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**ROYAL GOVERNMENT OF BHUTAN**
Ministry of Agriculture and Forests
Department of Livestock
**NATIONAL CENTRE FOR ANIMAL HEALTH**
Serbithang: Thimphu

# 2019-20

# ANNUAL REPORT

## LABORATORY SERVICES UNIT


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## 1. BACKGROUND

The Laboratory Services Unit (LSU) is one of the four functional technical units under the National Centre for Animal Health, Serbithang. It functions as the national veterinary referral laboratory in the country and is mandated with providing referral laboratory diagnostic services in the country. In addition to the basic laboratory tests like microscopy and the rapid tests, the unit has the capacity for advanced diagnostic tests such as Enzyme-linked immunosorbent assay (ELISA), Fluorescent antibody test (FAT) and molecular assays for emerging and re-emerging infectious disease like Foot and Mouth Disease (FMD), Highly Pathogenic Avian Influenza (HPAI), Classical Swine Fever (CSF), African Swine Fever (ASF), Brucella, Porcine Reproductive & Respiratory Syndrome (PRRS) and Rabies. In addition to the livestock and the pet animals, the diagnostic services are also being provided to other agencies like Nature Conservation Division (NCD) for wild life and Bhutan Agriculture and Food Regulatory Authority (BAFRA) for regulation of import and export.

The laboratory is equipped with real time Polymerase chain reaction (rtPCR) technology. The unit has Bio-safety level 2 plus for secure handling of high-risk pathogens. In addition, the unit is responsible for monitoring and evaluating Bio-safety in the veterinary laboratories in the country. The unit is also responsible for coordinating collaboration of advance level diagnostic research with international reference laboratories and institutes. It is also mandated to carry out laboratory-based surveillances/researches.

## 2. MANDATES

The main mandates of the Laboratory Services Unit are:

1. Providing referral veterinary laboratory diagnostic services to the clients
    a. Provide routine veterinary laboratory diagnostic services, support clinical services, animal health programs and One-Health activities in the country;
    b. Serve as the national referral laboratory for diagnosis of animal diseases in the country
2. Major Livestock Disease Surveillance/Survey
    a. To lead/coordinate and conduct laboratory-based animal health research activities in the country
3. Coordination of Biosafety and Bio-security programmes
    a. Implement and monitor bio-safety measures and good laboratory practices in all veterinary laboratories in the country
4. Strengthening and enhancement of laboratory diagnostic capacities
    a. To serve as National referral laboratory for antimicrobial resistance (AMR) monitoring in animals in the country
    b. Co-ordinate the Quality assurance systems (QAS) in all the animal health laboratories-participate in regional proficiency testing (PT) for specific diagnostic methods and conduct PT for regional, satellite and district laboratories;
    c. To technically backstop regional, satellite and district laboratories in the country
    d. Introduction of new diagnostic tests/upgradation of diagnostic tests for the emerging and re-emerging diseases in the country
    e. To liaise, collaborate and establish efficient laboratory networks with the outside agencies like National Food Testing Laboratory (NFTL), BAFRA; Clinical

Laboratory, Jigme Dorji Wangchuck National Referral Hospital (JDWNRH); Royal Centre for Disease Control (RCDC), Department of Public Health (DoPH); and Wildlife Clinic, Nature Conservation Division (NCD), Department of Forests and Park Services (DoFPS);

f. To liaise, collaborate and establish efficient laboratory networks with the international reference laboratories such as OIE and WHO Referral Laboratories;

5. Laboratory skill enhancement
    a. To develop human resource capacity by conducting the diploma course in laboratory technology in collaboration with other relevant institutions.
    b. Conduct refresher course and up-gradation courses for laboratory technicians

## 3. HUMAN RESOURCE CAPACITY

The human resource capacity in the Laboratory Services Unit as of 30th June 2020 are summarized below (Table 1):

**Table 1: Over all human resource capacity of LSU for FY 2019-20**

| Specialization | Sections | Number |
|---|---|---|
| Animal Health Specialist-I (Parasitologist) | Parasitology | 1* |
| Animal Health Specialist-III (Pathologist) | Pathology | 1 |
| Animal Health Specialist-III | Molecular biology/ Microbiology/Immunology | 1 |
| Laboratory Officer | Bacteriology/Molecular/Bio-safety & Bio-security/Biochemistry & toxicology | 2 |
| Sr. Laboratory Technician | Parasitology/Serology & Virology /Bacteriology | 3 |
| Assistant Laboratory Technician | Serology & Virology/Hematology/Bio-chemistry& Toxicology/Pathology | 6 |
| Laboratory Attendant | General | 1 |
| **Total** | | **15** |

*Superannuated on September 2019

## 4. DIAGNOSTIC CAPACITIES

The unit has six sections i.e. Bacteriology, Serology/Virology/Molecular biology, Toxicology & Bio-chemistry, Parasitology, Post-mortem and Histo-pathology and Haematology Section. The different sections under the LSU are equipped with advanced diagnostic facilities. The summary of diagnostic tests and capacities available in each section are as follows:

## 4.1 Parasitology Section

The section provides routine diagnostic services for parasitic disease and recommends control guidelines and advisory services to the government livestock farms, dzongkhags, private livestock agencies and also wild life clinic. It also provides other professional backstopping to RLDCs, SVLs and DVHs/DVLs. Besides the routine activities, the section regularly conducts research and surveillance pertaining to parasitic diseases in collaboration with government farms, RLDCs and the Dzongkhags. The section is also responsible to provide refresher/in-service courses for field staffs and trainings to the farmers with regard to parasitic diseases and control programs.

The personnel involved in Parasitology section were as follows:

1. Ms. Tshewang Dema, Assistant Laboratory Technician
2. Ms Ugyen Pema, Assistant Laboratory Technician

The following are the lists of diagnostic services that are being provided by the section:

- Identification of parasites through direct technique;
- Identification of parasites through qualitative tests (Sedimentation and Floatation methods);
- Identification of parasites through quantitative tests (Stoll method);
- Urine sedimentation test for nematodes;
- Skin scraping examination using 10% KOH digestion method;
- Blood parasite examination;
- Pepsin digestion test;
- Fecal culture (simple tube method, culture tube method, Baermann's method);
- Tick identification (stereo-zoom method);
- Post-mortem recovery of helminths, post mortem worm count;
- Microfilaria identification from blood (modified Knott's method);
- Worm staining & preservation;
- ELISA for Fasciola;
- Isolation and identification of Taeniid eggs

## 4.2 Bacteriology Section

The section provides routine diagnostic services for microbial diseases (bacteria & fungi) in the livestock through culture & identifications. The section has capacity for second stage bio-chemical tests and identification of important bacterial pathogens like *Salmonella*, and *B. anthracis*, etc.

The personnel involved in the section were as follows:

1. Dr. RB Gurung, Specialist III, LSU
2. Ms. Puspa Maya Sharma, Laboratory Officer
3. Mr. Tenzinla, Sr. Laboratory Technician
4. Ms Tshewang Dema, Assistant Laboratory Technician

The section has the following diagnostic capacities:

- Bacterial culture and identification using sheep blood agar, MacConkey agar and other selective media and various bio-chemical tests;
- Fungal culture and identification using Sabouraud agar;
- Staining techniques - Grams, Giemsa, Methylene blue, Ziehl-Neelsen/Acid fast, Leishman, Lactophenol, Spore staining and Capsule staining;
- Species identification of important bacterial pathogens in Bhutan – *Salmonella sp., E. coli, Staphylococcus spp., Bacillus anthracis, Clostridium sp., Pasteurella, Pseudomonas sp., Erysipelas rhusiopathiae*, *Brucella sp*., *Aeromonas hydrophila* and *Streptococcus sp.*
- Enumeration of bacteria - total aerobic count by pour plate technique and spread plate technique, total coli count by pour plate technique and spread plate technique, Most Probable Number (MPN) technique;
- Detection of *Mycobacterium species* by acid-fast technique;
- Agglutination tests: Slide agglutination test (SAT), Tray agglutination test (TAT) and Micro-titre plate agglutination test (MAT);
- Detection of mastitis in milk samples through the California mastitis test (CMT), Cell count and White side test (WST);
- Antimicrobial susceptibility test (AST), disk diffusion method;
- Intra-dermal test for bovine tuberculosis (TB) using purified protein derivatives (PPD).

### 4.3 Haematology Section

The section conducts the basic haematological tests to support clinical diagnosis in the animals.

The personnel involved in the section were as follows:

1. Dr. NK Thapa, AHS-III
2. Ms. Tshewang Dema, Assistant Laboratory Technician

The hematological parameters and tests commonly conducted in this section are:

- Haemoglobin estimation (Hb);
- Packed Cell Volume (PCV);
- Total Red Blood Cell Count (TRBCC);
- Total White Blood Cell Count (TWBCC);
- Differential Leukocyte Count (DLC);
- Erythrocyte Indices – MCV, MCHC and MCH;
- Erythrocyte Sedimentation Rate (ESR);
- Wet film examination for blood parasites like microfilaria and trypanosome;

### 4.4 Bio-chemistry & Toxicology Section

The section conducts basic tests for clinical bio-chemistry in serum and also qualitative analysis of urine to support the clinical diagnosis. The section also conducts basic toxicological tests especially, screening of important mycotoxins in the animal feeds.

The personnel involved in Parasitology section were as follows:

1. Dr. NK Thapa, AHS III
2. Ms. Dechen Wangmo, Laboratory officer
3. Ms. Ugyen Pema, Asst. Laboratory Technician

The following are the diagnostic capacities available in this section:

- Rapid tests for Aflatoxin in animal feed
- Quantitative estimation of mycotoxins (Aflatoxin, Ochratoxin, Fumonisin) in animal feeds;
- Mineral estimation for Ca, Mg and P in the serum;
- Qualitative urine analysis;
- Qualitative and quantitative bio-chemistry;

### 4.5 Molecular biology, Serology & Virology Section

The section performs tests on both routine basis and also on the samples referred by the Regional/District/Satellite Laboratories in the country
This section is equipped with advanced diagnostic facilities such as real time PCR, ELISA and has the capacity to undertake rapid diagnosis of emerging diseases including the highly pathogenic avian influenza, IBD, NCD and Rabies etc.

The personnel involved in the sections were as follows:

1. Dr RB Gurung, Animal Health Specialist – III
2. Ms Puspa Maya Sharma, Senior Laboratory Officer
3. Ms Dechen Wangmo, Senior Laboratory Officer
4. Mr Purna Bahadur Rai, Senior Laboratory Technician
5. Mr Dawa Tshering, Senior Laboratory Technician
6. Ms Kelzang Lhamo, Assistant Laboratory Technician

The diagnostic capacities available in this section are:

- Rapid antigen detection tests for Avian Influenza type A, H5, Newcastle disease (ND) virus, Infectious Bursal Disease (IBD), Foot and Mouth Disease (FMD) and Rabies;
- FAT for Rabies;
- Antibody ELISA for FMD, Brucellosis, Rabies, ND, IBD, CSF, Infectious bovine rhinotracheitis (IBR), Leptospirosis, Contagious Bovine Pleuropneumonia (CBPP), Contagious Caprine Pleuropneumonia (CCPP), Porcine reproductive and respiratory syndrome (PRRS), Johne's Disease (JD), Avian leucosis complex (ALC) and Peste des petits ruminants (PPR);
- Antigen ELISA for CSF and PPR;
- Typing ELISA (sandwich) for FMD;
- Conventional PCR for *Brucella*, FMD serotyping;
- Real-time PCR for AI Type A, (H5, N1, H7, N8) FMD, CSF, ASF, PRRS (EU and NA), Pigeon Paramyxovirus (PPMV) and ND;
- Agglutination tests - HA/HI for ND and H7N9;
- Slide agglutination test for *Salmonella* and *Mycoplasma*;

- Rose Bengal plate test (RBT) for *Brucella*.

### 4.6 Post-mortem & Pathology Section

The section has Post mortem and Histo-pathology section which provides necropsy and histo-pathological diagnosis.

The personnel involved in the section were as follows:

1. Dr. NK Thapa, Animal Health Specialist – III
2. Ms. Pasang Bida, Assistant Laboratory Technician
3. Ms. Ugyen Pema, Assistant Laboratory Technician
4. Mr. Tenzinla, Sr Laboratory Technician

The section is responsible for following diagnostic capacities:
- To conduct post-mortem examination and diagnosis in poultry, ruminants, canine, feline, equine, swine species and wild animals including reptiles and fish;
- To perform histo-pathological examination and diagnosis through processing and examination of slides (H&E, Grams, ZN, pigment staining and pearls staining);
- To perform immuno-histochemistry

### 4.7 Bio-safety and Bio-security section

The section is mandated to implement and monitor bio-safety measures and good laboratory practices in all veterinary laboratories in the country. Thus, this section is an aide-de-section for all other sections.

