

(Epi-week 26-38)

Contents

Highlights on National Early Warning and Alerts Response Surveillance (NEWARS):	2
	2
1. National Early Warning Alert and Response Surveillance (NEWARS)	3
1.1 Reporting status of health centers under 20 Dzongkhags	3
1.2 Status of Notifiable Diseases/Syndromes reported by health centers	4
1.3 Descriptive analysis of most common notifiable diseases	5
1.3.1 Respiratory Illness (ARI and SARI) syndrome	5
1.3.2 Diarrheal syndrome: (Acute Watery Diarrhea [AWD] and Acute Bloody Diarrhea [ABD])	6
1.3.3 Fever with Rashes syndrome:	7
1.4 Immediately Notifiable Diseases/syndromes:	8
1.5 Events/Outbreaks Reported	9
2 Sentinel surveillance	10
2.1.1 Drug-Resistant Surveillance for Tuberculosis:	10
2.2 COVID-19 Integrated Influenza surveillance:	11
2.2.1. COVID-19 Integrated Influenza Surveillance	11
2.2.2. Influenza	13
2.2.1 Influenza like Illness and SARI Surveillance	15
2.3.1. laboratory-based surveillance for vaccine-preventable diseases and	17
2.4. Sentinel Surveillance for Diarrheal Etiologic Agents:	19
2.5. Food safety surveillance:	21
2. 6. Drinking Water Quality Surveillance	22
2.6.1 Bacteriology test report of Urban Drinking Water Quality Monitoring (UDWQM):	22
2.6.2. Chlorination Report	22
2.8 Drug Quality Monitoring:	23
2.9. National External Quality Assessment Scheme for Malaria Microscopy:	24
2.10. National Toxicology Center	27

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

a) NEWARS:

- Overall reporting rate for notifiable diseases had increased compared to the previous quarter
- Of 52 samples collected from suspected measles and rubella cases, three samples tested positive for measles IgM and two tested positive for rubella IgM.
- Forty-one Dengue fever cases, three malaria, three bacterial meningitis, and seven acute encephalitis syndromes were reported during the quarter
- iv) Highest number of diseases outbreak reported was of Hand Foot and Mouth Diseases (47), it was reported from almost all the Dzongkhags. Three outbreaks of Acute Gastroenteritis, was reported. A dengue outbreak was reported from the Phuntsholing. All outbreaks were investigated by the local health centers with the recommendation from RCDC.

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. The overall reporting rate was inconsistent with the last quarter. Overall 84.0% of reports were received in the NEWARS of which 62.0% were reported on time, 12.0% were reported late and the rest were not reported (**Figure 1**).



Figure 1: Dzongkhag-wise weekly reporting status for 3rd quarter 2022

3

BUM (Bumthang, CHU (Chukha), DAG (Dagana), GAS(Gasa), LHU (Lhuntshe), MON (Mongar), Par (Paro), PEM (Pemagatshel), PUN (Punakha), SJK (Samdrupjongkhar), SAM (Samtse), SAR (Sarpang), THI (Thimphu), TRG (Trashigang), TRY (Trashiyangtshe), TRO (Trongsa), TSI (Tsirang), WANG (Wangduephodrang), ZHE (Zhemgang) 1.2 Status of Notifiable Diseases/Syndromes reported by health centers:

Among 11 weekly reportable diseases/syndromes, the highest number of cases were reported were ARI- 44445 (82.0%) followed by AWD- 7668 (14.0%), (**Table 1**). The total number of cases reported was higher in this quarter compared to the previous quarter.

