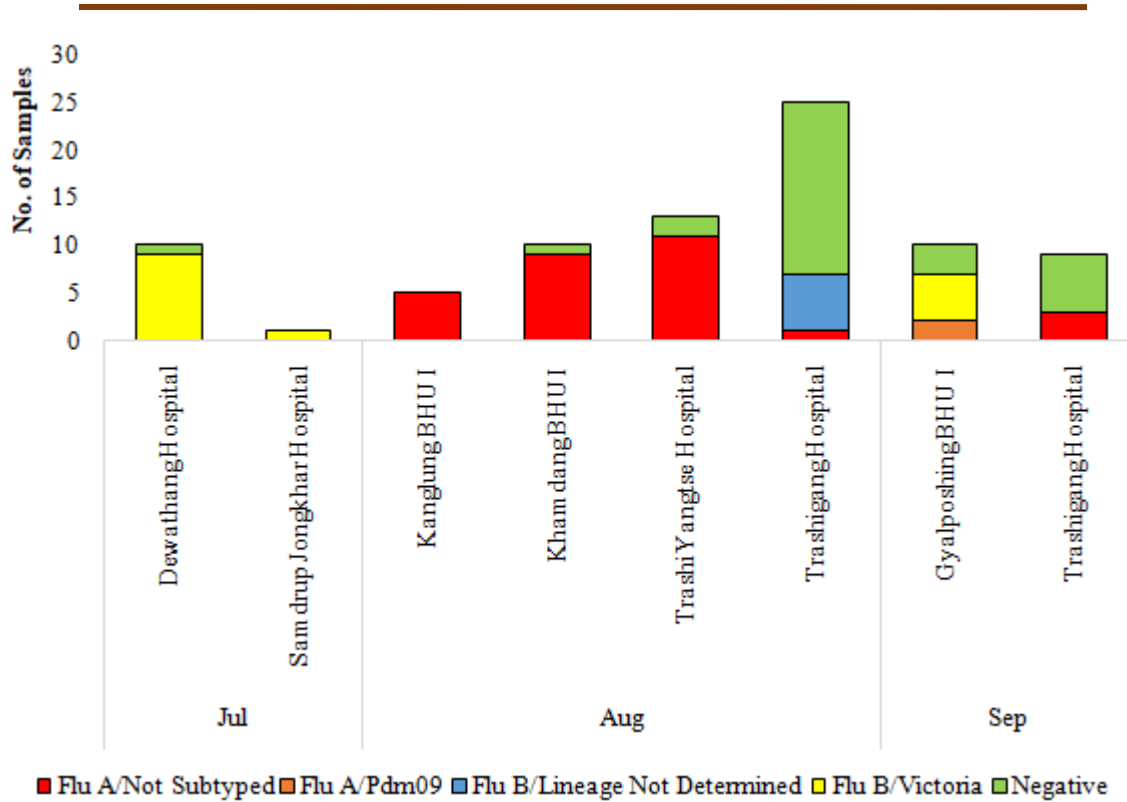
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**Table 5:** Summary table for Influenza subtypes and sample tested in quarter, 2023

Sentinel sites/ Hospitals	ILI									SARI							Grand Total	
	A/ H3	A/N ot Sub type d	A/ Pd m09	A/ Pd m09 & B/ Vic	B/li nea ge not det er mi ne d	B/ Vi c	S A R S- C o V- 2	Neg	ILI Total	A/ H3	A/ N ot S u bt yp ed	A/ Pd m 09	B/li nea ge not deter mi ned	B/ Vic	S A R S- C o V- 2	Neg		SA RI Tota l
Monggar ERRH	1	2			5			9	17						3	3	20	
JDWNRH										1		1	1		1	56	59	
Paro Hospital	15		7					26	48	4		1			5	59	64	
Punakha Hospital	11	1	2		2	6	10	60	82						1	3	3	
Trongsa Hospital	22				3	2	17	136	163			1				9	10	
Phuentsholing Hospital	23		5	1		9	4	43	81	11		4		3	3	61	79	
Gelephu CRRH	8	2	1		3	3	4	24	41	2	1	3	3	1	2	60	70	
Trashigang Hospital	25				6		13	89	120							2	2	
Tsirang Hospital	2		3		3		13	45	53	1			1		2	20	22	
Samdrup Jongkhar Hospital	4		6			5		51	66				1			4	5	
Samtse Hospital	27		38		19	43	25	121	248			1				5	6	
Dewathang Hospital	14				3	1		8	26									
Haa Hospital								2	2									
Samdrupchhol ing BHU I							1	3	3									
Chhuzergang BHU							1	1	1									
Sarpang Hospital																2	2	
<b>Grand Total</b>	<b>152</b>	<b>5</b>	<b>62</b>	<b>1</b>	<b>44</b>	<b>69</b>	<b>88</b>	<b>618</b>	<b>951</b>	<b>19</b>	<b>1</b>	<b>11</b>	<b>6</b>	<b>4</b>	<b>14</b>	<b>284</b>	<b>325</b>	<b>1276</b>

**2.2.3 ARI Outbreak during 3rd quarter, 2023**

A total of 83 acute respiratory infections (ARI) outbreak samples were received at least from seven different health centers during the 3<sup>rd</sup> quarter, of 2023, which detected 62.7% (52/83) Influenza positive. Influenza A subtype was the most predominant flu virus for the outbreak. More outbreak samples were received in August month (n=53) than in July and September month (**Figure 13**).