The personnel involved in the section were as follows:

1. Ms. Dechen Wangmo, Laboratory Officer

The section is responsible for the following:
- Planning, Coordination and Implementation of Biosafety and Bio-security plans
- Technical monitoring of Biosafety and Biosecurity measures
- In house training
- Reporting and Monitoring
- Samples referral to collaborating laboratories
- Compilation of routine and research laboratory test kits, reagents, consumables procurement
- Ensuring functionalities of the equipment

## 5. OVER ALL ACHIEVEMENTS OF FY 2019-20

During the fiscal year, 2019 - 2020, a total of 6,312 numbers of various laboratory samples were received or collected and 12,790 laboratory tests were performed for routine tests, disease outbreaks, disease screening, surveillance and researches (Table 2).

**Table 1: Summary of sample received and test performed during FY 2019-20**

| Section | Samples Processed | Tests conducted | Samples discarded |
|---|---|---|---|
| Toxicology | 40 | 98 | 12 |
| Bio-Chemistry | 41 | 858 | 2 |
| Parasitology | 2149 | 2909 | 0 |
| Clinical path/Haematology | 235 | 1095 | 0 |
| Bacteriology | 544 | 3,976 | 0 |
| Post Mortem | 176 | 172 | 4 |
| Histopathology | 872 | 1217 | 0 |
| Serology | 1664 | 1877 | 0 |
| Virology | 456 | 400 | 0 |
| Molecular | 135 | 188 | 0 |
| **Total** | **6,312** | **12,790** | **18** |

About 18 samples were discarded due to improper submission of samples and mainly due to lack of diagnostic kits especially in Toxicology due to travel restrictions

About 49% of the samples comprised for routine, 37% for research, 7% for disease outbreaks, 6% disease screening, 1% regulatory for BAFRA, and <1% for clinical cases (Fig. 1)

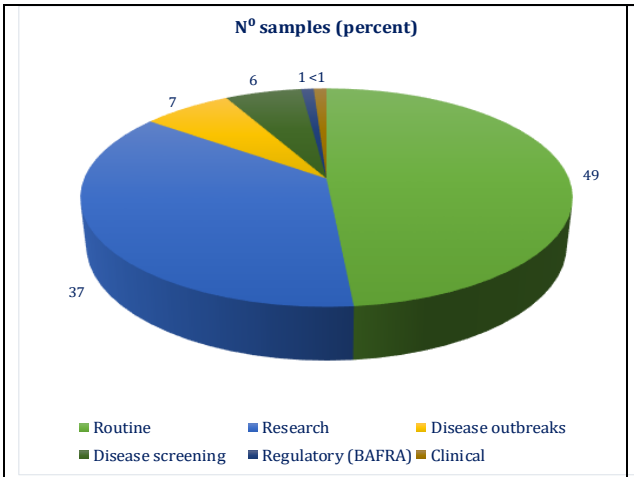


*Figure 1: Various categories of samples received and processed*

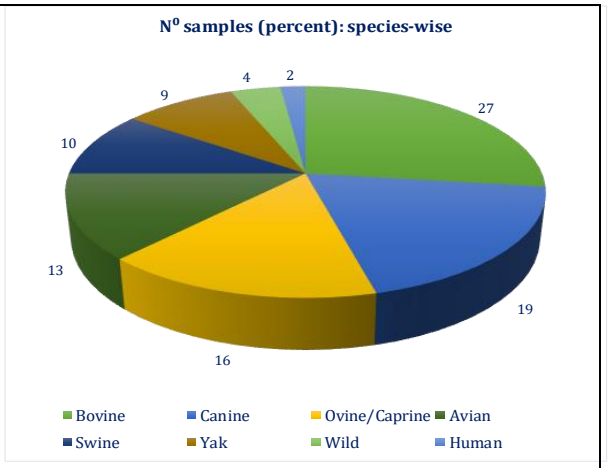


*Figure 2: Species-wise sample category*

The samples comprised of various species includes, 27% from bovine, 19% canine, 16% ovine/caprine, 13% avian, 10% swine, 9% yaks, 4% from wild animals, and 2% from humans (Fig. 2).

## 6. ACHIEVEMENTS OF INDIVIDUAL SECTIONS

### 6.1 Pathology section

A total of 250 animal carcasses and 810 tissue samples were received and examined in the pathology section in Table 3.

**Table 3: Samples and tests performed in the Pathology section**

| Specimen type | Number | Test type | Number |
|---|---|---|---|
| Tissue, organs | 872 | Histopathology- H & E Staining | 1217 |
| Biopsy | 3 | Giemsa staining | 3 |
| Carcass | 176 | Acid Fast staining | 7 |
| | | Post-mortem/Necropsy | 172* |
| **Total** | **1051** | | **1399** |

* 4 unfit for necropsy

**Significant findings**

**Histopathology**: Common cases diagnosed were IBD, ALC, Pneumonic Pasteurellosis and PPR in Takin.

**Post-mortem:** Erysipelas in swine, and ALC, Newcastle disease and Infectious Bursal Disease in poultry, and Canine distemper in dogs.

### 6.2 Parasitology section

A total of about 2,149 samples were received and 2,909 tests were performed by the section. The details of tests performed by this section are shown in Table 4.

**Table 4: Sample and test performed in Parasitology section**

| Specimen type | Number | Test type | Number |
|---|---|---|---|
| Faecal samples | 1737 | Direct examination, Sedimentation, Stoll's dilution, Floatation | 1777 |
| Soil samples | 399 | Test validation for taeniid egg isolation from soil | 8 |
| Skin scrapping | 2 | 10% KOH digestion | 2 |
| Blood smear | 10 | Giemsa staining | 10 |
| Intestine | 1 | Direct smear | 1 |
| **Total** | **2149** | | **2909** |

During the year, the section commonly detected parasitic infestations through the microscopic detection of eggs of *Fasciola, Coccidia, and Ascaris* in bovine and Taeniasis in stray dogs.

### 6.3 Bacteriology Section

About 544 different samples were received/collected and 3,976 different tests were conducted. The detail of the samples tested in the bacteriology section is as shown in Table 5.

**Table 5: Sample and test performed in Bacteriology section during 2019-20**

| Specimen type | Numbers | Test type | Numbers |
|---|---|---|---|
| Blood smear | 10 | Culture | 1023 |
| Whole blood | 3 | Gram stain | 270 |
| Aspirate fluid | 2 | Motility | 256 |
| Urine | 2 | Sensitivity test | 82 |
| Thoracic fluid | 2 | CMT | 80 |
| Organs | 38 | White Side test | 80 |
| Cloacae swab | 73 | Cell count | 77 |
| Buccal swab | 16 | Bio-chemical test | 1554 |
| Wound swab | 9 | Acid Fast stain | 1 |
| Vaginal swab | 20 | Methylene Blue Stain | 4 |
| Ear swab | 10 | Inoculation test | 140 |
| Saliva swab | 1 | Giemsa stain | 1 |
| Pus swab | 6 | Pour Plate Techniques | 280 |
| Ocular swab | 4 | LPCB Stain | 58 |
| Milk | 124 | Microbial Library for *Staph. aureus* | 40 |
| Nasal swab | 52 | Microbial Library for *E. coli* | 30 |
| Impression smear | 3 | | |
| Froth swab | 4 | | |
| Tracheal swab | 1 | | |
| Foot swab | 1 | | |
| Skin scraping | 53 | | |
| Soil Sample | 90 | | |
| Aborted Foetus | 5 | | |
| Ear Tip in 50% Glycerol | 1 | | |
| Ice cream | 1 | | |
| Cheese | 7 | | |
| Yogurt sample | 4 | | |
| Yogurt culture | 2 | | |
| **Total** | **544** | | **3976** |

**Significant findings**

Significant findings include *Streptococcus equi* from Strangles in horses, *Streptococcous uberis, S. agalactiae* & *S. dysgalactia* from mastitis in cattle of NJBC, and *Pasteurella* from the Takins. Furthermore, *Clostridium* was isolated from cheese samples.

### 6.4 Bio-Chemistry & Toxicology section

In the Toxicology section, 40 feed samples were screened against Aflatoxins. Serum biochemistry was performed in 20 samples. Also, 21 urine samples were screened against various parameters to assess the health of the animals at NJBC, Samtse.
Details of samples and tests conducted in this section are presented in Table 6.

**Table 6: Sample type and the tests conducted in Bio-Chemistry & Toxicology section**

| Specimen type | Number | Test type | Number |
|---|---|---|---|
| Feed | 40 | Aflatoxin | 98 |
| Serum | 20 | Mineral bio-chemistry | 210 |
| Urine | 21 | Urine biochemistry | 30 |
| **Total** | **81** | | **338** |

**Significant findings**
While conducting Aflatoxin test in the animal feed, only one tested positive from a private poultry farm in Thimphu.

### 6.5 Haematology section

Basic haematological tests were also conducted to support the clinical diagnosis in the animals. Details of samples and tests conducted in these sections are presented in Table 7.

**Table 7: Sample type and the tests conducted in Haematology section**

| Specimen type | Number | Test type | Number |
|---|---|---|---|
| Blood smear | 8 | PCV | 212 |
| Whole blood | 227 | Hb | 212 |
| | | DLC | 231 |
| | | TRCC | 200 |
| | | TWCC | 200 |
| | | Knott's test | 20 |
| **Total** | **235** | **Total** | **1,095** |

### 6.6 Serology Section

The section received/collected 1664 samples and carried out 1877 different types of tests as described below in table 8.

**Table 8: Sample type and the tests conducted in Serology section**

| Specimen type | Numbers | Test type | Numbers |
|---|---|---|---|
| | | RBT | 383 |

| | | | |
|---|---|---|---|
| | | IBR ELISA | 13 |
| | | Brucella ELISA | 252 |
| | | *Mycoplasma gallisepticum* Ab | 11 |
| | | PPR ELISA | 59 |
| | | CSF ELISA | 2 |
| | | PPR rapid | 18 |
| | | BDV ELISA | 13 |
| Serum | 1662 | *Mycoplasma synoviae* Ag rapid | 12 |
| | | *Salmonella pullurum* Ag rapid | 11 |
| | | Brucella Agglutination (Human) | 33 |
| | | RAPINA | 99 |
| | | Rapid EIV | 5 |
| | | IBD ELISA | 30 |
| | | CCHF ELISA | 936 |
| Swabs/Organs | 2 | Rapid IBD | 2 |
| **Total** | **1664** | | **1877** |

**Significant findings**

Important findings in serology includes *Brucella* antibody from cattle in NJBC, Samtse, and CRC, Wangkha; CSF in pigs; CCHF in goats; IBD, *Mycoplasma* and *Salmonella* in poultry; and PPR in Takin. The section also performed proficiency testing for RBT for Brucellosis for four RLDCs.

### 6.7 Virology section

The section received/collected 456 samples and carried out 400 different types of tests as described below in table 9.

**Table 9: Sample type and the tests conducted in Virology section**

| Specimen type | Numbers | Type of test | Numbers |
|---|---|---|---|
| | 15 | FAT | 15 |
| Serum | 47 | FMD NSP(Rapid) | 47 |
| Cloacae swab | 43 | Rapid AI | 6 |
| Swabs/Organs | 2 | Rapid NDV | 2 |
| Bursa swab | 11 | Rapid IBD | 2 |
| Tracheal swab | 57 | NDV | 47 |
| Cloacae swab | 35 | AIV | 45 |
| Eye swab | 1 | CDV | 1 |

| | | | |
|---|---|---|---|
| Bursa swab | 11 | CPV | 1 |
| Serum | 234 | IF for CCHF | 234 |
| **Total** | **456** | | **400** |

**Significant findings**
Important findings in virology include rabies detection by FAT in canines and Canine distemper virus.

### 6.8 Molecular diagnostic section

The section received/collected 456 samples and carried out 400 different types of tests as described below in Table 10.

**Table 10: Sample type and the tests conducted in Virology section**

| Specimen type | Numbers | Test type | Numbers |
|---|---|---|---|
| Swabs/Organs | 13 | PCR PRRS-NA | 15 |
| Bacteria colony | 13 | PCR PRRS-EU | 15 |
| Organ | 4 | Conventional PCR Brucella. | 15 |
| Bacteria colony | 4 | Conventional PCR Brucella. | 6 |
| Bacteria colony | 6 | Conventional PCR-Anthrax | 6 |
| Swabs/Organs | 9 | Conventional PCR erysipelas | 8 |
| Organ | 25 | PPMV RT PCR | 11 |
| Organs/Swab | 10 | CSFV PCR | 25 |
| Swabs/Organs | 19 | PCR FMD | 12 |
| Organs/Swab | 24 | PCR AI | 19 |
| Organs/Swab | 3 | PCR NDV | 36 |
| Swabs/Organs | 5 | PCR PPMV | 5 |
| | | PCR-ASF | 15 |
| **Total** | **135** | | **188** |

**Significant findings**
Important findings in molecular biology include PPMV in pigeon, NDV in poultry, FMDV in bovine, and CSF in swine.