DZO	ABD	AWD	AJS	ARI	MUM	FWR	FDP	TPF	SAR	RKS
BUM	17	290	31	1713	1	0	2	1	13	2
CHU	46	376	0	2806	0	6	0	72	1	11
DAG	7	219	0	1918	0	10	1	0	14	0
GAS	14	38	0	96	0	0	0	0	0	0
HAA	1	102	0	609	0	10	10	0	0	0
LHU	20	113	0	734	0	0	0	2	1	1
MON	68	422	58	2014	0	8	0	1	23	8
PAR	236	1040	0	2938	1	0	0	0	3	2
PEM	6	260	1	1594	0	3	2	9	2	2
PUN	27	173	0	227	1	4	0	0	4	0
SJK	29	322	0	3739	0	2	76	5	16	0
SAM	6	499	0	5402	1	3	0	8	68	4
SAR	47	387	0	6108	2	57	0	0	26	2
THI	83	1374	27	4985	0	3	0	6	48	1
TRG	69	575	42	1714	2	12	16	17	4	12
TRY	106	282	0	1103	2	13	0	0	14	9
TRO	35	357	17	1267	0	4	3	1	16	0

Table 1: Notifiable diseases/syndromes reported by Dzongkhags

Total	878	7668	199	44443	10	145	112	124	292	58
ZHE	6	97	0	838	0	10	1	2	3	4
WNG	35	559	1	2043	0	0	1	0	5	0
TSI	20	183	22	2595	0	0	0	0	31	0
			KCDC .	JUK V LILLI	AINCE DU	JLLEIIN				•
RCDC SURVEILLANCE BULLETIN										

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 44937 cases of respiratory illness were reported, almost all cases were ARI (99.0%) while only 1.0% were SARI cases. The trend of ARI was found consistently 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 district, Sarpang and Samtse reported the maximum number of ARI cases (**Figure 2C**).



A: Incidence by Epi-week

B: Incidence by age group



C: Incidence by district



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

A total of 8546 cases of diarrheal cases were reported (AWD: [7668] 90.0% and ABD: [878] 10.0%). The trend for diarrheal diseases was found at-par 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**). Diarrheal diseases were reported from all the dzongkhag while Thimphu and Paro reported maximum AWD (**Figure 3C**).



A: Incidence by Epi-week

B: Incidence by age groups



C: Incidence by district

Figure 3: Diarrheal syndrome (AWD and ABD) incidence by Epi-week, age group and place

1.3.3 Fever with Rashes syndrome:

A total of 145 cases of fever with rash (FWR) syndrome were reported in the quarter (**Figure 4A**). The trend was found higher compared with the previous quarter. A majority of FWR were reported in the age group < 14 years (**Figure 4B**). Among the dzongkhag, Samtse, Sarpang Trashigang and Trashiyangtse reported maximum number of the cases (**Figure 4C**).



A: Incidence by Epi-week

B: Incidence by age groups



C: Incidence by district



1.4 Immediately Notifiable Diseases/syndromes:

A majority of the immediately notifiable diseases/syndromes reported were Dengue fever cases, seven acute encephalitis syndromes, three malaria, three measles cases and two rubella were reported during the quarter. Dengue cases were reported from 10 districts with a maximum number of cases reported from Phuntsholing, Chukha (**Figure 5**).



8

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

1.5 Events/Outbreaks Reported

Highest number of diseases outbreak reported was of Hand Foot and Mouth Diseases (47), it was reported from almost all the Dzongkhags. Three outbreaks of Acute Gastroenteritis, was reported. A dengue outbreak was reported from the Phuntsholing. (**Figure 6**). 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 6: Distribution of events by dzongkhag *ARI=Acute Respiratory Infection

2 Sentinel surveillance

2.1.1 Drug-Resistant Surveillance for Tuberculosis:

A total of 320 patient samples were received at National Tuberculosis Reference Laboratory (NTRL) for culture and drug susceptibility testing for anti-tuberculosis drugs. Of the 297 samples, 234 (73.1%) were pulmonary tuberculosis (PTB) cases, 32 (10.0%) were extra-pulmonary tuberculosis cases and 54(17.0%) were pulmonary samples received for TB screening for VISA. In addition, 99 follow-up samples were received for culture from MDR-TB patients under treatment.

Among the PTB cases, new smear positive (NSP) constituted 42.0% (n=98) of the cases, followed by 51.3% (n=120) of new smear negative cases, 3.4% (n=8) did not have record of case type and 3.4% (n=8) were previously treated cases (**Figure 7**).