**Figure 13:** Influenza positivity among ARI outbreak samples in 3<sup>rd</sup> quarter of 2023

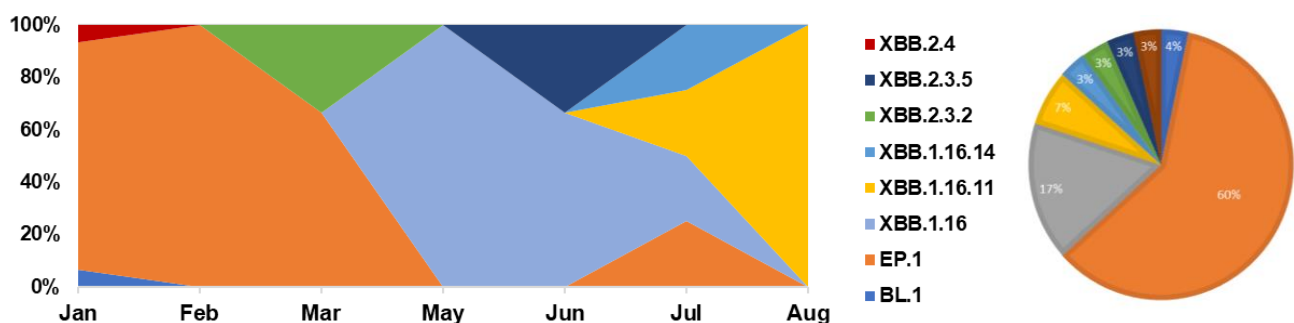
### 2.2.2.3. SARS-CoV-2 Genomic Sequences

The Royal Centre for Disease Control (RCDC) conducts Whole Genome Sequencing (WGS) on a subset of samples collected from COVID-19 Integrated Influenza Surveillance sites as part of COVID-19 Genomic Surveillance. This initiative aims to monitor the variants of the SARS-CoV-2 virus circulating in the country.

Between January 2023 and August 2023, a total of 30 samples were subjected to WGS and subsequently uploaded to the Global Initiative on Sharing All Influenza Data (GISAID). Through this analysis, eight distinct pangolin lineages were identified: BL.1, EP.1, XBB.1.16 (Variants under Monitoring), XBB.1.16.11, XBB.1.16.14, XBB.2.3.2, XBB.2.3.5, and XBB.2.4 (Variants of Interest). The EP.1 lineage predominated in January 2023, but was gradually substituted by the XBB.1.16 lineages. In this quarter, five samples were subjected to Whole genomic sequencing as part of COVID-19 Genomic surveillance. XBB.1.16 lineages were predominant in this quarter which aligns with global trend (**Table 6 & Figure 14**).

**Table 6:** SARS-CoV-2 lineages detected by Month in 2023

Variants	Jan	Feb	Mar	Apr	May	June	July	Aug	Total
BL.1	1								1
EP.1	13	2	2				1		18
XBB.1.16					2	2	1		5
XBB.1.16.11							1	1	2
XBB.1.16.14							1		1
XBB.2.3.2			1						1
XBB.2.3.5						1			1
XBB.2.4	1								1
Total Sequence	15	2	3		2	3	4	1	30



**Figure 14:** Lineage relative frequencies of SARS-CoV-2 in Bhutan in 2023

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

During the third quarter of the year, a total of 184 MR samples were received. Out of the total, two measles IgM Positive were detected from Trongsa and Paro.

The PCR positive sample has been sent to Regional Referral Laboratory (RRL) Thailand for further confirmation and genotyping sequencing. All four samples received for Japanese Encephalitis was negative. All ten Pertussis samples were negative (nine from JDWNRH and one from Monggar) (**Table 7**).

**Table 7:** Number of samples received from health center’s for MR and JE surveillances

Surveillance	Site/ Hospital	Number of samples received
<b>MR</b>	Chhukha	22
	Dagana	1
	Paro	76
	Pemagatshel	1
	Punakha	1
	Samdrup Jongkhar	15
	Samtse	1
	Sarpang	3
	Thimphu	29
	Trashigang	1
	Trongsa	34
<b>AES (JE)</b>	JDWNRH	03
	Gelephu	01
<b>Pertussis</b>	JDWNRH	09
	Monggar	01

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

We received five samples for AUFI surveillance, forty nine samples for dengue and three samples for scrub typhus confirmation from 58 sentinel sites. Samples received for the purpose of surveillance, diagnosis and confirmation of independent tests are reflected below.

**Table 8:** Samples received and tested for AUFI, dengue and scrub typhus surveillances

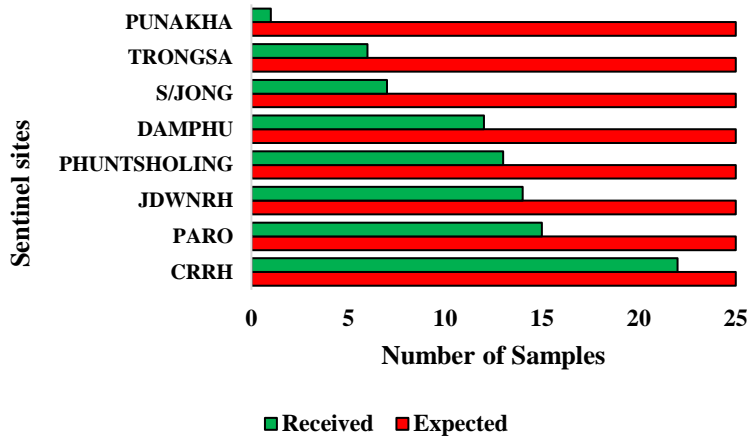
Surveillance /Test requested	Site/ Hospital	No. of samples received and tested	Positive test result							
			DE NV NS1	DE NV IgM	Scrub typhus IgM	Leptospira IgM	JE IgM	CHIKV IgM	Brucella IgM	
<b>AUFI</b>	Punakha	03	00	01	00	00		N T	NT	00
	Trongsa	02	00	00	00	00		N T	NT	00
<b>Dengue Confirmation</b>	Sarpang	04	00	00	NA	NA		N A	NA	NA
	Gedu	02	00	00	NA	NA		N A	NA	NA
	Tsirang	01	00	00	NA	NA		N A	NA	NA
	Gidakom	02	00	00	NA	NA		N A	NA	NA
	Pling	19	07	00	NA	NA		N A	NA	NA
	Panbang	03	00	00						
	Gelephu	09	06	00						
	SamdrupCholing	03	00	00						
	SamdrupJonkhar	05	03	00						
	<b>Scrub Typhus</b>	Nganglam	03	NA	NA	00	NA		N A	NA

DENV: Dengue virus, CHIKV: Chikungunya virus, NA: Not applicable, NT: Not Tested

### 2.5. Sentinel Surveillance for Diarrheal Etiologic Agents

This quarter, 90 samples were received from eight sentinel sites (**Figure 15**). Most of the samples received were from the CRRH. Of these, 51 (57.0%) were females and 39 (43.0%) were males.