### 6.9 Bio-safety and Bio-security section

The section is mandated to implement and monitor bio-safety and bio-security measures in the daily laboratory activities. Thus, this section is an aide-de-section for all other sections. Followings are the activities completed by this section:

- Routine Bio-safety monitoring in the laboratory;
- Developed SOP for Laboratory Waste Management with the support of Fleming Fund;
- Co-ordinate the maintenance of laboratory equipment;
- Developed the incident report form, weekly equipment inspection form, equipment maintenance form, and also laboratory auditing checklist;
- Visited NVH, Motithang, for monitoring and evaluation of biosafety activities in the laboratory;
- Maintained monthly temperature for fridges, incubators, and deep freezers;
- Issued laundry basket and container for all the sections;
- The section conducted internal auditing for the national laboratory to as a part of monitoring of bio-safety and bio-security measures;
- Conducted refresher training on biosafety & biosecurity for the staff of NVL and BPU.

National Veterinary Hospital, Motithang was visited as a follow up visit. There were a significant improvement and necessary changes made as per the recommendations provided earlier. However, it was noticed that the focal person for bio-safety & biosecurity was not nominated, also SOP for equipment maintenance and monitoring of laboratory visitors were yet to be developed.

## 7. INTRODUCTION OF NEW TESTS

During the financial year 2019–2020, the following new diagnostic technologies for important diseases were established:

a) Serological tests
   - Introduction of ELISA for CCHF
   - Introduction of RAPINA test for Rabies

b) Cell culture facilities established; Acquired $CO_2$ incubator

c) Bacterial techniques
   - Isolation, identification & Antimicrobial sensitivity testing of *Streptococcus agalactiae*
   - SOP developed for isolation, identification & Antimicrobial sensitivity testing of *Campylobacter* and *Enterococci*

d) Parasitological techniques: Tick identification and validation of isolation of Taeniid eggs from environmental soil samples.

## 8. SAMPLES REFERRED TO INTERNATIONAL LABORATORIES

Due to travel restriction and cancellation of flights in light of COVID 19, samples could not be referred outside to international laboratories (Table 11).

**Table 11: Sample referred to international laboratories**

| Species of animals | Sample type | Samples referred to | Numbers of samples | Remarks |
|---|---|---|---|---|
| Other(soil) | DNA extracted in | NIID, Japan | 1 | Anthrax |

| | | | | |
|---|---|---|---|---|
| | FTA card | | | |
| Takin | Serum, Ocular & Nasal swab in PBS, Organs in PBS & whole blood in FTA card | NIAH, Bangkok | 35 | PPR from Takins at JDWNP (Could not be referred due to travel restriction and cancellation of flights) |

## 9. LABORATORY QUALITY ASSURANCE

### 9.1 Proficiency testing on Rose Bengal Test for screening of Brucellosis in animals

Proficiency testing (PT) on Rose Bengal Test (RBT) was conducted by National Veterinary Laboratory, National Centre for Animal Health, Serbithang as a part of National External Quality Assurance System for regional laboratories (2019-20)

#### 9.1.1 Introduction

Proficiency testing (PT) is a part of laboratory quality assurance system (QAS) to ensure a test procedure consistently produces quality result. Proficiency testing along with various other components of QAS such as record keeping, quality control, training, evaluation, calibration, monitoring, taking corrective actions and competency assessment will contribute to quality management of a laboratory. Staff performing test must be qualified, their competency documented, trained in the areas of specific requirement, should be able to perform intended test and evaluate result. Proficiency testing samples are sent to participating laboratories for a specific testing method and results are reported to the coordinating laboratory for analysis. The coordinating laboratory then collates the results and ranks participating laboratories based on their testing performance. Details of performance of participating laboratories shall be anonymous. This anonymity allows participating laboratories to see trends in their own testing performance and to compare with other laboratories. The coordinating laboratory shall individually convey performance of each participating laboratory with details of their strength, weakness and recommendation.

#### 9.1.2 The disease: Brucellosis

Brucellosis is an infectious zoonotic disease caused by bacteria of the genus Brucella. Usually, Brucellosis in cattle is caused by *Brucella abortus*, *Brucella suis* in swine and *Brucella melitensis* in sheep and goats. Brucellosis cause abortion or birth of weak calves and infertility. Brucellosis is commonly transmitted to susceptible animals by direct contact with infected animals or with an environment contaminated by discharges from infected animals. As the disease primarily localizes in the udder and/or reproductive organs of animal, the milk, aborted foetuses, placental membranes, fluids and other reproductive tract discharges of an infected animal are highly contaminated with infectious Brucella organisms. The disease may also be spread when wild animals or animals from an affected herd mingle with Brucellosis-free herds. The general rule is that Brucellosis is carried from one herd to another by an infected or exposed animal.

Brucellosis poses serious public health risk when humans are infected. Human infection with Brucella organisms usually occurs through occupational contact with discharges from infected animals, particularly through calving, but also through slaughtering or ingestion of unpasteurised dairy products.

### 9.1.3 The test: Rose Bengal Test (RBT)

The Rose Bengal Test (RBT) is a rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum. Serum (25-30 µl) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter. The mixture is rocked gently for 4 minutes at ambient temperature and then observed for agglutination (1). Any visible reaction of agglutination is considered to be positive. The test is very sensitive, especially in vaccinated animals and positive samples should be retested by a confirmatory test such as the complement fixation test (CFT) or enzyme-linked immunosorbent assay (ELISA). False-negative reactions may occur and can be detected by retesting animals at intervals over a period of at least 3 months.

### 9.1.4 Importance of proficiency testing

From the data collected during proficiency testing, laboratory managers can identify staff that may require further training. This therefore leads to more consistent working practices throughout the laboratory. Regular testing also keeps the team focused on how routine procedures should be carried out. Proficiency testing can provide an opportunity to further educate staff in the potential areas of testing. Training allows staff to appreciate their contribution to the output quality of their laboratory. Staff can then also appreciate their role in the success of their laboratory. Anyone who is trained and is regularly involved in routine testing needs to be included in proficiency testing.

### 9.1.5 Brucella RBR-PT for fiscal year 2019-20

During the fiscal year 2019-20, as a part of national external quality assurance (NEQAS) in laboratory test performance, the National Veterinary Laboratory, National Centre for Animal Health, Serbithang organized a round of proficiency testing with regional animal health laboratories at Regional Livestock Development Centres on Rose Bengal Test for screening Brucellosis in cattle. The main objective of this PT was to assess the performance of different regional laboratories in screening of Brucellosis in cattle. Details of coordinating and participating laboratories in PT are as follows:

1. Coordinating laboratory
    a. National Veterinary Laboratory, National Centre for Animal Health, Serbithang
2. Participating laboratories
    a. Regional Livestock Development Centres, Kanglung
    b. Regional Livestock Development Centres, Tsimasham
    c. Regional Livestock Development Centres, Wangdue
    d. Regional Livestock Development Centres, Zhemgang

### 9.1.6 Proficiency testing panel

Twenty serum samples collected and archived at National Veterinary Laboratory, National Centre for Animal Health, Serbithang were identified. These samples were tested with RBT and ELISA to determine the level of agglutination reactivity in RBT and optical density (OD) values in ELISA. Accordingly, samples were classified as negative or positive. Depending on the level of reactions among positive samples (n =11), they were further classified as + (positive), ++ (positive) and +++ (strong positive). Panel also included a set of negative samples (n = 9), one positive control and one negative control. Antigen (Rose Bengal stained *B. abortus* suspension) was also supplied along with serum samples.

### 9.1.7 Test procedure

Following steps are the guide to perform RBT at each participating laboratory.

- Bring antigen and test/panel sera to room temperature (20°C ± 3°C)
- Pipette 25 µl of panel serum and place on agglutination plate (white smooth tile) leaving about 4 cm distance between each serum sample
- Similarly, pipette 25 µl of positive and negative control serum and place on the agglutination tile
- Pipette 25 µl of RBT antigen and place next to each serum sample and controls. The antigen and serum should not be mixed while placing on tile
- Once all antigen and serum samples are placed on tile, start mixing with clean tooth pick in a circular fashion to develop a uniform border line of the mixture.
- Match stick also can be used for this purpose. When match sticks are used, use only its tail end
- Set timer as soon as mixing is started
- Ten samples can be tested at one go to minimise delay in time between the addition of antigen to the first and last serum
- Hold the plate and oscillate gently for about 4 min; 20-25 oscillation is good to mix the antigen and serum properly
- Read the results under bright light depending on the level of reaction (agglutination or no agglutination)
- Read the results within 10 min after mixing of antigen and serum
- Read the results of control sera first, then the panel sera
- Record result in the provided form (Appendix 1)
- Negative and positive control serum should be used for each batch of panel serum tested

Table 1: Distinction of degrees of reaction

| Reactivity | Description | Interpretation |
|---|---|---|

| | | |
|---|---|---|
| 0 | No agglutination, no flakes | Negative (N) |
| + | Barely perceptible agglutination. May be doubtful | Positive (P/D) |
| ++ | Fine agglutination, definite flakes and some clearing | P |
| +++ | Coarse clumping, definite clearing | SP |

Note: 0, N (Negative); +, P/D (Positive/Doubtful); ++, P (Positive), +++, SP (Strong positive)

### 9.1.8 Analysis

**Collation of RBT results**

The participating laboratories were coded as laboratory code 1, 2, 3 and 4 to maintain the anonymity of test results among all participating laboratories. The test results of all participating laboratories were collated and compared with the results of coordinating laboratory. The results were collated as reported by participating laboratories. (Table 2)

Table 2: Collated RBT result of coordinating and participating laboratories

| | NVL, NCAH | | LAB CODE 1 | | LAB CODE 2 | | LAB CODE 3 | | LAB CODE 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| SI no | Result | Intpn | Result | Intpn | Result | Intpn | Result | Intpn | Result | Intpn |
| 1 | ++ | P | ++ | P | ++ | P | ++ | P | ++ | P |
| 2 | + | P | ++ | P | ++ | P | + | P | 0 | N |
| 3 | + | P | ++ | P | ++ | P | + | P | + | D |
| 4 | +++ | SP | +++ | SP | +++ | SP | +++ | SP | ++ | P |
| 5 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 6 | +++ | SP | +++ | SP | +++ | SP | +++ | SP | +++ | SP |
| 7 | +++ | SP | ++ | P | ++ | P | + | P | 0 | N |
| 8 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 9 | + | P | + | P | ++ | P | + | P | + | D |
| 10 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 11 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 12 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 13 | + | P | + | P | ++ | P | + | P | 0 | N |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | +++ | SP | ++ | P | ++ | P | + | P | 0 | N |
| 15 | + | P | +++ | SP | +++ | SP | ++ | P | + | D |
| 16 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 17 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 18 | 0 | N | 0 | N | 0 | N | 0 | N | 0 | N |
| 19 | 0 | N | + | P | ++ | P | + | D | 0 | N |
| 20 | + | P | ++ | P | +++ | SP | + | P | + | D |

**Analysis of test result**

Analysis of test results is shown in Table 3. Positive samples: Three laboratories (Lab code 1, 2 and 3) identified all 11 true positive samples as positive, thus had estimated diagnostic sensitivity of 1.0. Whereas, one laboratory (Lab code 4) diagnosed 4 true positive samples as negative (false negative) resulting into estimated diagnostic sensitivity of 0.64.
Negative samples: Only one laboratory (Lab code 4) identified all 9 true negative samples as negative and received an estimated diagnostic specificity of 1.0. Whereas, three laboratories (Lab code 1, 2 and 3) diagnosed one true negative sample as positive (false positive) resulting into the estimated diagnostic specificity of 0.89.
An ideal test is the one with diagnostic estimates of 1.0 (sensitivity and specificity). Unfortunately, there is no commercial test available with diagnostic estimate as 1.0. The diagnostic estimates reported here for all participating laboratories and coordinating laboratory are only relative estimates. However, these estimates are useful in recognizing the strength and weakness in the testing capacity of each laboratory and provide directions for improvement.

**Laboratory 1, 2 and 3**

a. Did not have issue in identifying true positive samples as positive irrespective of samples having different intensity of agglutination/reactions
b. Had difficulty in correctly identifying different intensity of agglutination among positive samples
c. Had difficulty in identifying true negative sample as negative
d. There is a need to improve on reducing the rate of false positive test result

**Laboratory 4**

a. Did not have issue in identifying true negative samples as negative
a. Had difficulty in identifying true positive samples as positive
b. There is a need to improve on reducing the rate of false negative test result

Table 3: Calculation of diagnostic estimates (sensitivity and specificity)

| Sl. No. | Parameters | LAB CODE 1 | LAB CODE 2 | LAB CODE 3 | LAB CODE 4 |
|---|---|---|---|---|---|
| 1 | True positive | 11 | 11 | 11 | 7 |
| 2 | True negative | 8 | 8 | 8 | 9 |
| 3 | False positive | 1 | 1 | 1 | 0 |
| 4 | False negative | 0 | 0 | 0 | 4 |
| 5 | Sensitivity | 1.00 | 1.00 | 1.00 | 0.64 |
| 6 | Specificity | 0.89 | 0.89 | 0.89 | 1.00 |

### 9.1.9 Conclusion

The result analysis was performed based on the ability of participating laboratory to correctly identify true positives and positive and true negatives as negative. Owing to the small size of PT panel, analysis on the ability of laboratories to correctly identify different intensities of reaction among positive samples were not performed. Although RBT is a very sensitive test, it is also a highly subjective test in terms of result interpretation. Performing and interpreting RBT requires high level of experience. Therefore, performing this test and accurately interpreting result can be gained only regular practice. This applies to all the participating laboratories and work towards improving their performance.