Figure 7: Classification of Pulmonary TB samples

2.1.2 Drug Sensitivity Test

Drug sensitivity test report was available for 109 samples using Line Probe Assay and 49 samples had reports for Liquid DST. A total of 8 multi-drug resistant tuberculosis (MDR-TB) cases were detected among patients with complete drug susceptibility report. Four of the MDR-TB cases were from new smear positive pulmonary samples, two MDR-TB cases were detected from NSN samples, one from previously treated case and one MDR-TB case was detected from EPTB sample. MDR-TB cases were highest in the age group of 20-29 years (3/8) (**Figure 8**). Five MDR-TB cases with second line LPA reports were sensitive to both Fluoroquinolones and aminoglycoside.



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

2.2 COVID-19 Integrated Influenza surveillance:

2.2.1. COVID-19 Integrated Influenza Surveillance

A total of 2,257 (5.0 %) new cases of COVID-19 were detected during third quarter of 2022 through enhanced surveillance. A total of 45,785 samples were tested for COVID-19 of which 6,480 was tested by Rapid Antigen test, and 39,305 by RT-PCR assay. The highest number of

cases were detected in epi-week 37 (311), followed by week 29 (259) and the trend declined gradually over the week (**Figure 9**). All cases were detected from community, close contact and in-coming travelers (imported cases). The cases were reported from all the districts except Trashigang and Trashiyangtse. Chukha (682) reported highest cases followed by Thimphu (542) and Paro (341) (**Figure 10**).

The mostly affected age group for COVID-19 was 25 - 49 years (59.9 %), followed by 20 - 24 years (11.9 %) and 50 - 59 years (10.7 %). More males (66.7 %) were affected more than the females (33.3 %) (Figure 11).



Figure 9: Weekly SARS-CoV-2 positives and its positivity rate against total samples tested in the country



COVID-19 Positives in third quarter of 2022 (July - September)

Figure 10: COVID-19 positives reported from district Hospitals in third quarter

2.2.2. Influenza

A total of 532 samples (ILI- 408, SARI- 124) were tested for Influenza and SARS-CoV-2 through multiplex RT-PCR (Flu SC2) and detected 37 Influenza positives with positivity rate 6.9 % and 17 SARS-CoV-2 positives. Influenza subtype Flu A/H3 (14) and Flu A/H1pdm09 (1) was found most predominating strain (**Figure 11, and Figure 12**). Samtse Hospital (185) and Trongsa Hospital (131) has sent more samples compared to rest of the sentinel hospitals (**Table 2**).

The mostly affected age group for Influenza was 10 - 14 years (30.0 %), followed by 5 - 9 years (25.0 %). Males (60.0 %) were affected more than the females (40.0 %) (**Figure 13**).



Figure 11: Influenza subtype and SARS-CoV-2 positives



Figure 12 : Influenza positives with subtypes by districts in third quarter, 2022

14



Figure 13: COVID-19 and Influenza by age group and gender for third quarter, 2022

		IU				S/		
SI. No.	Hospital Sites	SARS-CoV-2	FLU A/H3	A/Not subt	yp ILI Total	FLU A /H1pdm09	SARI Total	Grand Total
1	Gedu					1	9	9
2	Наа				1			1
3	JDWNRH				2		30	32
4	Lungtenphu	3			11			11
5	Mongar ERR	1			12		7	19
6	Paro	2			14		37	51
7	Phuntsholing			17	24			24
8	Punakha		1		8		1	9
9	Samdrup Jongkhar		11	6	25		2	27
10	Samtse	2			159		26	185
11	Trashigang				3			3
12	Trongsa	7	1		120		11	131
13	Tsirang	2	1		17		1	18
14	Wangdue				2			2
15	Gelephu CRR				10			10
	Grand Tota	I 17	14	23	408	1	124	532

Table 2: Summary table for Influenza subtypes and sample tested for third quarter, 2022

2.2.1 Influenza like Illness and SARI Surveillance

The weekly aggregate report for Influenza-like illness (ILI) and severe acute respiratory infection (SARI) cases are being reported weekly from respective sentinel sites. A total of 1277 ILI cases were reported during 3rd quarter. The ILI case surge was observed in week 35, 37 and week 38. This was mostly contributed by Flu outbreak in Samdrup Jongkhar. Trongsa (237) and Samdrup

Jongkhar (629) Hospitals has reported more ILI cases compared to other sentinel sites. Punakha Hospital has not reported since week 1 - 38, 2022 (**Figure 14**).