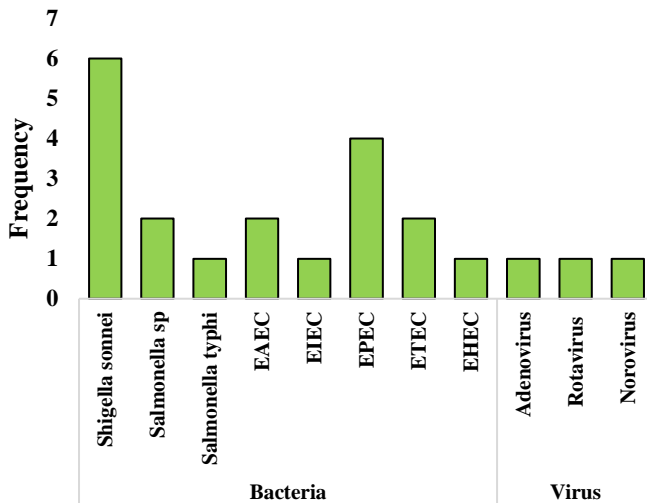




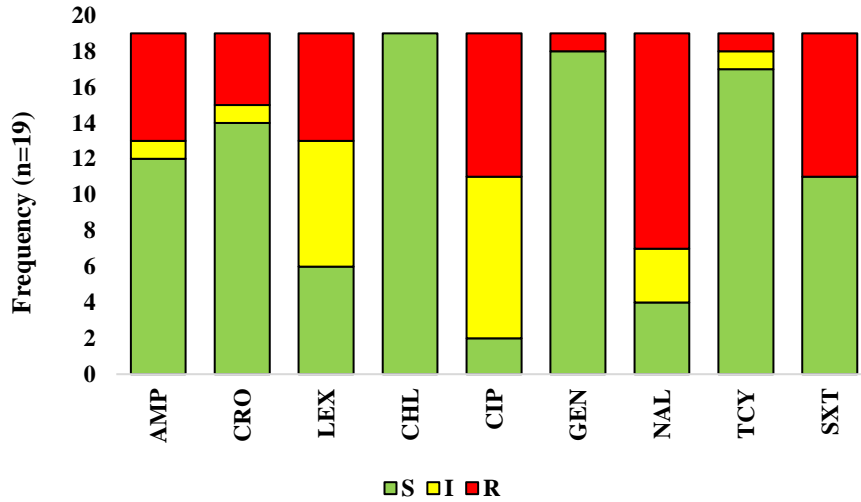
**Figure 15:** Number of fecal specimens collected from sentinel sites

The sample’s character consists of loose (50.0%), watery (43.0%) and (7.0%) bloody. The mean age was 25 years and the mean of diarrheal duration is 44 hours for this quarter. Of the total, 20.0% required hospital admission while the rest were treated in the OPD. Of all, four diarrheal cases were found linked to having consumed a suspected food.

The most detected enteropathogens were *Shigella sonnei* (n=6), and Diarrheagenic *E.coli*(n=10) (Figure 16). The antimicrobial-resistant pattern for the isolated bacterial pathogens is provided in (Figure 17).