### 9.1.10 Reference

1. Morgan W.J.B., MacKinnon D.J., Lawson J.R. & Cullen G.A. (1969). The rose bengal plate agglutination test in the diagnosis of brucellosis. Vet. Rec., 85, 636– 641.

### Appendix 1: Result recording sheet

| TEST RESULT REPORTING FORMAT FOR PT ON BRUCELLA RBT |
|---|

| | | | |
|---|---|---|---|
| | Lab name | Your laboratory name | |
| | Lab code | Mentioned in the cryo box of PT panel | |
| | | RBT | |
| | | Test performed on: | Test performed date |
| | | Antigen supplier: | NCAH, Serbithang |
| | Tube ID: | Result | Interpretation (N/D/P/SP) |
| Positive control | | + | P |
| Negative control | | 0 | N |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 1: Enter the raw results (0, +, ++, +++) depending on the intensity of the reaction | | | |
| 2: N = Negative; Positive (P/WP) for + or doubtful (D); Positive (P) for ++ and Strong positive (SP) for +++ | | | |
| FOR THIS PROFICIENCY TESTING, REPORT ONLY NEGATIVE (N), POSITIVE (P) AND STRONG POSITIVE (SP). IF POSSIBLE “D” FOR DOUBTFUL | | | |
| Comments: | | | |
| Name and designation of the official performing test | | Signature | |

## 9.2 Assessment of the National Antimicrobial Resistance Surveillance System in Food and Agriculture sectors

**Towards global surveillance of Antimicrobial Resistance**

**Mission Report 2-4 September 2019**

| **Assessors** | |
|---|---|
| Dr Shawn Ting | FAO Regional Office for Asia and the Pacific |
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| Ms Rangsiya Prathan | Chulalongkorn University, Thailand |

Food and Agriculture Organization of the United Nations
Rome, 2019

### 9.2.1 Executive summary

The ATLASS mission to Bhutan was conducted from 2-4 September 2019 with the following objectives: 1) map the structure of the national antimicrobial resistance (AMR) surveillance system in the food and agriculture sectors, including laboratory capacities and network; 2) identify prioritized steps for action and advocacy to strengthen the national AMR surveillance system through a stepwise approach; 3) establish the baseline country capacities for AMR monitoring and surveillance in the food and agriculture sectors; and 4) describe the linkages with AMR surveillance in other sectors in support of the One Health approach.

AMR surveillance in the animal sector is coordinated by the Disease Prevention & Control Unit (DPCU) based in the National Centre for Animal Health (NCAH). There have been several prevalence studies on AMR in terrestrial animal and animal products between 2007 and 2017. NVL conducts passive AMR surveillance for clinical specimens submitted by veterinarians. Active AMR surveillance has been planned. There has been a WHO Advisory Group on Integrated Surveillance of Antimicrobial (AGISAR) project to test for resistance in *Salmonella* spp in humans, livestock and food, as well as in *E. coli* in pigs.

Antimicrobial usage (AMU) surveillance in the animal sector is coordinated by the Drug, Vaccine and Equipment Unit (DVEU), based in the NCAH. AMU results are submitted to the World Organisation for Animal Health (OIE) annually using reporting option 1.

Antibiotic residue surveillance in the animal sector is coordinated by the National Food Testing Laboratory (NFTL) under the Bhutan Agriculture & Food Regulatory Authority (BAFRA). There are limited samples collected from poultry, pork and fish samples to test for drug residues using rapid test kits, and is performed on an ad-hoc basis.

Overall, the AMR surveillance system of Bhutan was assessed to be at country Stage 1 (Limited capacity) on the Progressive Improvement Pathway (PIP). The following strengths were identified

1. "Governance" pillar:

   o Institutional arrangements for AMR are clearly identified and formalized [i.e. Inter-Ministerial Committee for One Health (IMCOH), National AMR Technical Committee (NATC), Technical Working Groups] and

   o National Action Plan has performance indicators, timelines, and lead/partner agencies identified.

2. "Data collection and analysis" pillar

o Implementation of electronic database [i.e. Laboratory information management system (LIMS) and WHONET] to manage data generated from AMR surveillance system.

3. "Data production" pillar
   o All laboratories have agreed to use the same standards and methods for AST, which will enable a high level of harmonization of data generated from the AMR laboratory network.

4. "Communication" pillar
   o There is regular release of surveillance results through reports (including AMR surveillance results) to the Department of Livestock every quarter, and an annual report is publicly available online.

To achieve country PIP Stage 2 (Moderate capacity) for the AMR surveillance system, Bhutan should prioritize actions to improve AMR data collection and analysis, particularly, to implement active AMR surveillance in the poultry sector. This is foreseen to progress very soon, with the implementation of AMR surveillance work through the Fleming Fund Country Grant which will commence in late 2019.
To achieve country PIP Stage 3 (Developed capacity) for the AMR surveillance system, the following second-line priorities were identified:

• "Governance" pillar
  - Establish a specific list of antibiotics for different groups of organisms (e.g. one for gram-negatives and a separate list for gram-positives) relevant to the country, and consider inclusion of resistance phenotypes of public health concern such as ESBL *E. coli*, Vancomycin-resistant Enterococci (VRE), and Methicillin-resistant Staphylococcus aureus (MRSA), and;
  - Develop strategy to expand AMR surveillance to include additional livestock species (e.g. cattle).

• "Data collection and analysis" pillar:
  - Expand active AMR surveillance to cover more surveillance sites;
  - Involve RLDCs in passive AMR surveillance;
  - Strengthen skills of central epidemiology unit (i.e. Disease Prevention & Control Unit) specific for AMR epidemiology through appropriate training and knowledge-building; and
  - Perform regular analysis of AMR data at pre-defined intervals [e.g. analyse trends, emerging resistance, increasing minimum inhibitory concentrations (MIC)]

• "Data production" pillar:
  - Improve the capacity of all laboratories involved in the AMR surveillance network to obtain at least PIP stage 3;
  - Build capability for *Campylobacter*, *Enterococcus* spp, as well as detecting and characterizing specific resistance phenotypes, such as ESBL *E. coli*, VRE and MRSA; and

- Develop capability for MIC testing.

- "Communication" pillar:
- Use surveillance data for risk assessment and convey outcome to relevant parties;
- Train relevant personnel in risk assessment, if needed; and
- To intentionally distribute a summary of surveillance results to field actors (farmers, attending farm vets) at regular intervals.

As the national reference laboratory for AMR, the National Veterinary Laboratory (NVL) was assessed and classified to be at lab PIP Stage 2. The following strengths were identified for NVL:

- SOPs for bacterial isolation and identification were updated, with the latest updated in 2018
- Antibiotic disk diffusion test is performed according to recommendations from Clinical and Laboratory Standards Institute (CLSI).
- There are sufficient facilities and equipment for bacteriology, antibiotic susceptibility testing (AST), basic molecular techniques [thermocycler and real-time polymerase chain reaction (PCR)] and storage (-80$^0$C freezer)
- There is strong collaboration with national and international institutions
- Data management and transmission is well performed. Computerized systems including WHONET and LIMS are used for data collection, together with paper documentation. Data captured and analysed in WHONET by NVL is also shared with relevant laboratories.

The following recommendations will allow NVL to advance to lab PIP Stage 3:
• *Technical capacities*: Improving access to quality reagents for AST.
• *Quality assurance*: AST should be concurrently performed for the test isolate and the appropriate strain (as recommended by CLSI) to ensure that AST results obtained are valid, and NVL should participate in proficiency testing for AST.

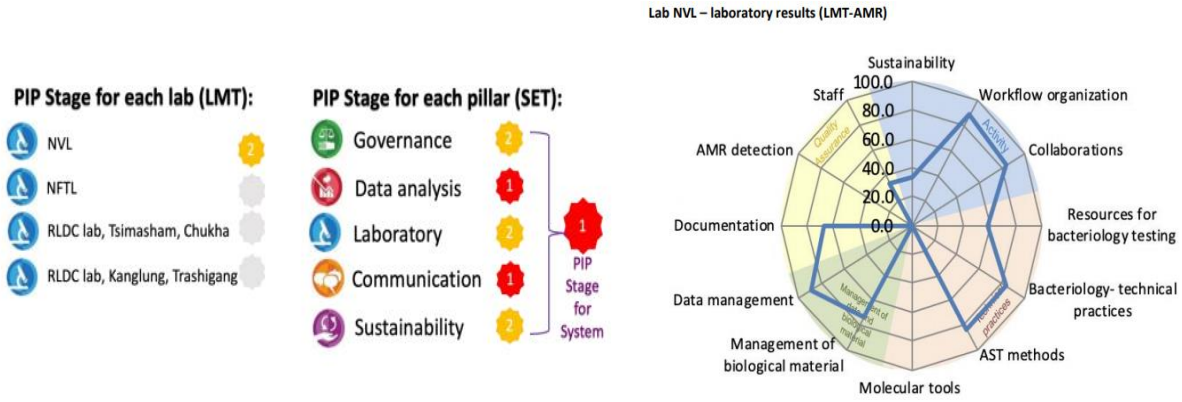



*Figure 4. Progressive Improvement Pathway (PIP) Stage for the Laboratory Mapping Tool (LMT) and Surveillance Evaluation Tool (SET) of the FAO ATLASS Assessment. NVL is at lab PIP Stage 2, while Bhutan is at country PIP Stage 1.*

### 9.2.2 Conclusion

In conclusion, Bhutan is at country Stage 1 on the ATLASS PIP but will likely move forward towards PIP Stage 2 with the implementation of planned AMR surveillance under the

Fleming Fund Country Grant. Furthermore, specific actions to advance four of the five pillars (Governance, data collection and summary, data production, and communication), will move the country further up towards PIP Stage 3 (developed capacity).

## 10. BIOSAFETY & BIOSECURITY MONITORING

### 10.1 Laboratory Auditing

Under the Bio-safety monitoring and evaluation program, Bio-safety unit carried out auditing of the Laboratory services unit on monthly basis. During the monitoring and evaluation program, the feedbacks were also collected and necessary advice/recommendations were provided thereof.

The unit also visited the laboratory of National Veterinary Hospital (NVH), Motithang, in January 2020 as a part of monitoring program.

#### 10.1.1 Objective of the visit

The visit was conducted to monitor the bio-safety practices followed at the veterinary laboratories.

#### 10.1.2 Methods

a. Discussion on Bio-safety and Good Laboratory Practices of the Centre.
b. Discussion on lab related issues encountered and suggestions.
c. Inspection to the lab

A. Laboratory staffs working and In-charge:

a. Dr. NimaWangdi- Lab In-charge
b. Mrs. Punya Mata, Sr. Lab Technician-Haematology section
c. Mrs. Pema Tshomo, Sr. Lab Assistant-Parasitology Section
d. Mrs. KinzangPalden, Lab Technician-Biochemistry Section

#### 10.1.3 Improvement from last visit

a. It was great to know that there was **Lab Incharge** appointed who can look after any matter related with daily laboratory work hindrance faced by Lab workers and also in providing all necessity supports that might be needed while doing their regular works in maintaining Quality work and Biosafety measures which are very essential for the safety of lab workers.
b. It was found that the laboratory room and working bench were kept clean and not congested like last time. All equipment was arranged properly with **Equipment ID No.** with **Biohazard signs** for that equipment dealing with infectious samples and all forms were properly kept in **labelled tray and files.**
c. All the chemicals and reagents containers were labelled and stored in designated shelves.
d. All the lab coats and lab shoe were kept in proper designated area.
e. **Biosafety sign** was posted on entrance door.

f. There were designated **sharp containers** for disposing sharps and non-infectious waste bin was also labelled.

g. There is proper record keeping for sample tested.

h. Emergency exit well defined.

i. Eating and drinking is prohibited.

j. There is restriction for outsider from entering the lab.