A total 203 SARI cases were reported from sentinel sites during the 3^{rd} quarter of 2022. Trashigang (60) and Gelephu (60) hospitals have reported more SARI cases compared to other sentinel sites. All sentinel sites has reported except Tsirang and Punkha hospital has note reported since week 1 – 39, 2022 (**Figure 15**).



Figure 14: Weekly ILI cases reported from Sentinel sites



16

Figure 15: Weekly SARI cases reported from Sentinel sites

2.3.1. laboratory-based surveillance for vaccine-preventable diseases and

During the third quarter of the year, 52 samples were received for MR testing, seven for AES and three for *Bordetella pertussis*. IgM ELISA performed on these samples resulted in three positive each for *measles* and two *rubella*. Detection by PCR and further genotyping of these samples are pending. IgM ELISA for *Japanese encephalitis* (JE) virus on samples received for AES and *Bordetella pertussis* performed did not yield any positive results (**Table 4**).

Table No 3: Number of samples received from health centers for MR, JE, and Pertussis surveillances

Surveillance	Site/ Hospital	Total samples	Positive	Positive
		received		
	Paro	21	2 (measles)	1 (rubella)
	JDWNRH	12	1 (measles)	0
	Sarpang	2	0	1 (rubella)
	Punakha	2	0	0
Measles/	Wangduephodrang	2	0	0
Rubella	Samtse	03	0	0
	Gedu	2		0
	Haa	1	0	0
	Trashigang	3	0	0
	Samdrupjongkhar	2	0	0
AES (JE)	JDWNRH	7	0	0
Pertussis	JDWNRH	3	0	0

2.3.2 Surveillances for Dengue, Acute Undifferentiated Febrile Illness (AUFI) and other confirmatory tests

There were 6 samples received from AUFI sentinel sites (Bajo Hospital 5Trongsa 1). All tested negative to the panel of tests described in (**Table. 4**). IDSL received 52 samples for Dengue surveillance out of which 15 samples were positive to either Dengue NS1 or IgM (**Table. 4**).

Surveillanc	Site/	No. of Positive test result								
e /Test	Hospital	samples	DEN	DEN	Scrub	Leptosp	JE	CHIK	Bruce	
requested		received	V	V	typhus	ira IgM	IgM	V	llaIg	
		and tested	NS1	IgM	IgM			IgM	Μ	
AUFI	Trongsa	01	00	00	00	00	00	00	NA	
sentinel	Bajo	05	00	00	00	00	00	00	NA	
e										
	Phuntsho ling	16	11	00	NA	NA	NA	NA	NA	
	Tsirang	20	00	01	NA	NA	NA	NA	NA	
Dengue	CRRH	07	00	00					NA	
Confirmati	Samdrup	01	00	00						
on	jongkhar									
	Samtse	03	00	00						
	Gedu	05	00	01	NA	NA	NA	NA	NA	
	1									
Scrub										
typhus confirmati on	Samtse	03	NA	NA	02	NA	NA	NA	NA	
	RBA Thimphu	01	NA	NA	00	NA	NA	NA	NA	
Brucellosis										
Confirmati	Samtse	08	NA	NA	NA	NA	NA	NA	04	
on										

Table 4: Samples received and tested for dengue and AUFI sentinel surveillances

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

2.4. Sentinel Surveillance for Diarrheal Etiologic Agents:

A total of 205 samples were received from ten sentinel sites (**Figure 16**) for this quarter. Most of the samples received were from the JDWNRH, Trongsa, and Tsirang.