**Figure 16:** Proportion of pathogens isolated

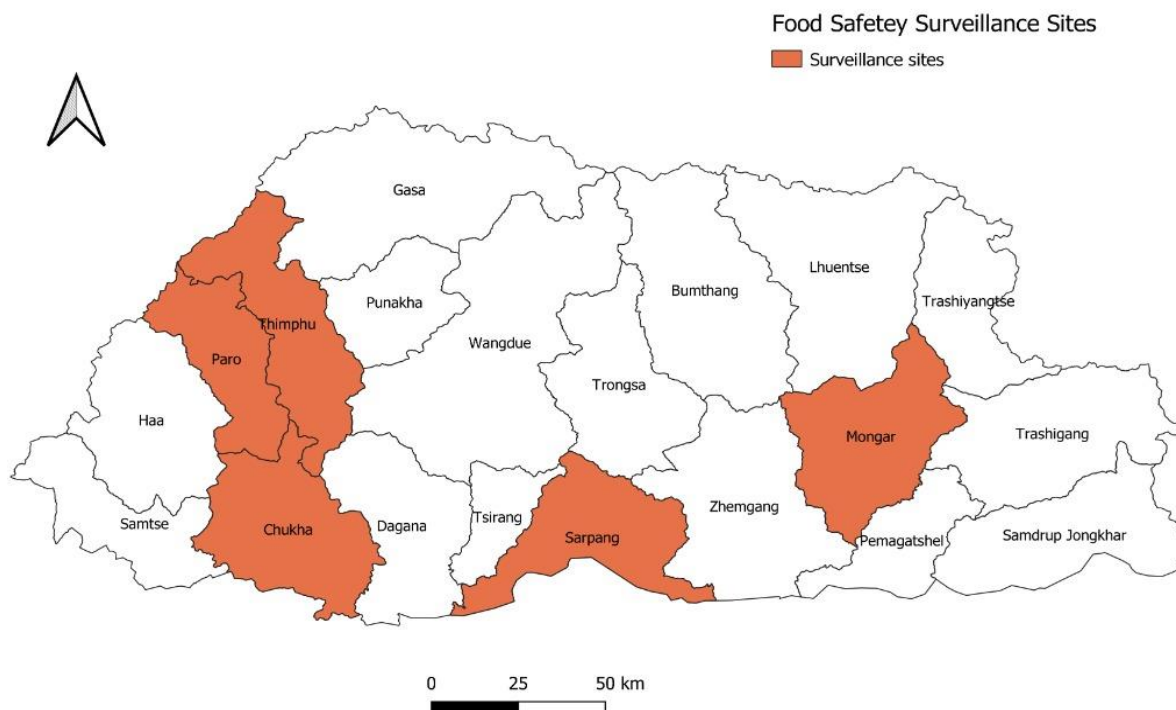


AMX (Amoxicillin), CZO (Cephazolin), CRO (Ceftriaxone), LEX (Cephalaxin), CHL (Chloramphenical), CIP (Ciprofloxacin), GEN (Gentamycin), NAL Nalidic Acid, TCY (Tetracycline), SXT (Trimethoprim and sulfamethaxazole), EAEC (Enteroaggregative *E-coli*) EPEC (Enteropathogenic *E-coli*)

**Figure 17:** Antibiotic susceptibility pattern for bacterial pathogens

### 2.6 Food safety surveillance

It is accepted internationally that food safety surveillance systems have a dual purpose; the first is to detect, control and prevent foodborne disease outbreaks. During the 3<sup>rd</sup> quarter of 2023, from the food safety surveillance conducted by Food and Nutrition Laboratory, a total of 59 ready to eat food samples were received from the surveillance sites (**Figure 18**). Of the five sites, Thimphu dzongkhag collected 20 samples, followed by Sarpang with 16 samples, 13 samples from Chhukha and 10 samples each from Paro and Mongger for the third quarter 2023.



**Figure 18:** Food safety surveillance sites

The food samples were tested for physical parameters, such as pH, weight of the samples and sample type (solid/liquid or semi-solid), indicator organisms (total aerobic plate count, yeast and mold count, total *Enterobacteriaceae*, total coliform count and total listeria count) and pathogenic organisms (*Staphylococcus aureus*, *Bacillus cereus*, *Salmonella*, *Shigella* and *Campylobacter*).

Of the 59 samples tested 15.3% (n=9) of the samples had indicator test organism violation (Total plate count of  $>10 \log 5$  and *E.coli* of  $>10 \log 2$ ). The total plate count and *E.coli* counts are the two commonly used as indicator test for the foods. It also serves as the surrogacy for hygiene indicator in food safety. The common pathogenic organism isolated was *Staphylococcus aureus* and *Bacillus cereus*. *Staphylococcus aureus* are commonly found as normal flora in human skin and respiratory tract. Moreover, *S. aureus* can contaminate the food after cooking due to unhygienic practices. Also the toxins, which are heat and acid stable, can cause foodborne intoxication. During this quarter five food samples were contaminated with *S. aureus* and 8.5 % with *B. cereus*, respectively. During the sample period three events of food-borne outbreaks were

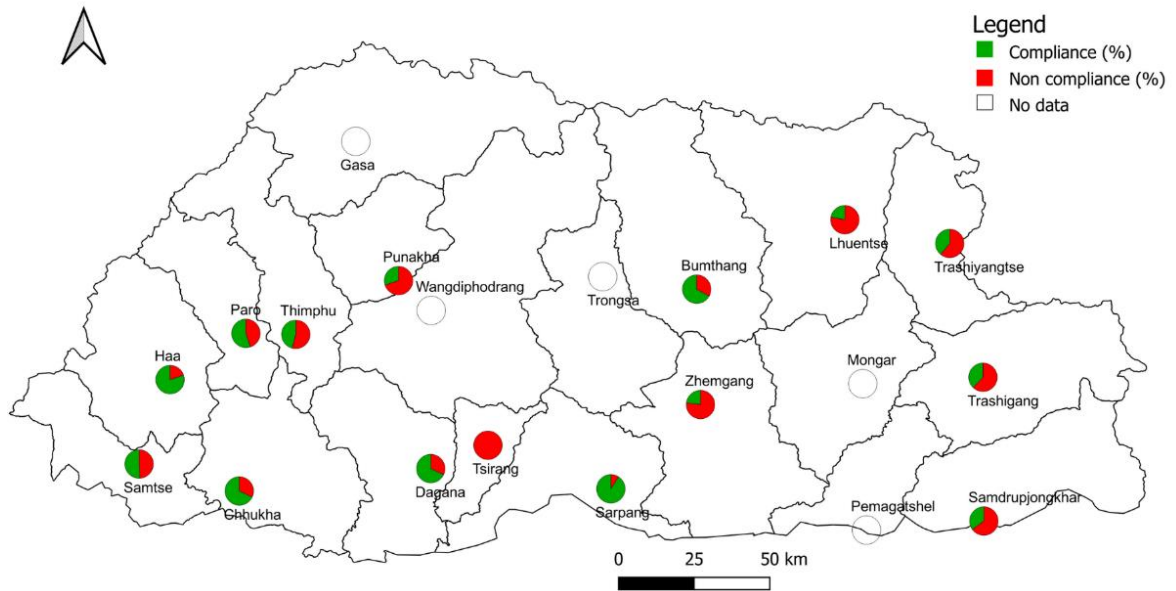
reported, however no food samples were collected from these outbreaks, as there were no left-over samples for laboratory investigation.