### 10.1.4 Observation/Recommendation:

a) There was **No Biosafety Focal Person** appointed so that he/she can implement Biosafety practices in their lab and also NCAH can share any resources related with Biosafety in future.

b) All the laboratory staff working including Lab Technician and Lab attendant needs to be given at least **Awareness Training on Biosafety protocols** annually.

c) There were **No proper rules for restricting the entry of outsider** from entering the Lab for e.g. Visitor forms to be filling up and approved by the lab Incharge.

d) There was **No written signage** restricting outsider and also signage for "**NO EATING AND DRINKING IN LAB"**.

e) It was found that the Lab has **Autoclave machine** but it's **Not Functioning** at the moment. As per **WHO waste management guideline** it says all the infectious waste should be autoclaved before disposal be it blood, serum, swab or any sharp waste. Once the Bacteriology section is set up there will be need of separate Autoclave for disinfecting all the media cultures etc.

f) After A**utoclave** is repaired there will be need for maintaining **Register** for entering whether the machine is used. *NCAH can provide the sample of **QC Log book** after Biosafety focal are appointed. And also, appropriate autoclave bags or container should be used.*

g) National Veterinary Hospital should use Proper Colour Coded Waste Bins as per WHO laboratory Biosafety manual guideline. Under Fleming Fund activities, the SOP for Waste Management was developed and soon it will be provided to NVH for reference.

h) Use of Proper Personal Protective Equipment is mandatory so therefore it is important that all the staffs working in the lab should wear them while working in the lab. And inform the management or Incharge to provide PPE as and when needed.

i) Development of Operating Procedure for all the equipment is necessary as it will guide all the lab workers in how to operate the particular machine. For example if someone who is looking after the section is absent for a week instead of leaving the work pending someone working in lab can do his/her job.

j) There is need for **separate refrigerator** for keeping **reagents and samples** respectively. It was found that there was only one freezer with no thermometer to maintain the temperature.

k) There is need for maintaining **forms** for **Temperature log chart, incubator log chart, equipment registers, visitor forms and Autoclave log book** with respective files. ***NCAH can provide all the forms once Biosafety Focal is appointed.***

l) Currently the lab is using **70% Alcohol for disinfection**, it is advised that **1-2% Virkon and Bleaching powder** can also be used for disinfecting equipment and floor.

m) **First Aid Kits and Spill Kits** need to be purchased in times of any accident.

### 10.1.5 Conclusion

Most of the recommendations made during earlier visit were followed up. However, few of the recommendations were still pending. As discussed and decided, Biosafety Focal person will be appointed to carry out daily Biosafety activities in their centre and also it will be easier for NCAH to share any materials and to provide training if there is any training conducted in NVL in future. The above recommendations and findings need to be followed and is suggested to contact Bio-safety related issues with the Bio-safety Section at NCAH/LSU. The monitoring and evaluation of the basic Biosafety practices will be conducted regularly.

## 10.2 Monitoring equipment functionality

During the year, various laboratory equipment was maintained as described in Table 12.

**Table 12: Details of equipment rectified**

| SI No. | Name of equipment | Remarks |
|---|---|---|
| 1 | Automatic tissue processor (HP) | Issues rectified |
| 2 | Deep Freezer (DF01-corridor) | Issues rectified |
| 3 | Fume hood (motor malfunctioned) | Issues rectified |
| 4 | Biosafety class II -Master mix and molecular | Issues rectified but was faulty again |
| 5 | Refrigerator R-2 | Issue rectified |
| 6 | Biosecurity signage prepared 2 nos. | Installed at the entry gate |
| 7 | Autoclave (BPU) | Rectified |
| 8 | Cold room & warm room (BPU) | Rectified |

## 10.3 Incidence Monitoring and Reporting

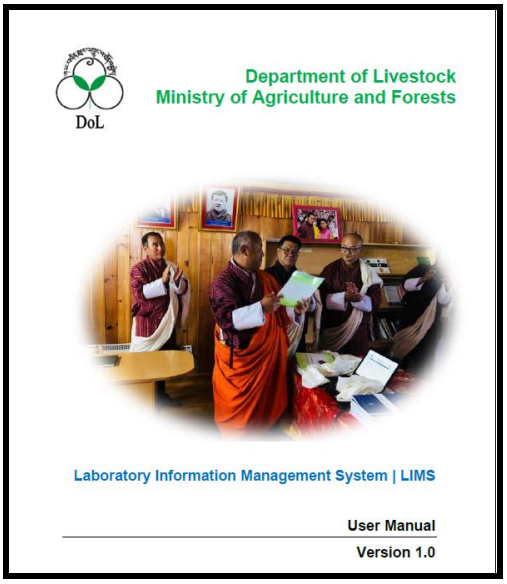
The incidences as encountered and reported are listed below in Table13.

**Table 23: Details of incidence reported and monitoring**

| Date | Incident | Action |
|---|---|---|
| 17.07.2019 | Exhaust fan of fume hood burnt and minor cut received by the technician | First aid provided |

# 11. LABORATORY INFORMATION MANAGEMENT SYSTEM (LIMS) – TRAINING MODULE DEVELOPMENT

Laboratory Information Management System (LIMS) is the online database system designed to efficiently manage the information of all the veterinary laboratory activities in the country. It has the features for online entry of sample details, test result, diagnosis and recommendation. The system helps the veterinary laboratories to track samples from submission to testing and reporting. This database enables real-time tracking of sample testing status through a paperless system. Besides data storage and test result dissemination, customized analysis can also be performed to provide decisions required in policy interventions. This system is intended for all the laboratory facilities under the Department of Livestock (DoL) viz. National Centre for Animal Health (NCAH), Regional Livestock Development Centres (RLDCs), Satellite Veterinary Laboratories (SVLs) and Dzongkhag Veterinary Laboratories (DVLs).




The generic functionality of any LIMS database can roughly be divided into five laboratory processing phases:

a) Reception and login of a sample and its associated customer data
b) Assignment, scheduling, and tracking of the sample and the associated analysis
c) Processing quality controls associated with the sample and equipment
d) Storage of data associated with the sample analysis
e) Inspection, approval and compilation of report or further analysis

The LIMS was launched online during July 2019 and the users were to be trained on it's usage. Accordingly, the training manual was developed. However, the training as proposed under Fleming fund could not be conducted due to the restriction of mass gathering.

## 11. ASSESSMENT OF ELECTRONIC RECORD SYSTEM

Assessment of electronic record system like Veterinary Information System (VIS), Transboundary Animal Disease (TAD) Info and Laboratory Information Management System (LIMS) was carried out by Advance Technical Corp, USA, voluntarily.

## 11.1 Summary of Recommendations

### 11.1.1 Urgent Priority

- Either attain direct access to VIS and LIMS databases or move VIS and LIMS to a server hosted by the Kingdom of Bhutan;
- Either attain direct access to VIS and LIMS source code or form an escrow source-code agreement with the vendor stating that the Department of Livestock will have full rights to VIS and LIMS should the vendor cease supporting either application;
- Determine vendor's commitment to attaining subject matter expertise and focus on veterinary medicine.

### 11.1.2 High Priority

- Initiate deep discussions about the future of LIMS and determine the costs in revamping the design to be more focused on operations;
  - Department of Livestock must have access to update reference tables (such as tests, specimens, etc.)
  - LIMS design has to be more cognizant of laboratory workflow and requirements (non-Pathology Cases do not require diagnoses)
  - Design of LIMS has to flow more smoothly and be more intuitive
- Determine a Department of Livestock staff member who can become more acquainted or even trained IT software development;
- NVH re-evaluate staffing needs in cost-sharing with clients, if a practice management system is deployed to optimize operations.

### 11.1.3 Medium Priority

- Plan for a complete overhaul of VIS as a secondary dataset derived from operations;
- Evaluate funding options with JICOA and / or Canadian partnerships;
- Establish the Department of Livestock's relationships with local World Bank representatives so that they are better aware of the impact of DoL programmes and needs;
- Include in DoL literature the idea of supporting sustainability by recommending practices that increase yield without increasing land consumption.

### 11.1.4 Options for LIMS and VIS

By our estimate based on the current rate of LIMS development, it will take an additional six months to get LIMS into reasonable shape for the handling of current operations. However, the design of LIMS is fixed based upon perceived laboratory requirements from two years ago. The needs of the laboratory will continue to evolve and LIMS will not have the capability to meet emerging requirements. For example, image capture, instrument interfacing and even the ability to directly email results are not part of LIMS. Their addition would require more customization and development.

The key problem is that the DoL will always be playing "catch up" with LIMS because LIMS has been created as a one-off custom programme.

## 12. ONE HEALTH ADVOCACY MEETING ON ANTIMICROBIAL RESISTANCE – OBSERVING WORLD ANTIBIOTIC AWARENESS WEEK (WAAW)

November 18th – 24trh is observed as *World Antibiotic Awareness Week* (WAAW) globally. The goal of the week was to raise awareness of the health risks posed by antibiotic resistance and to promote good practice in this area of concern, to limit the emergence and spread of resistant bacteria throughout the world. The theme is "The future of antibiotics depends on all of us".

Antibiotics have helped in saving millions of lives, reducing the disease burden in people and animals, contributed to improved food production and safety. Unfortunately, it's inappropriate use has led to the emergence and spread of antimicrobial resistance (AMR) in several microorganisms, complicating the management of many infectious diseases in human and animals. AMR also endangers food production.

Globally, about 70% of the antibiotics manufactured by pharmaceutical companies are used in veterinary practices that include production animals, companion animals and wildlife. This means a substantial amount of antibiotics are pumped into our food chain and environment. Weak compliance to regulations of prudent use of antibiotics will expose humans and animals to sub-therapeutic dose and facilitate resistance development.

It is also known that AMR has adverse effects in the functioning of human, animal and plant health systems. Hence, it requires multisector involvement (one health) to tackle the issue. Therefore, it is essential to preserve the efficacy by using responsibly and prudently in whatever areas we use.

To mark the WAAW, a half-day workshop on "**Advocacy on Antimicrobial Resistance to the One Health Stakeholders"** was held at National Veterinary Hospital, Motithang on 20th November 2019. The main objectives of the workshop were to create awareness to various stakeholders, on the AMR and to encourage the prudent usage of antibiotics in respective areas.


His Excellency Lyonpo Yeshey Penjor, Hon'ble Minister of Ministry of Agriculture and Forests, graced the occasion as Chief Guest. The program was attended by WHO Representative to Bhutan and Expert from the World Organisation for Animal Health (OIE).

About 64 participants attended the advocacy program representing various agencies such as Department of Livestock, Bhutan Agriculture & Food Regulatory Authority, Department of Agriculture & Department of Forests & Park services under Ministry of Agriculture and Forests and Department of Medical Services and Department of Public Health under Ministry of Health, Drug Regulatory Authority and officials from Thimphu Dzongkhag Administration. The program was organised with the generous fund support from WHO and OIE.

## 13. FLEMING FUND COUNTRY GRANT

The Fleming fund country grant was implemented with an inception phase of six months (April to September 2019) was initiated with a focus on strengthening the governance, v.i.z. office setups and infrastructure development. The phase, however, had to be postponed till December 2019.

### 13.1 Infrastructure development/Office set up

During the inception phase, mainly infrastructure development was considered. The details are listed below in Table. 14

**Table 34: Details of Infrastructure development**

| Sl. | Particulars | Location |
|---|---|---|
| 1 | • Installation of Reverse Osmosis (RO) Plant | NCAH, Serbithang |
| 2 | • Installation of benchtop<br>• Installation of the Climate control unit (AC)<br>• Installation of emergency shower<br>• Hand sanitizing station<br>• Installation of water storage tanks | LSU, NCAH Serbithang |
| 3 | • Installation of benchtop<br>• Installation of the Climate control unit (AC) | RLDC Kanglung |
| 4 | • Installation of benchtop<br>• Installation of the Climate control unit (AC) | RLDC, Tshimasham |
| 5 | • Installation of benchtop<br>• Installation of the Climate control unit (AC) | NFTL, Yusipang |

During the inception phase, various office equipment was also procured and distributed to the surveillance laboratories. The details are listed below in Table 15.

**Table 45: Details of office equipment**

| Sl. No. | Item | Quantity |
|---|---|---|

| | | |
|---|---|---|
| 1 | Lap top (mid-end) | 3 |
| 2 | Lap top (high end) | 3 |
| 3 | Desktop | 8 |
| 4 | Multi-function printer | 5 |
| 5 | Projector | 1 |
| 6 | Lamination machine | 1 |
| 7 | Office furniture | 1 |
| 8 | Visitor chair | 30 |
| 9 | Steel almirah | 2 |
| 10 | Computer table | 8 |
| 11 | Stationaries | 1 |

## 13.2 Detailed Technical Implementation

The detailed implementation of the technical activities started in January20 with sensitization workshops and other workshops and training as follows:

### 13.2.1 Sensitization workshop on Antimicrobial Resistance (AMR) and Fleming Fund Project Activities

The antibiotics have saved millions of lives since they were first discovered. In animals, we use antibiotics for improving welfare and for enhancing production. However, the indiscriminate use of these antibiotics has led to the emergence of resistant bacteria in which antibiotics no longer work also known as antimicrobial resistance (AMR). The UK Government has established the Fleming Fund to respond to the global threat of drug-resistant infections. The Fleming Fund is critical to achieving the resolution of the 68th World Health Assembly, 2015 (WHA A68/20); 84$^{th}$ World Animal Health Assembly (WAHA 2016); and in realising the 'Political Declaration of the High-Level Meeting of the United Nation General Assembly (UNGA) on Antimicrobial Resistance, 2016'. The overall goal of the grant is to avert the human and economic burden of antimicrobial resistance (AMR).

Bhutan is one of the recipients of Fleming Fund Country Grants to improve the diagnosis and surveillance of AMR in both human health and the animal health sector thereby to inform policy and practices at national and international levels. The human health component implemented by Department of Medical Services which benefits five laboratories (surveillance sites) and animal health component is implemented by the Department of Livestock with four laboratories benefitting (National Centre for Animal Health, National

Food Testing Laboratory and two Regional Livestock Development Centres Tshimasham and Kanglung).