The sample's character consists of loose (75.0%), watery (24.0%) and bloody (1.0%). The mean age of the patients enrolled was 19 years. The mean duration of illness was 39 hours. Of 205 cases,

31.0% required hospital admission while the rest were treated on OPD basis. Of all, nine diarrhea cases were found linked to having consumed a suspected food. Among the cases recorded, 56.0% were male and 44.0% were females.

The proportion of enteric pathogens isolated during the quarter is shown in (**Figure 17**). The antimicrobial-resistant pattern for the isolated bacterial pathogens is provided in (**Table 5**).



Figure 16: Number of fecal specimens collected from sentinel sites



Figure 17: proportion of enteric pathogens isolated

Pathogen	AMP	CZO	CRO	LEX	CHL	CIP	GEN	NAL	TCY	SXT
Aeromonas species (n=3)	3	3	2	3	0	1	1	1	0	1
Salmonella species (n=5)	2	2	1	2	0	1	1	2	1	1
Shigella sonnei (n=6)	6	6	6	6	0	6	0	6	6	6
Shigella flexneri (n=1)	0	1	0	0	0	0	0	1	0	1
EAEC (n=18)	7	7	2	4	0	4	2	4	1	1
EPEC (n=3)	0	0	0	0	0	0	0	0	0	0
ETEC (n=5)	1	1	0	1	1	0	0	0	1	0

Table 5: Anti-bio gram (Resistant pattern) for enteric pathogens:

S: Susceptible; I: Intermediate; R: Resistant

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

2.5. Food safety surveillance:

Food-borne diseases are caused by a wide range of microbiological and chemical or toxins with different levels of severity, which range from mild sickness to life-threatening illness. 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 3rd quarter of 2022 food safety surveillance conducted by Food and Nutrition Laboratory, RCDC a total of 50 ready to eat food samples were received from the surveillance sites (**Figure 18**).



Figure 18: Food safety surveillance sites

Total Plate count of aerobic microorganisms, total *Enterobacteriaceae* count, total coli form count and yeast mould count are used as indicators to access the food quality and also used as a hygiene indicator. Of the total 50 samples 32% (n=16 samples) (Total plate count of >10 log 5 and *E.coli* of >10 log 2). presented with indicator test organism growth.

Twenty eight percent of the samples were contaminated with pathogenic organism, the commonly isolated pathogen includes *Staphylococcus aureus* and *Bacillus ceres*. And 6% of the samples were contaminated with both *S aureus* and *B. cereus* organism.

During the same period a suspected food poisoning was reported from Thimphu. The samples collected and the outbreak was suspected due to *Staphylococcus aureus* toxins (intoxication).

2. 6. Drinking Water Quality Surveillance

2.6.1 Bacteriology test report of Urban Drinking Water Quality Monitoring (UDWQM):

In the third quarter of 2022, 494 out of 774 drinking water samples were tested by the urban health centers. Out of which 55.1% of the tested samples were found fit for consumption and the rest 44.9% were found to be contaminated by fecal coliform hence, it is unfit for drinking. 14 out of 34 health centers have not reported for this quarter (**Figure 19**)



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

2.6.2. Chlorination Report

(Royal Centre for Disease Control, Samtse Hospital, Bumthang Hospital, Gelephu CRRH, Wangduephodrang Hospital) out of 6 health centers monitoring residual chlorine have reported for this quarter. In this quarter, 60 samples were tested and 96.7% of drinking water has been found inadequately chlorinated and only 3.3% has been adequately chlorinated (**Figure 20**).