## 2.7 Urban Drinking Water Quality Monitoring (UDWQM)

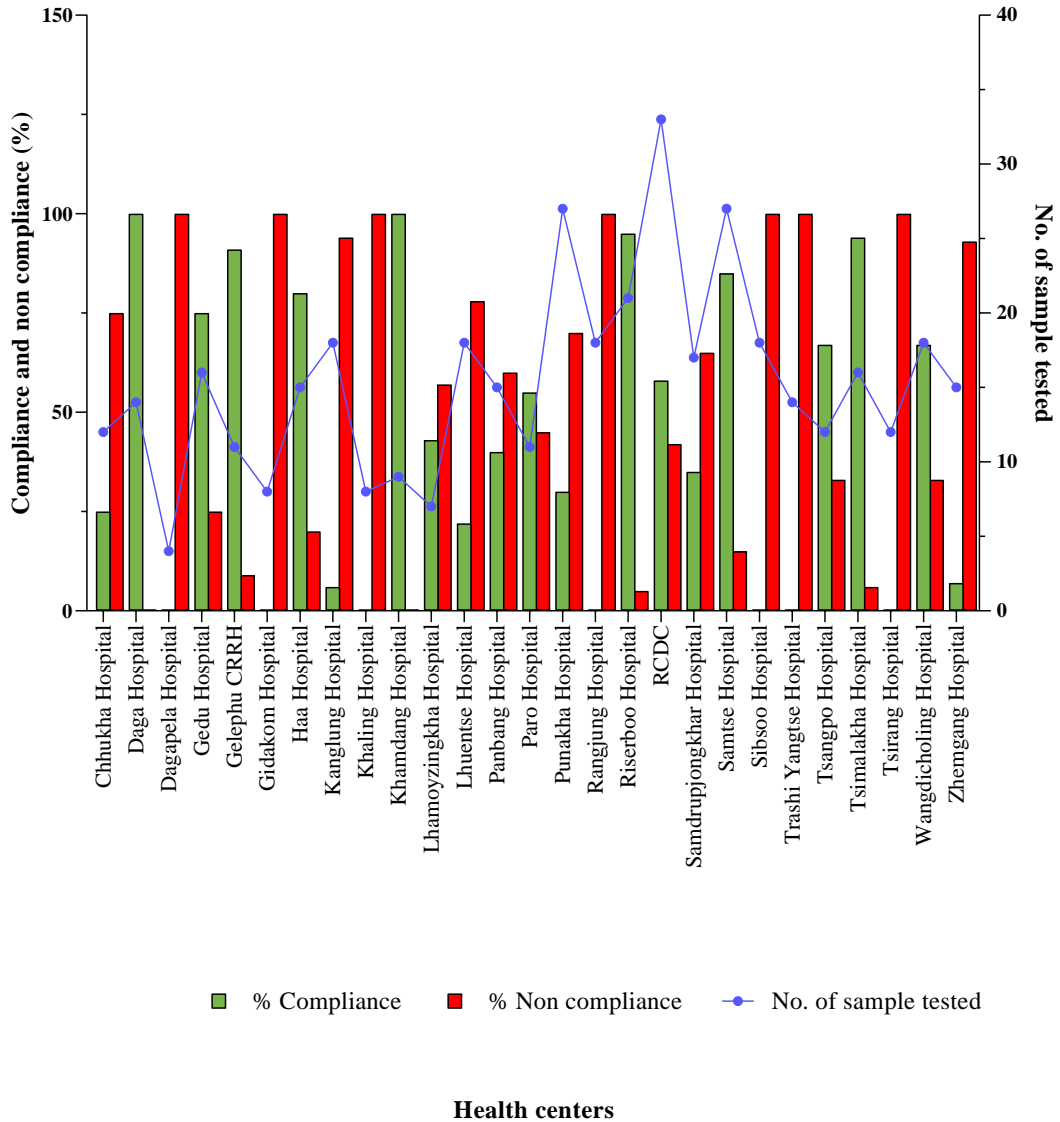
### 2.7.1 Bacteriology test (Thermotolerant coliform) Report

A total of 414 samples were collected and tested for microbial analysis from 27 urban health centers for the 3<sup>rd</sup> quarter of 2023. The results were compared to Bhutan’s drinking water quality standard 2016 to determine the compliance. The drinking water samples with no microbial contamination (0 CFU/100mL) are considered safe and are considered compliant with the standard. The result showed that the mean compliance rate across the health centers was 44.0 %.

entails the microbial data compliance for 20 districts. As shown in the figure, a significant number of water samples did not comply with the standard in most of the dzongkhags. Further surveillance sites from some of the dzongkhags failed to report for this quarter (**Figures 19 & 20**).



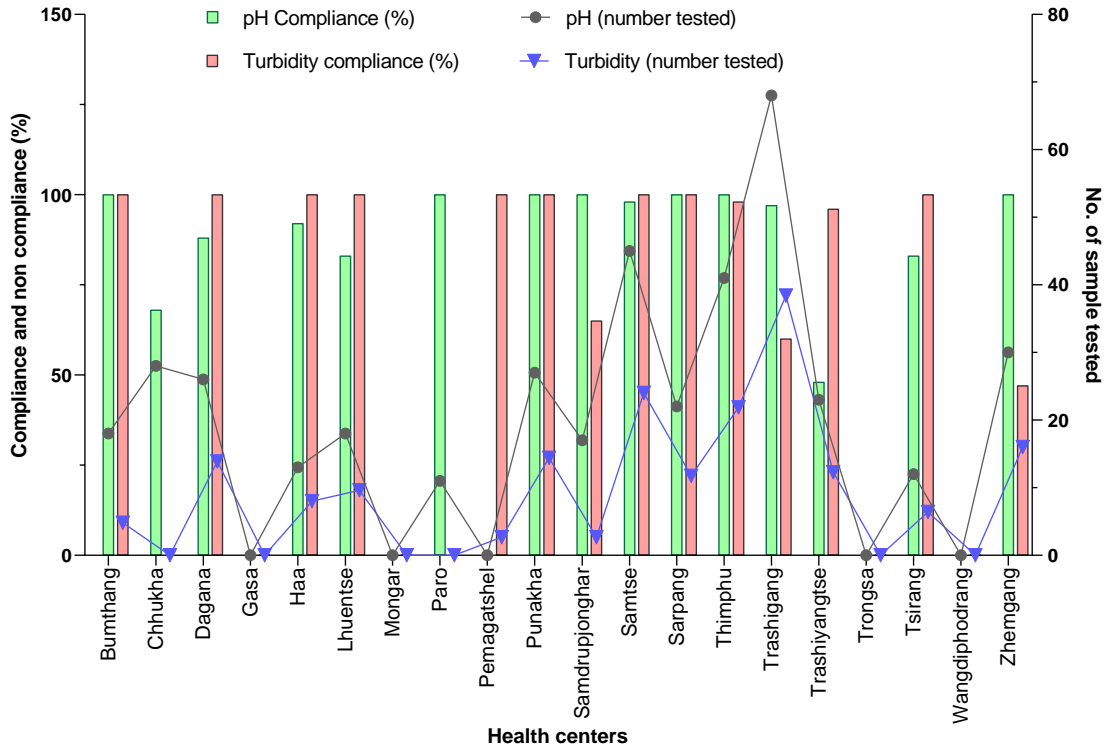
**Figure 19:** Microbial test result compliance in the urban area of 20 Dzongkhag