In addition to the country grant, the Fleming Fund also supports Fleming Fellowship Scheme to provide continuing professional development and leadership training opportunities for relevant fellows. Seven Fleming Fellowships were provided to Bhutan out of which three are for the animal health.

The inception phase of the project (Country Grant) was implemented from April 2019 to November 2019 where the infrastructure development and other preparatory works were carried out in the beneficiary laboratories. Following the finalization of Detail Technical Implementation Plan (DTIP) in December 2019, one-day sensitization workshop was conducted on 3rd January 2020 at Royal Thimphu College to inform all the stakeholders on activities that shall be implemented through 18 months period. The meeting is expected to enhance coordination and collaboration among the various stakeholder including the policymakers.

Dr. Tashi Samdup, the Director-General of Department of Livestock, graced the opening ceremony as Chief Guest. About 31 participants including the representatives from the Fleming Fund Project Implementation Unit, Department of Medical Services, Department of Livestock (Animal Health Division, National Centre for Animal Health, National Veterinary Hospital, Regional Livestock Development centres) and Bhutan Agriculture & Food Regulatory Authority attended the one-day sensitization workshop.

### 13.2.2 Workshop on development of Standard Operating Procedures (SOPs)

Bhutan has received the UK government-based Fleming Fund Grant to improve the diagnosis of antimicrobial-resistant infections with an emphasis on antibiotics, to improve data management, surveillance and to inform policies/practices at national and international levels. The grant is aimed to improve the diagnosis and surveillance of antimicrobial resistance (AMR) in both human and animal health sectors. Under the Department of Livestock, National Centre for Animal Health (NCAH) is being identified as the beneficiary institute and four laboratories (National Veterinary Laboratory (NCAH); National Food Testing Laboratory (BAFRA); Regional Livestock Development Centre (RLDC) (Kanglung); and Regional Livestock Development Centre (RLDC) (Tshimasham) as surveillance laboratories for the project.

One of the main aims of the Fleming Fund Project is to strengthen microbiology laboratory capacity for AMR diagnostics at the surveillance laboratories. The capacity of these laboratories needs to be enhanced and facilitated to identify, isolate and perform Antibiotic Sensitivity Test (AST) on WHO identified GLASS (Global AMR surveillance system) pathogens such as *Campylobacter spp, Enterococci spp, Salmonella spp and E. coli.* To produce valid test results, Standard Operating Procedures (SOP) are required for these bacteria.

Hence, a "workshop on the development of SOPs" was conducted at Phuentshogling from 14-19th January 2020. 12 participants representing the Technical Working Group (TWG) for Animal Health, representation from the Department of Livestock (Animal Health Division, National Centre for Animal Health, National Veterinary Hospital, Regional Livestock Development centres), Department of Public Health (Royal Centre for Disease Control) and Bhutan Agriculture & Food Regulatory Authority (Quality Control & Regulatory Division, National Food Testing Laboratory) attended the workshop. During the workshop following SOPs were developed:

A. Iotation and identification of who glass pathogens
    i. *Campylobacter*
    ii. *Enterococci*
    iii. *Escherichia coli*
    iv. *Salmonella*
    v. Antimicrobial susceptibility Testing, (Disk Diffusion Method)
    vi. ESBL detection method
B. Isolate transportation
C. Laboratory Waste Management

### 13.2.3 Workshop to develop surveillance plan & sampling protocols for AMR surveillance in poultry under Fleming fund country grant

To enable implementation of the surveillance, several activities have been identified in the detail technical implementation plan (DTiP) of the project. One of the important activities on DTiP is to develop a surveillance plan for active AMR surveillance in broiler and layer

chickens. Therefore, a workshop was conducted from 2nd to 6th February 2020 in Hotel Holiday Home, Paro with relevant officials from different stakeholders. In total, 12 participants comprising of TWG members and the representative from relevant stakeholders took part in the workshop. During the workshop following were developed:

a) AMR Surveillance plan for in broiler and layer chickens (DTiP no 3.6.5),
b) Sampling protocol (DTiP no. 3.6.1),
c) SOPs for sample collection, packaging and transportation (DTiP no. 3.6.2).

The workshop was organized by NCAH, Serbithang as the beneficiary institute for Fleming fund project in collaboration with Project Management Unit (PMU), Fleming Fund.

### 13.2.4 Microbiological Hands-on Training for Culture, Identification and Antimicrobial Susceptibility Testing (AST) for *E. coli* and *Salmonella*

One of the main aims of the grant is to improve the diagnosis and surveillance of antimicrobial resistance (AMR) in both human and animal health sectors. Under the Department of Livestock, National Centre for Animal Health (NCAH) is being identified as the beneficiary institute and four laboratories (National Veterinary Laboratory (NCAH); National Food Testing Laboratory (BAFRA); Regional Livestock Development Centre (RLDC) (Kanglung); and Regional Livestock Development Centre (RLDC) (Tsimasham) as surveillance laboratories for the project.

One of the objectives of the Fleming Fund country grant project is to strengthen microbiology laboratory capacity for AMR diagnostics at the surveillance laboratories. The capacity of these laboratories needs to be enhanced and facilitated to identify, isolate and perform Antibiotic Sensitivity Test (AST) on WHO identified GLASS (Global AMR surveillance system) pathogens such as *Campylobacter* spp., *Enterococci* spp., *Salmonella* spp. *and E. coli.*

Hence, five days long hands-on training on culture, identification and AST for *E. coli* and *Salmonella* spp. were conducted at (NCAH), Serbithang, from 6-10th January 2020 for laboratory technicians from Regional Veterinary Laboratories (RLDCs), National Veterinary Hospital (NVH) and National Food Testing Laboratory (NFTL). The training was conducted by a microbiologist from NCAH.

During the training, following activities were carried out:

- Preparation of bacteriological media;
- Perform culture and identification of *E. coli* and *Salmonella;*
- Antimicrobial susceptibility test (AST) - disk diffusion methodology.

Figure: A. Sheep blood agar (SBA) plates; B. Culture method for *Salmonella* identification; C. Pure colonies of ATCC 13076 control organism (*Salmonella enteritidis*); D. Pure colonies in SBA; E. Observing the zone diameter on Muller Hinton agar; F. Explaining the zone diameter on Muller Hinton agar (AST)

### 13.3 Fleming Fellowship

The UK Government's Department of Health and Social Care has established the Fleming Fund to respond to the global threat of antimicrobial resistance (AMR). In Bhutan, the Fleming Fund activities comprise of Country grant and the Fellowship program. The Fellowship programme, through open competition, has selected three animal health professionals to develop and enhance skills on AMR surveillance, develop one health approaches on AMR and support country grant activities.

*The Bhutan fellows at the Bhutan Country Grant launch, with the UK High Commissioner to India and the Minister of Health, Royal Government of Bhutan.*

Following are the Fellows selected through open competition:

a) Antimicrobial Resistance (AMR) Surveillance Fellowship: Dr Ugyen Namgyel (Sr. Veterinary Officer within the Animal Health Unit of Regional Livestock Development Centre, Wangdue Phodrang);
b) Antimicrobial Resistance (AMR) Laboratory Fellowship: Ms Puspa Maya Sharma (Sr. Laboratory Officer / Microbiologist at the National Veterinary Laboratory, National Centre for Animal Health);

c) Antimicrobial Consumption/Usage (AMU/C) Fellowship: Dr Pema Tshewang, Dy Chief Veterinary Officer, National Veterinary Hospital, Motithang.

The fellows received a briefing on one health activities, training on R statistical tools, AMR testing and culture methodology. The fellows visited the host institute, Doherty Institute/University of Melbourne from 10th – 28th February 2020, to strengthen the AMR and AMU surveillance in food animals.

## 14. ASSISTANCE IN ESTABLISHMENT OF COVID-19 TESTING FACILITY AT MONGGAR REFERRAL HOSPITAL

The National Centre for Animal Health mobilized two laboratory officials, Ms Puspa M Sharma, Sr. Lab officer and Ms Kelzang Lhamo, Asst. Lab technician, along with human health officials and the necessary equipment/facilities on an emergency basis to establish a new COVID-19 testing laboratory at Mongar Referral Hospital, the eastern part of the country on 17th March 2020. The officials optimized the real-time PCR machine for COVID-19 tests, performed RNA extraction and PCR for suspect samples of COVID 19, performed rapid tests from suspect samples and trained two human health officials from Monger Referral Hospital on RNA extraction, master mix preparation, use of Quant Studio 5 real-time PCR machine, result-analysis and storage and handling of positive samples and suspect samples.


Figure A: RNA Extraction room  Figure B: PCR room


Figure C: Laboratory official at COVID-19 Testing laboratory; Figure D: With PPE, ready for extraction.

## 15. REFRESHER TRAINING ON LABORATORY BIOSAFETY & BIOSECURITY

A one-day refresher training on **Biosafety & Biosecurity** was conducted at National Centre for Animal Health, Serbithang for the laboratory personals of Laboratory Services Unit and Biological Production Unit on 25th June 2020. The main objectives of the training were to ensure regular implementation of Good Laboratory Practices (GLP) in the laboratory and also to monitor Bio-safety and biosecurity measures in the laboratory.

About 15 laboratory personnel participated in the training. The training programme was conducted by pre-test with awards followed by video shows on wearing PPE, -working safely in BSC, chemical spills management. The presentation was also made on Good Laboratory Practices (GLP) and also the participants were sensitized on the newly developed SOP on Laboratory Waste.

## 16. TRAINING-WORKSHOP ON TICK IDENTIFICATION USING MORPHOLOGICAL KEYS

**Summary**

The national-level tick identification training was conducted from 25-27th September 2019 at the National Centre for Animal Health (NCAH), Serbithang, Thimphu. The Department of Ecosystem and Public Health (EPH), Faculty of Veterinary Medicine, University of Calgary, Canada organized the training in collaboration with the NCAH as a part of the knowledge exchange program. University International Grants that fosters international activities of the University of Calgary funded the training workshop. Dr. Susan Cork who is the founding Head and Professor with the Department of Ecosystem and Public Health and Dr R.B Gurung who is the Programme Director of the NCAH coordinated the entire training workshop that was attended by four veterinarians, two laboratory technologists and 15 laboratory technicians. Dr Cork also conducted a seminar on "One Health approaches to control vector-borne diseases" to the training participants on 25th September 2019.

The technical training was led by Dr Tim J Lysyk and Dr Jamyang Namgyal. The former is a noted Canadian Entomologist who has worked as a Research Scientist with the Agriculture

and Agri-Food Canada for decades while the latter is a Bhutanese veterinarian who is currently an MSc researcher under Dr Cork's supervision.

The technical sessions covered the following topics: 1. general history and classification of ticks; 2. basic biology and ecology of ticks; 3. life cycle of *argasid* and *ixodid* ticks; 4. collection and preservation of ticks; 5. step for tick identification using dichotomous, polychotomous and electronic keys; 6. a general overview of tick-borne diseases in animals; and 7. tick-induced paralysis. In the practical sessions, the participants examined a wide range of tick specimens Namgyal had collected from eastern Bhutan. The skills imparted in the practicals were: sorting the sex and the life stages (i.e., larva, nymph and adult); identifying the genus and then identifying species using genus-specific keys containing a detailed description of species. Namgyal also delivered a seminar on "Tick habitat distribution modelling" which is a part of his MSc research.

## 17. ANIMAL DISEASE SURVEILLANCE AND RESEARCHES

### 17.1 Retrospective study on mortality of pigs at regional pig breeding centre (RPBC), Yusipang

*Dr. NK Thapa[1], Ms. Puspa Maya Sharma[1], Mr. Tenzinla[1], Tshewang Dema[1], Ms. Menuka Rai[2] Mr. Choedup Gyeltshen[2] and Dr. RB Gurung[1]*

*[1]Lboratory Services Unit, National Centre for Animal Health, Serbithang*
*[2]Regional Pig Breeding Centre, Yusipang*

***Summary***

*The overall mortality rate in pigs at Regional Pig Breeding Centre, Yusipang for the year 2018-19 was* (6.78 ± 2.26) (Mean ± SE). *Highest mortality rate was highest in adult (13.3%) followed by suckler (5.9%), weaner (5%) and grower (2.9%). The three main categories of animal reared are Great Grand Parent, Grand Parent and Parent Stock.*

*Seasonally, highest mortality was recorded during October to January in the sucklers and in weaners during December to February. The high mortality in suckler and weaners during winter could be attributed to cold stress. In growers, the mortality was almost uniform though out the seasons and considerable mortality in adult was recorded from April to August.*

*About 97.6% of sucklers and 67.7% of weaners were found suddenly dead and very less frequent cases of other conditions like weakness, diarrhoea, fever were recorded. Similarly, majority of the growers died suddenly (31%) followed by lameness (24.1%), Blue ear (20.7%). In the adult group, highest case reported was associated with Chronic illness and weakness (27.3%) followed by digestive related illness (21.2%) which includes, off feed,*

*poisoning and vomiting, high respiration (15.2%) and reproductive problems (12.1%) which includes, vaginal discharges, mastitis, metritis and dystocia. As per the necropsy diagnosis, highest cause of death was due to hepatic disorder (28.9%) followed by cardiopathy (15.6%), respiratory infection and septicaemia (12.5%) each.*

*The main isolates in the bacterial infection and septicaemia were Escherichia coli, Erysipelothrix rhusiopathiae, Klebsiella, Streptococcus and Staphylococcus. Salmonella was isolated from diarrhoeal cases in the piglet during the month of May 2019. From the samples of the animals with reproductive disorder, Staphylococcus hyicus, Streptococcus, Corynebacterium, Actinomyces, Actinobacillus and Escherichia coli were isolated during September 2019.*

*Molecular analysis conducted against African Swine Fever (ASF), Classical Swine Fever (CSF), Porcine Respiratory and Reproductive Syndrome (PRRS) and Brucellosis confirmed negative.*

*For any farm, three main steps in biosecurity measures are segregation, cleaning and disinfection. Hence, RPBC Yusipang should have adequate space for isolation of the sick animals, proper water supply for cleaning and adequate stock of disinfectants for routine disinfection. In addition, proper health monitoring of the animals needs to be enhanced.*

## 1.0 Introduction

The farm was established during 2016 in Yusipang for maintaining the Great Grand Parents (GGP), Grand Parents (GP) and Parent Stock (PS) to supply genetically improved piglets and PS. The new stock of white pig breeds comprising of Landrace and Yorkshire were imported from privately owned farms in Thailand during July 2016. Though the pigs passed the 15 days of quarantine observation at Paro International Airport, various degrees of mortalities were recorded in the farm. Hence, a retrospective study was conducted to understand the cause(s) of mortality and recommend control measures.