Figure 20: Residual Chlorine test report for 5 health centers in urban area

2.8 Drug Quality Monitoring:

A total of 48 samples were tested at National Drug Testing Laboratory in the 3rd quarter 2022. The samples were tested as per their pharmacopeial claim. From the 48 samples tested, nine samples were found to be non-compliant. Accordingly, the test reports were communicated to DRA for their necessary regulatory action (**Table 6 and Figure 21**)

Dzongkhag	Complies	Does not comply	Total
Chhukha	0	1	1
Dagana	11	5	16
Thimphu	16	2	18
Trashiyangtse	11	1	12
Total	38	9	47

Table 6: Distribution	of samples collected
-----------------------	----------------------



Figure 21: List of test parameters analyzed

2.9. National External Quality Assessment Scheme for Malaria Microscopy:

2.9.1. Reporting status of health centers

In the 3rd quarter, a total of 199 health centers has participated in malaria blinded rechecking. The overall reporting rate with 16.4 % were reported on time, 26.8% were reported late and rest were not reported (**Figure 22**)



Figure 22: Monthly reporting status for 3rd quarter 2022

2.9.2. Blinded rechecking of malaria slides:

Total of 880 malaria slides were received at National Malaria Reference Laboratory for blinded rechecking. From the total slides examined, 2 malaria-positive slides are detected (0.3%). 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 was 100.0%, species identification was 100.0%, stages identification was 100.0% and parasite density determination was 100.0% (**Table 7 and 8**)

Third Quarterly report on Malaria Blinded Rechecking 2022								
Month	July	August	September	Total				
Health center participated in blinded rechecking	31	32	26	89				
Total slides received for blinded rechecking	351	336	193	880				
Total positive detected	1		1	2				
Total Nmps detected	350	336	192	878				
	Total slide Examine880							

Table 7: Report on Malaria Blinded rechecking for 2nd quarter 2022

Table 8: Report on performance score for Blinded rechecking

Performance score on blinded rechecking							
Month	July	Beare					
Sensitivity (True positive detection)	100		100	100			
Specificity (True negative detection)	100	100	100	100			
Malaria parasite detection	100	100	100	100			
Mp Species Identification	100		100	100			
Mp Stages Identification	100		100	100			
Parasite density	100		100	100			

In the second quarter, total of 503 malaria slides were received at National Malaria Reference Laboratory for blinded rechecking. From the total slides examined, two malaria-positive slides are detected (0.4 %). 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 was 100.0%, species identification was 100.0%, stages identification was 75.0%, parasite density determination was nil, quality of blood film was 88.0% and quality of stain was 62.0% (**Table 7 and 8**)

Table 7: Report on Malaria Blinded rechecking for 2nd quarter 2022

Second Quarterly report on Malaria Blinded rechecking 2022	2			
Month	April	May	June	Total
Health center participated in blinded rechecking	17	24	23	64
Total slides received for blinded rechecking	109	172	222	503
Total positive detected	1	0	1	2
Total Nmps detected	108	172	221	501
	Total	slide Exar	nine	503

Table 8: Report on performance score for Blinded rechecking

Performance score on blinded rechecking				Quarterly Score
Month	April	May	June	
Sensitivity (True positive detection)	100		100	100
Specificity (True negative detection)	100	100	100	100
Malaria parasite detection	100	100	100	100
Mp Species Identification	100		100	100
Mp Stages Identification	50		100	75
Parasite density	0		0	0
Quality of blood smear	88	87	89	88
Quality of stain	57	59	70	62

2.10. National Toxicology Center

2.10.1. Determination of Mercury in dry fish and the human health risk assessment in Thimphu,Bhutan

Introduction

Dry fish is the most affordable and abundantly available source of proteins to the Bhutanese population. It is considered a delicacy and are consumed by all ages including children in Bhutan. Annually, 372 metric ton of dry fish are imported from the neighboring country India and there has been a steep increase in the consumption of dry fish. However, the concentration of heavy metals like mercury above the permitted level is a global public health concern. Mercury is the most commonly found contaminant in the dry fish and do not have any physiological role in the human body and even a trace amount is detrimental to the human health. Mercury is a neurotoxicant and is of greatest concern due to its impacts on cognitive and behavioral functions. Mercury causes acute and chronic toxicity to the CNS, CVD, respiratory system, hepatic, renal and musculoskeletal system. Currently, there is no data available and we aim to determine the mercury in dry fish and assess the potential health risk in the Bhutanese population.