**Figure 20:** Microbial test result compliance of 27 health centers in an urban area

**2.7.2 Physical test**

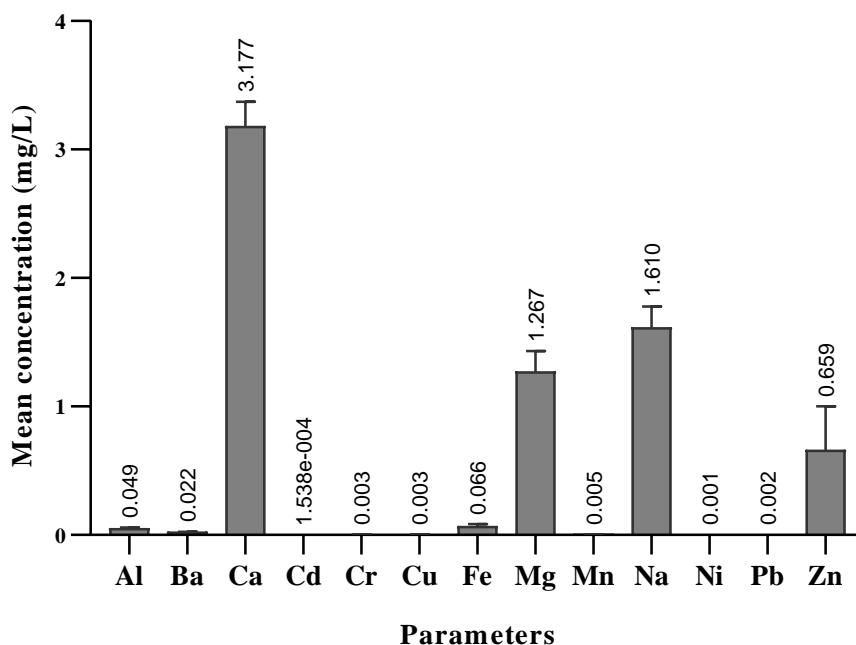
Regarding the assessment of physical parameters, including pH and turbidity, data was received from health centers across 15 dzongkhags for pH and 16 dzongkhags for turbidity (**Figure 21**). The compliance rate for the physical parameter shows a comparatively better trend when compared with the microbial parameters, with a mean compliance rate of 90.0% for both pH (recommended value; 6.5-8.5) and turbidity (recommended value; <5NTU).



**Figure 21:** Physical test compliance rate from 20 dzongkhag

**2.7.3 Chemical test**

A total of 23 samples were tested for chemical parameters at RCDC from various water sources and sampling points. The mean concentration for some of the chemical parameters is given in **Figure 22**. As shown in the figure all the chemical parameters of the concern are well below their recommended level in drinking water as per BDWQS and WHO guideline for drinking water.



**Figure 22:** Chemical test results for water samples tested at the National water reference laboratory

### 2.8. Drug Quality Monitoring

A total of 17 medicines were tested at National Drug Testing Laboratory in the 3rd quarter 2023. The samples were tested as per their pharmacopeia claim. All the samples were found to be within specification (**Table 9**)

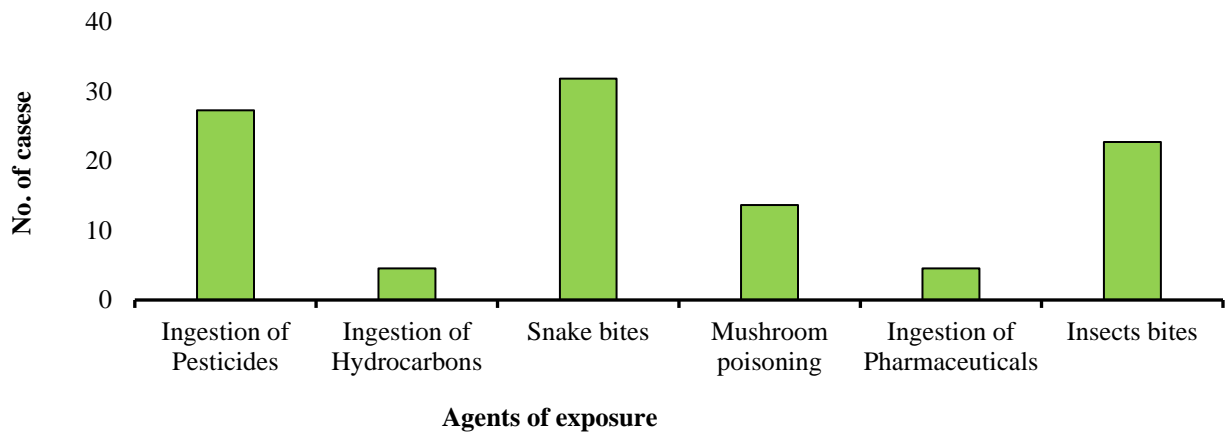
**Table 9:** Types of samples tested

Type of Samples	Complies	Does not comply	Total
Complain	1	0	1
Registration	16	0	16
<b>Total</b>	17	0	17

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

The Poison Information and Toxicology Laboratory at RCDC operates 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 July to September 2023. A total of 22 cases

were reported in NTESIS. The mean age of the cases was  $34.45 \pm 23.81$  years. Males were still the most exposed as compared to females (12 vs 10). Among the total cases, snake envenomation (31.8%) was the predominant exposure followed by ingestion of pesticides (27.3%) and insect bites especially by neurobi fly (22.7%). Poisoning with other agents constitutes a total proportion of 22.7 % which includes ingestion of hydrocarbons, pharmaceutical drugs, and mushroom poisoning (**Figure 23**).