## 2.0 Methodology

A brief meeting with the farm management was conducted on 06/09/2019 to discuss on the issues and work out the modalities. During the meeting, it was decided to conduct the study as follows:

1. To conduct a retrospective study by compiling and analysing the mortality causes based on the record for past one year;
2. Inspect the farm for:
    a. Assessing the bio-security and biosafety practices practiced;
    b. Assessing the overall health condition of the animals and clinical examination of the affected animals;
    c. Collect and analyse the relevant samples for important diseases;
    d. Sanitation and hygiene practices;
    e. Treatment register/record;

3. To design a check list for daily health monitoring of the animals;
4. Review the breeding practices.

**3.0 Findings and Discussions**

***3.1 Biosafety and Biosecurity:*** The food dip at the entrance of the shed was filled with water but without disinfectant solution. The foot dips of only the nursery shed was found with proper disinfectant solution where as other foot dips were empty.

***3.2 Overall health condition****:* Overall health conditions of the animals were apparently good except for few with chronic conditions such as weakness and lameness in adult section. No mortality was reported on the day of the visit.

About eight numbers of sows had history of pyometra and also with the history of abortion. Most of the animals had history of cheesy exudates. Three of the sows were observed with the whitish to greyish discharges from vagina. Vaginal swabs were collected from all the affected animals to isolate the pathogens.

The farm had a history of Erysipelas outbreak and also Salmonellosis in piglets during May 2019. However, there was no report of cases of Erysipelas in the farm during this visit. No mortality was reported during the visit.

In the weaner section, 2 piglets were observed with watery diarrhoea. The faecal samples were collected for laboratory analysis. In addition, 2 environmental faecal samples were collected from 2 of the pens for parasite examination.

***3.3 Sanitation and hygiene practices***- It was informed that the floor of the sheds is being washed sometimes, however, the pigs are not washed with water.

***3.4 Treatment records-*** The treatment register is maintained at the office and no separate record of follow up treatments are being maintained especially for the antibiotics. The treatment record/information were not available at the individual pens.

***3.5 Breeding practices-*** Artificial insemination is being practiced currently in the farm. The semen is collected and inseminated in the females instantly. The semen is not stored however the semen is being processed, especially strained in a tap water washed muslin cloth.

**4. Retrospective mortality rates in different categories of animals (2018-19)**

***4.1 Overall mortality***

As per the record, the highest mortality was observed in the adult category (13.3%) followed by suckler (5.9%) weaner (5.0%), grower (2.9%). The overall mortality percentage during the year was (6.78 ± 2.26) (Mean ± SE), (Table 1).

*Table 1: Mortality rates in various categories*

| Sl no | Category | Opening balance | New addition/born | Total stock | Nos. dead | Mortality rate (%) |
|---|---|---|---|---|---|---|
| 1 | Suckler | 27 | 663 | 690 | 41 | 5.9 |
| 2 | Weaner | 164 | 455 | 619 | 31 | 5.0 |
| 3 | Grower | 634 | 363 | 997 | 29 | 2.9 |
| 4 | Adult | 106 | 135 | 241 | 32 | 13.3 |
| | **Total** | **931** | **1616** | **2547** | **143** | **6.78** |

***4.2 Seasonal pattern***

Highest mortality in sucklers was recorded during October to January However, considerable mortality was recorded during July also. In weaners, mortality was recorded higher during December to February. The high mortality in suckler and weaners during winter could be due to cold stress. In growers, the mortality was almost uniform though out the season and considerable mortality in adult was recorded from April to August (Figure 1)

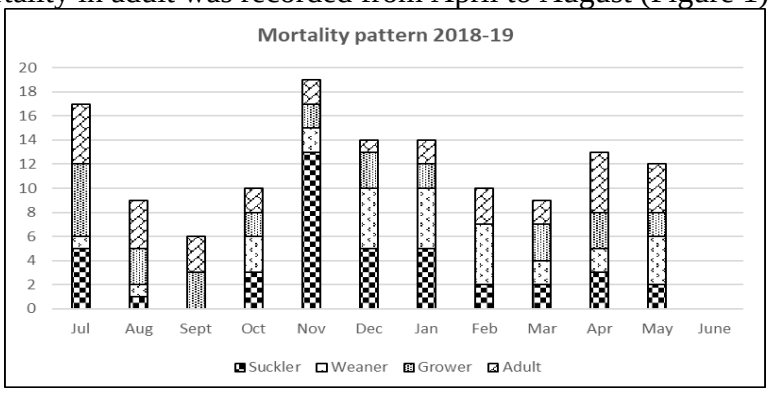



*Figure 1. Seasonal pattern of mortality 2018-19*

**4.3 Clinical signs**

The signs observed in the affected animals are summarized and as recorded 97.6% of the sucklers and 74.2% of the weaners were found to die suddenly and very less frequent cases of other conditions like weakness, diarrhoea, fever were recorded (Table 2).

*Table 2: Clinical signs of the affected sucklers and weaners*

| *Sl. no* | *Clinical Conditions* | *Sucklers* | *Weaners* |
|---|---|---|---|
| 1 | Sudden death | 41/42 (97.6%) | 23/31 (74.2 %) |
| 2 | Weakness | - | 3/31 (9.7%) |
| 3 | Others (diarrhoea, fever, epistaxis) | 1/42 (2.4%) | 5/31 (16.1%) |

Similarly, majority of the growers died suddenly (31%) followed by lameness (24.1%), Blue ear (20.7%) (Table 3).

*Table 3: Clinical signs of the affected grower (Total number of growers 997)*

| *Sl no* | *Conditions* | *Nos.* | *Percentage (%)* |
|---|---|---|---|
| 1 | Lameness | 7 | 24.1 |
| 2 | Blue ear | 6 | 20.7 |
| 3 | Sudden death | 9 | 31.0 |
| 4 | Weakness | 2 | 6.9 |
| 5 | Vomiting/off feed | 2 | 6.9 |
| 6 | Others | 3 | 10.3 |

As per the record, various clinical signs were exhibited by the adult animals. For analysis, they were grouped into various conditions and the highest case reported was associated with Chronic illness and weakness (27.3%) followed by digestive related illness(21.2%) which includes, off feed, poisoning and vomiting, high respiration (15.2%) and reproductive problems (12.1%) which includes, vaginal discharges, mastitis, metritis and dystocia (Table 4).

*Table 4: Clinical signs in the affected adults*

| **Clinical conditions** | **Nos.** | **Percentage (%)** |
|---|---|---|
| Chronic illness/weakness | 9 | 27.3 |
| High respiration | 5 | 15.2 |
| vomiting/off feed/poisoning | 7 | 21.1 |
| Lameness/paralysis | 2 | 6.1 |
| Sudden death | 3 | 9.1 |
| Reproductive disorder | 4 | 12.1 |
| Others | 3 | 9.1 |
| **Total** | **33** | **100** |

**4.4 Necropsy findings**

The necropsy diagnosis was also compiled and grouped into various categories as below.

*Table 5: Various causes of death as per the necropsy diagnosis*

| **Sl. No.** | **Conditions** | **Nos** | **Percentage (%)** |
|---|---|---|---|
| 1 | Anaemia | 12 | 9.4 |
| 2 | Ascites | 1 | 0.8 |
| 3 | Bacterial infection | 4 | 3.1 |
| 4 | Cardiopathy | 20 | 15.6 |
| 5 | Gastro enteritis | 10 | 7.8 |
| 6 | Hepatic disorder | 37 | 28.9 |
| 7 | Respiratory infection | 16 | 12.5 |
| 8 | Renal disorder | 6 | 4.7 |
| 9 | Septicaemia | 16 | 12.5 |
| 10 | Shock | 1 | 0.8 |
| 11 | Stress | 4 | 3.1 |
| 12 | Toxaemia | 1 | 0.8 |
| | **Total** | **128** | **100** |

As per the necropsy diagnosis, highest cause of death was due to hepatic disorder (28.9%) followed by cardiopathy (15.6%), respiratory infection and septicaemia (12.5% each) (Table 5).

The main isolates in the bacterial infection and septicaemia were *Escherichia coli, Erysipelothrix rhusiopathiae, Klebsiella, Streptococcus and Staphylococcus. Salmonella* was isolated from diarrhoeal cases in the piglet during the month of May 2019.

### 4.5 Laboratory culture

From the samples collected from animals with reproductive disorder, bacteria could be isolated from almost all the animals with vaginal discharges. The bacteria included *Staphylococcus hyicus, Streptococcus, Corynebacterium, Actinomyces, Actinobacillus and Escherichia coli* (Table 6).

*Table 6: Animals with reproductive problems and the piglets with diarrhoeal signs*

| Sl. No. | Tag. No./sex/Age | Breed | History/Clinical signs | Status of treatment/Sample | Lab findings |
|---|---|---|---|---|---|
| 1 | 11492/F/A | Yorkshire | Vaginal discharges observed, had history of abortion | Under treatment with Nitrofurazone, Vaginal swab | *Staphylococcus hyicus* |
| 2 | 304403/F/A | Landrace | Vaginal discharges, infertility | | *Streptococcus* |
| 3 | 500996/F/A | Yorkshire | Vaginal discharges | Before service, it had signs of discharges, Vaginal swab | *Corynebacterium* |
| 4 | 7000005/F/A | Landrace | History of cheesy discharge from vagina | Vaginal swab | *Actinomyces spp* |
| 5 | 304350/F/A | Landrace | Vaginal discharges | Vaginal swab | *Proteus* |
| 6 | 700931/F/A | Landrace | Whitish discharges from vagina | Vaginal swab | *Escherichia coli* |
| 7 | 500958/M/A | Yorkshire | Lesions in the penis | Preputial swab collected | *Proteus* |
| 8 | 304327/F/A | Landrace | History of abortion | Vaginal swab | *Actinobacillus* |
| 9 | 11394/F/A | Yorkshire | History of abortion (3 times) | Vaginal swab | *Escherichia coli* |
| 10 | 701083/F/A | Landrace | Discharge from vagina | Vaginal swab | *Actinomyces* |
| 8 | 1182/F/W | Landrace | Watery | Rectal swab | *Streptococcus* |

| | | | | | |
|---|---|---|---|---|---|
| 9 | 1041/F/W | Landrace | diarrhoea Watery diarrhoea | Rectal swab | *Staphylococcus* |
| 10 | Weaner shed | NA | Faecal samples | 4 numbers from 2 pens | *Balantidium coli and Strongyles* |

Molecular test conducted against African Swine Fever (ASF), Classical Swine Fever (CSF) and Brucellosis tested negative. The tests were conducted to rule out the role of these diseases in recent mortality.

## 5.0 Recommendations

**5.1 Biosecurity measures-** In general, three main steps in biosecurity measures are Segregation, Cleaning and Disinfection.

*Segregation:* is the first and most important element of biosecurity which involves keeping potentially infected animals and materials away from uninfected animals. Segregation is regarded as the most effective step in achieving the required levels of biosecurity. This includes not only pigs, but also other species (including humans) that may be infected with pathogens and that can also infect pigs. Hence, isolation shed needs to be maintained for sick animals.

Currently the farm does not have isolation shed to segregate the affected animals from the healthy ones. Hence, a provision needs to be kept for isolation of the sick animals.

*Cleaning:* The most effective step in biosecurity is cleaning. Most pathogen contamination on physical objects is contained in faecal material, urine or secretions that adhere to the surface and therefore the cleaning will remove most of the contaminating pathogen. Any materials that must pass through the segregation barrier (in either direction) should be thoroughly cleaned. This step is very much essential as various bacteria has been isolated from the vaginal discharges of the animals.