Methods

A total of 18 different types of dry fish samples were purchased from the Centenary Farmer's Market. Samples were prepared according to the AOAC standard official methods. Briefly, 5 grams of dry fish tissue samples were weighed and transferred to the digestion vessel and 5 ml of nitric acid were added. Blank digest was carried out to validate the digestion procedure accordingly. Samples for mercury determination was carried out using direct mercury analyzer MA-3000 cold vapor atomic absorption spectroscopy as per the standard US EPA method 7473 (SW-846) [10]. In brief, 5 level of multi-standards of certified reference materials was used (5, 10, 25, 50 ppb) to constructed a calibration curve. Samples was diluted when the concentration of mercury in the samples were beyond the limit of quantification. Calibration curve with the correlation coefficient (\mathbb{R}^2) of 0.9999 was used to quantify the level of each heavy metals in the samples.

The estimated daily intake (EDI) and Target hazard quotient was calculated as per the US EPA, WHO and FAO's guidance for risk assessment.

The estimated daily intake of each heavy metal was calculated using the following equation:

EDI (mg kg⁻¹ day⁻¹) = $\frac{\text{FIR x C}}{\text{BW}}$

Where; FIR = Fish ingestion rate (kg person-1 day⁻¹); C = Metal concentration in fish (mg kg⁻¹), BW = Average body weight (kg).

The target hazard quotient (THQ) is an estimate of the non-carcinogenic risk level due to metal exposure. To estimate the human health risk from consuming metal contaminated fish, the target hazard quotient (THQ) was calculated by the following equations:

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD \times BW \times ATn} \times 10^{-3}$$

Where; EF = Exposure frequency (days year⁻¹); ED = Exposure duration (67 years), equivalent to the average lifetime; FIR = Fish ingestion rate (kg person-1 day⁻¹); C = Metal concentration in fish (mg kg⁻¹); RfD = Oral reference dose for mercury of 0.1 mg kg⁻¹ day⁻¹; WAB = Average body weight (kg); ATn = Average exposure time for non-carcinogens (365 days year⁻¹ × ED).

The oral reference dose (RfD) for mercury was retrieved from agency for toxic substance disease registry (ATSDR), US CDC.

Results and Discussion

Of the total of 18 samples, 100 % of the samples were detected with mercury in the range of 0.005-0.12 mg kg⁻¹ with the mean concentration of 0.043 ± 0.037 mg kg⁻¹. The concentration at 95th percentile was 0.115 mg kg⁻¹. Table 1.

 Table 1. Concentration of mercury in dry fish samples

n	MeHg (mg/kg)
1	0.089
2	0.120

3	0.052		
4	0.074		
5	0.052		
6	0.023		
7	0.036		
8	0.110		
9	0.082		
10	0.019		
11	0.028		
12	0.024		
13	0.011		
14	0.015		
15	0.006		
16	0.005		
17	0.007		
18	0.018		
mean	0.043		
SD	0.037		
95 th percentile	0.1115		
Min-Max Hg	0.005-0.12		

Human health risk assessment for exposure of mercury through the consumption of dry fish was conducted accordingly. The fish ingestion rate assumed here in the assessment were based upon the number of pieces of dry fish one may consume due to the unavailability of data. The average weight of one dry fish was 28.06 grams (**Picture 1**).



Picture 1: Dry fish used for the assessment

The estimated daily intake of dry fish for an adult consuming four pieces of dry fish (28.06 grams x 4 pieces = 112.24 grams) four times a week ($52.143 \times 4 = 208.57$ days) was 0.192 mg/day/kg body weight. Average body weight of 65 kg with a lifetime exposure of 67 years was considered for the current assessment.

Furthermore, the target hazard quotient (THQ) was calculated, which is the ratio of exposure to the toxic element and the reference dose (the highest level at which no adverse health effects are expected). The THQ of >1 refers to the consumers experiencing significant health hazards due to toxic metal exposure. In this assessment of the current scenario, we found a THQ of 1.09 showing concern for exposure of mercury via the consumption of dry fish.