**Figure 23:** Common agents of exposure

Among the proportion of poisoning cases reported, the highest were from Samtse dzongkhag (27.3%) and Chhukha dzongkhag (27.3%), followed by Samdrup Jongkhar (22.7%). The rest of the reports was made from Trongsa, Tashigang, Tsirang, and Dagana. Also, the majority of the poisoning cases via ingestion of pesticides and pharmaceutical drugs were recorded to be unintentional.

**2.9.2 Confirmation of drugs of abuse**

A total of four samples were received in this quarter for confirmation of Tramadol, benzodiazepines and its derivatives, delta 9 tetrahydrocannabinol, Ketamine, heroin, alcohol, and other Opiates in urine, blood, and beer samples. The samples were prepared using both liquid-liquid extraction and solid-phase extraction methods (**Table 10**)



**Table 10:** Types of drugs of abuse samples received and tested

SN	Samples	Drugs of abuse	Result
1.	Suspected spiked Beer sample and urine sample	Test for all drugs of abuse including tramadol, delta-THC, heroin, codeine, Methamphetamine, opiates, benzodiazepines, and ketamine	Negative
2.	Blood sample	Test for toxic alcohol (methanol), ethanol and 2-propanol	Negative
3.	Urine sample	Test for Tramadol and delta-THC	Positive for Tramadol

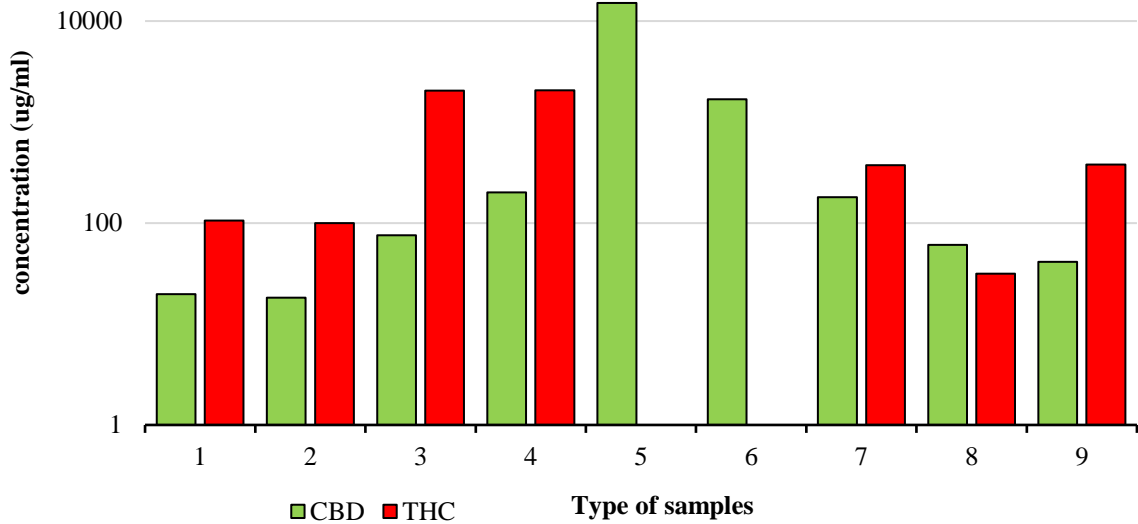
The analysis method was developed for each type of drug, validated using the certified reference standards, compared with the NIST 17.0 library, and use of internal standards/spiking method. Among these four samples, only one tested positive for tramadol when analyzed by Gas Chromatography Mass Spectrometry (GCMS).

### 2.9.3 Quantitative analysis of Cannabis products for CBD, THC content and heavy metals

CBD (Cannabidiol) and THC (Tetrahydrocannabinol) are both chemical compounds found in the cannabis plants. CBD is non-psychoactive and is commonly used for its potential therapeutic benefits, such as pain relief and reducing anxiety. THC, on the other hand, is psychoactive compound associated with cannabis use.

A total of nine samples containing cannabis were received. Types of products were in sprays, resins, oil-based, and powder forms. Both solid-phase and liquid-liquid extraction methods were carried out for the extraction of CBD and THC from the products. The analysis method was performed using GCMS.

The highest concentration of CBD analyzed in the sample was from an emulsion (oil-based product) with 15,163.9 $\mu$ g/ml and THC of <LOD. Only two samples that were of emulsion-based had a good amount of CBD above 1500  $\mu$ g/ml and THC with <LOD. The rest of the seven products contained higher amount of THC than CBD (**Figure 24**).



**Figure 24:** Quantitative analysis of Cannabis products for CBD, THC content, and heavy metals

#### 2.9.4 Quantitative analysis of cannabis products for heavy metals

The same samples mentioned above were further analyzed for heavy metals using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-EOS). Samples were prepared using acid digestion method and were analyzed for heavy metals such as Aluminum (Al), Boron (B), Barium (Ba), Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Strontium (Sr), Lead (Pb) and Zinc (Zn). Among these, lead and cadmium are the most toxic heavy metals that can have detrimental effects on human health and the environment.

The average concentration of lead from the samples was  $0.212 \pm 0.244$  mg/kg and cadmium with  $0.021 \pm 0.012$  mg/kg. Highest lead and cadmium analyzed were of 0.807 mg/kg and 0.041 mg/kg.

#### 2.9.5 Quantitative analysis of mercury in blood and urine samples

Metal Artisans are occupationally exposed to mercury during the use of these heavy metals in various crafts and trades. The exposure can occur through inhalation of mercury vapors, ingestion, or skin contact with mercury-containing materials. Artisans exposed to mercury are at risk of mercury poisoning, which can lead to a range of health problems, including neurological disorders, kidney damage, and respiratory issues.

This quarter, a total of 14 urine samples and 18 blood samples of artisans from Thimphu were received for the quantitative analysis of Hg.

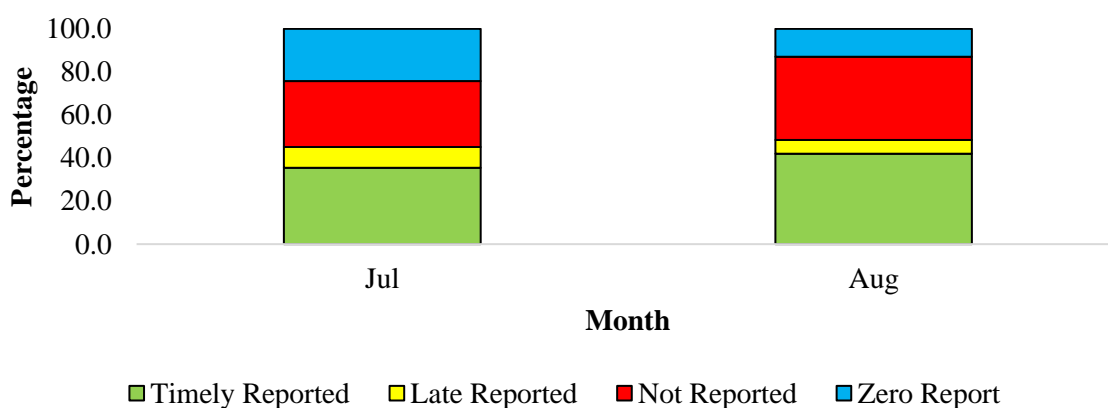
For the analysis of Hg in both blood and urine, Cold Vapour Atomic Absorption Spectroscopy (CVAAS) was used. The highest concentration of Hg determined in urine was 618.0  $\mu\text{g/ml}$  and the lowest with 4.94  $\mu\text{g/ml}$ . The normal reference range for blood and urine mercury levels measures to be 10.0  $\mu\text{g/ml}$  for both blood and urine mercury. From a total of 14 urine samples, nine samples have Mercury above the normal range showing a proportion of 64.28 %. There were eight males and two females.

The highest mercury concentration determined in blood samples was 390.06 $\mu\text{g/ml}$  and the lowest with 5.86  $\mu\text{g/ml}$ . A proportion of 88.8% of the samples showed a higher concentration of mercury in blood than the normal reference range of 10  $\mu\text{g/ml}$  raising a significant concern.

## 2.10 Blinded rechecking of malaria slides

### 2.10.1 Reporting status of health centers

A total of 84 health centers have participated in malaria-blinded rechecking. The overall average reporting rate with 38.7% were reported on time, 18.6 % were zero reported, 8.1% were reported late and the rest were not reported (**Figure 25**).



**Figure 25:** Monthly reporting status for 3<sup>rd</sup> quarter 2023

### 2.10.2 Blinded rechecking status

A total of 790 malaria slides were received at the National Malaria Reference Laboratory for blinded rechecking. From the total slides examined, eight malaria-positive slides were detected (1.0%).

The performance score on sensitivity was 100.0%, specificity was 100.0%, malaria detection was 100.0%, species identification was 100.0%, stages identification was 100.0%, parasite density determination was 100.0%, quality of blood film was 72.4% and quality of stain was 82.5% (**Table 11 & 12**)

**Table 11:** Report on Malaria Blinded rechecking for 3<sup>rd</sup>quarter 2023

Quarterly Report on Malaria Blinded Rechecking 2023			
Month	July	August	Total
<b>The health center participated in blinded rechecking</b>	44	40	84
<b>Total slides received for blinded rechecking</b>	381	408	789
<b>Total positive detected</b>	2	5	7
<b>Total Nmpps detected</b>	379	403	782
	<b>Total slide Examine</b>		1662

**Table 12:** Report on performance score for Blinded rechecking

Performance score on blinded rechecking			
Month	July	August	Quarterly Score
<b>Sensitivity (True positive detection)</b>	100.0	100.0	100.0
<b>Specificity (True negative detection)</b>	100.0	100.0	100.0
<b>Malaria parasite detection</b>	100.0	100.0	100.0
<b>Mp Species Identification</b>	100.0	100.0	100.0
<b>Mp Stages Identification</b>	100.0	100.0	100.0
<b>Mp Parasite density</b>	100.0	0.0	50.0
<b>Stain Quality</b>	61.9	59.8	60.8
<b>Blood film Quality</b>	90.6	89.1	89.8

### 2.10.3 Malaria Nested PCR

A total of 49 whole blood samples were received in this quarter for PCR confirmation of malaria diagnosis (Figure 4). Out of this, eight samples were received from passive case detection (PCD), 38 samples were tested from proactive case detection (PACD), and three samples were tested from Reactive case detection (RACD) (**Table 13**)

**Table 13:** PCR test result

Sample-based on case type	July	August
<b>Passive case detection (PCD)</b>	2	6
<b>Proactive case detection (PACD)</b>	38	0
<b>Reactive case detection (RACD)</b>	0	3
<b>Total</b>	<b>40</b>	<b>9</b>

**Sample Sources:** PCD typically involves patients who seek medical care and are tested for malaria. PACD could involve systematic screening or surveillance for malaria in a specific population. RACD often occurs in response to a confirmed malaria case to identify and treat other potentially infected individuals in the vicinity.

**Molecular Testing Method:** Nested PCR was used for detecting malaria parasites in these samples. Nested PCR is a highly sensitive technique commonly used to detect specific DNA sequences, which, in this case, would be DNA from the malaria parasite.

**Gel Electrophoresis:** Gel electrophoresis is often used in molecular biology to separate and visualize DNA fragments to check for the presence or absence of malaria DNA.