As observed and informed by the farm management, there is currently facing water shortage problem to carry out routine cleaning of the shed as well as animals. Therefore, there is a need to restore or secure a continuous water supply to the farm.

*Disinfection:* The final step of biosecurity is disinfection. Disinfection is important when performed consistently and correctly, but should be regarded as a final "polishing" step in biosecurity, used after effective and comprehensive cleaning. Disinfectants will not necessarily penetrate dirt in sufficiently high concentrations, nor will they be present for sufficient time to be effective. In addition, many disinfectants are inactivated by organic materials, such as wood or faecal material. Thus, although important, disinfection can be regarded as the least effective step in biosecurity.

Therefore, the disinfection should be carried out routinely with the appropriate disinfectant agents available in the farm or central store.

### 5.2 Breeding practices

The farm is currently using artificial insemination technology for breeding practices. While the technology is very useful in optimally utilizing quality semen and synchronising farm activities, standard protocols for semen collection, processing and insemination should be of strictly complied. With slightest disregard to standards and protocols can become a serious source of infection especially the infections associated with reproductive system in breeding sows. Therefore, extra care should be taken to ensure breeding practice using AI does not introduce any infection.

### 5.3 Further screening

This is the initial report where we mainly looked at the bacterial cause of mortality except PRRS, ASF and CSF. Additionally, farm biosecurity was also reviewed. The next step of investigation will be focused on Porcine Circovirus-2 (PCV-2) and *Mycoplasma hyopneumonae* infections. There are records that the earlier stock of pigs at the farm had outbreak of PCV-2. These two diseases are of significant importance in pig husbandry. The immunoassay kits for screening these diseases are hard to find and expensive to buy. The kits are not available at NCAH, Serbithang. When available the farm management shall be intimated of screening program.

### 5.4 Diagnostic kits

Considering the current stocks of pigs are of high value based on its breed line, we need to provide adequate resource for enhancing overall management for the farm. Adequate resource may include human resource, biosecurity, quality feed and diagnostic consumables. This arrangement will definitely reduce loss from disease and other conditions.

### 5.5 Stocking

Some of the pens appeared over crowded in the farm. There is high risk of spread of diseases, in overcrowded sheds. Therefore, the animals should be stocked as per the standards/guidelines of stocking.

### 5.6 Referring the health alarm List

In order to guide the routine health monitoring of the animals, the reference has been provided so that the concerned authorities or the health personal should be informed as soon as possible if any of the following signs are observed. This is important to reduce any time delay between an outbreak and effective response or treatment.

| | |
|---|---|
| Any age groups | The development of lameness in pens or groups of pigs |
| Blisters on the snout or excessive salivation in pens or groups of pigs | |
| Sows | Four or more sows off their feed with an elevated temperature |
| Four or more sows breathing rapidly and with obvious respiratory | |
| Four or more sows aborting within seven days | |
| Suckling herd | A noticeable rise in pre-weaning mortality over a week period |

| | |
|---|---|
| Growing-finishing herd | A noticeable rise in post weaning mortality |
| | Scour spreading through any age of pigs |
| | A marked rise in the number and severity of pigs coughing or with labored breathing |
| | Three or more unexpected deaths in one day |

### 5.7 Routine health inspection card

In order to keep track of the sick animals and for necessary follow up, following chart has been designed for routine inspection. This chart may be used for monitoring the health of the animals.

| Date | Animal details | Condition observed | Actions taken | Initial |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## 6.0 Conclusion

The hygiene and management of the farm needs to be improved. Biosecurity measures need to be strengthened especially with regular use of disinfectants in the foot dips. Since bacteria could be isolated from almost all the animals with reproductive disorders, it could be due to lack of isolation facilities and thus propagative infection persisted for quite some time.

For the suckler and the weaners, the highest mortality is recorded during the winter season. Proper care needs to be provided during these periods.

Currently the important diseases like African Swine Fever, Classical Swine Fever, Porcine Respiratory and Reproductive Syndrome and Brucellosis have been ruled out with molecular diagnosis. However, in future investigation needs to be expanded to other diseases like Porcine Circovirus-2 (PCV-2) and *Mycoplasma hyopneumonae* infections. There are records

that the earlier stock of pigs at the farm had outbreak of PCV-2. These two diseases are of significant importance in pig husbandry.

Therefore, in addition to proper biosecurity measures, routine health monitoring of the animals by inspection and proper treatment of animals needs to be carried out by the farm as per the design of the card provided.

## 17.2 Health Screening of Animals at National Jersey Breeding Centre with urine parameters

*Dechen Wangmo[1], Arpana Rai[2], Tshering Dem[2], Kiran gurung[2], Pema Wangchuck[2] , Purna Bdr. Rai[1], N.K Thapa[1]*

*[1]NCAH Serbithang, [2]NJBC, Samtse*

### Introduction

Analysis of urine is one of the approaches to assess the health of the animals. The measure of specific gravity will help in assessing the tubular function of the kidneys. It will reveal many of the diseases that could go unnoticed and undiagnosed because they generally do not produce pathognomonic clinical signs or symptoms. These diseases include diabetes mellitus, various forms of glomerulonephritis, and chronic urinary tract infections.

Observing the colour, transparency, microscopic and chemical characteristics of urine and urinary sediments coupled with microbial culture and sensitivity test is likely to identify the majority of the lower urinary tract disorders in domestic animals. It is a remarkable and readily available and an inexpensive tool for the diagnosis and management of numerous urinary tract abnormalities.
Hence, on a pilot scale, screening of animals through urine analysis was conducted at NJBC, Samtse, through random sampling.

### Material & Methods

During June 2019, 17 urine samples were collected randomly and tested with urine strip (Urine Insta Test, CORTEZ diagnostic Inc, USA) from various categories of animals (Milch cow-5 urine samples, Dry Cow-3 samples, Heifer-7 samples and Bull-2 samples) at National Jersey Breeding Centre Samtse. The test detects the presence of bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrite, leukocytes, ascorbic acid.

### Results & Discussions

The urine of all the animals had traces of protein and Leukocytes. However, the specific gravity, pH was within the normal range. Other parameters like bilirubin, ketone, blood protein. Urobilinogen, nitrite, ascorbic acid was negative (Table 16).

**Table 5: Summary of urine analysis conducted in animals of NJBC, Samtse.**

| Animal Category | Number of samples | Findings |
|---|---|---|
| Milking cow | 5 | All milking cow contains a trace of leukocytes & protein |
| Heifer | 7 | All heifer was found with a trace of leukocytes & protein |
| Dry cow | 3 | All dry cow was found with a trace of leukocytes & protein |
| Bull | 2 | All bull was found with a trace of leukocytes & protein |
| **TOTAL** | **17** | |

The results were almost similar for all the animals. This could be due to the same type of feed and fodders fed to the animals.

**Conclusion**

There were no significant findings except a trace of Leukocytes in all the animals. This indicates that the animals were healthy without any infection or metabolic conditions or renal ailments.

## 17.3 Laboratory analysis of Dog faecal samples & Scats from Yak rearing areas

**Introduction**

After the Highland Research & Development workshop held in Gelegphu in January 2020, Taenia prevalence study in yak rearing areas was conducted at NCAH, serbithang. Gid caused by *Taenia multiceps*, one of the several genera under Taenidae family, is a concern for yak rearing communities in the highland areas of Bhutan. Taenia causes neuropathy in young yak calves by lodging cyst (intermediate stage) of the dog tapeworm, *T. multiceps.* Yaks, cattle, sheep, goats are the intermediate hosts, whereas, dogs and wild canids are the definitive hosts (intestinal adult worm). The disease causes significant economic losses to yak herders due to mortality of young yaks.

**Methodology**

A semi-structured questionnaire was developed to interview farmers on husbandry practices, mortality, migratory pattern. It is a concurrent study- Taenia prevalence established through laboratory analysis of dog faecal samples and scat sample. Administration of semi-structured questionnaire to establish baseline data of mortality due to gid and also to establish husbandry practices.

The study was conducted in 10 Dzongkhags and 26 Gewogs. The samples include environmental faecal sample, scat samples, soil samples from the yak rearing areas. Sample analysis was done by F/S, Sequential sieving (100µ, 40µ, 21µ).

**Results**

Through laboratory analysis of faecal and scat samples, the overall positivity of Taeniid was 62/563, suspected 8/563 a, non-taeniid 169/563 (Figure 3).

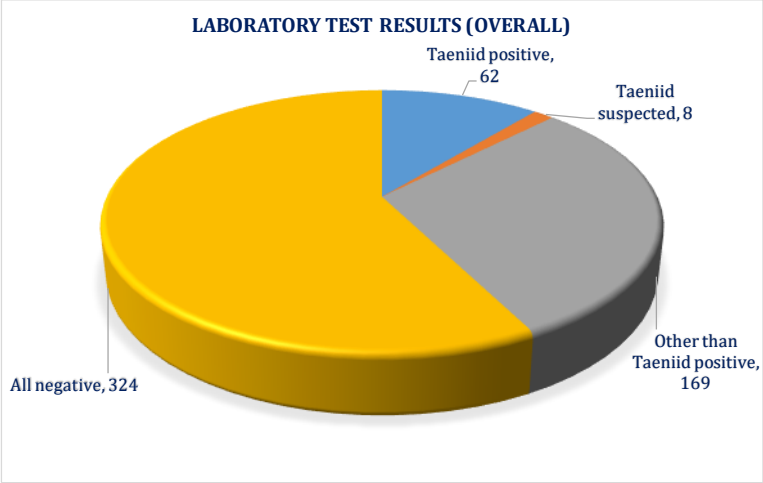



*Figure 3: Overall test results for Taeniid*

Gewog-wise comparison revealed highest Taeniid positive dog faecal samples from Merag, and scat samples from Kazhi (Figure 4).

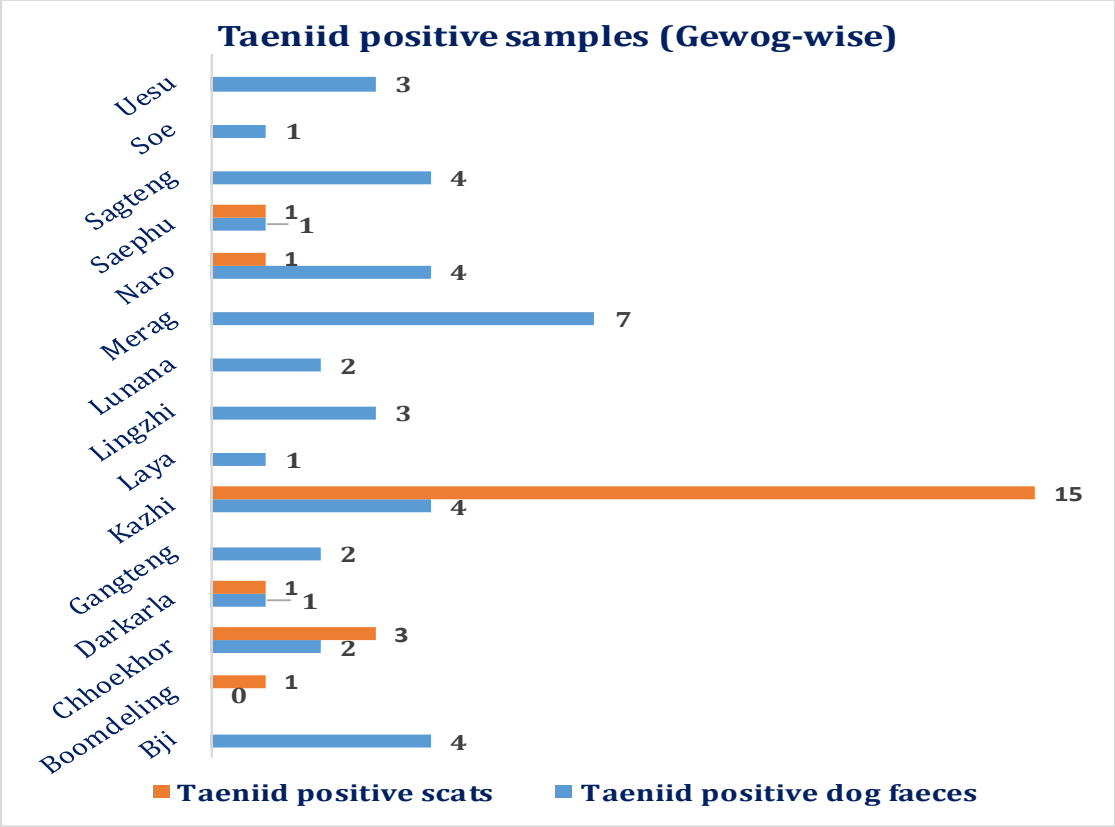



*Figure 1: Gewog-wise Taeniid positive samples*

When compared Dzongkhag-wise, Taeniid prevalence was highest in Wangdue Phodrang, followed by Thimphu, Trashigang, etc. (Figure 5).

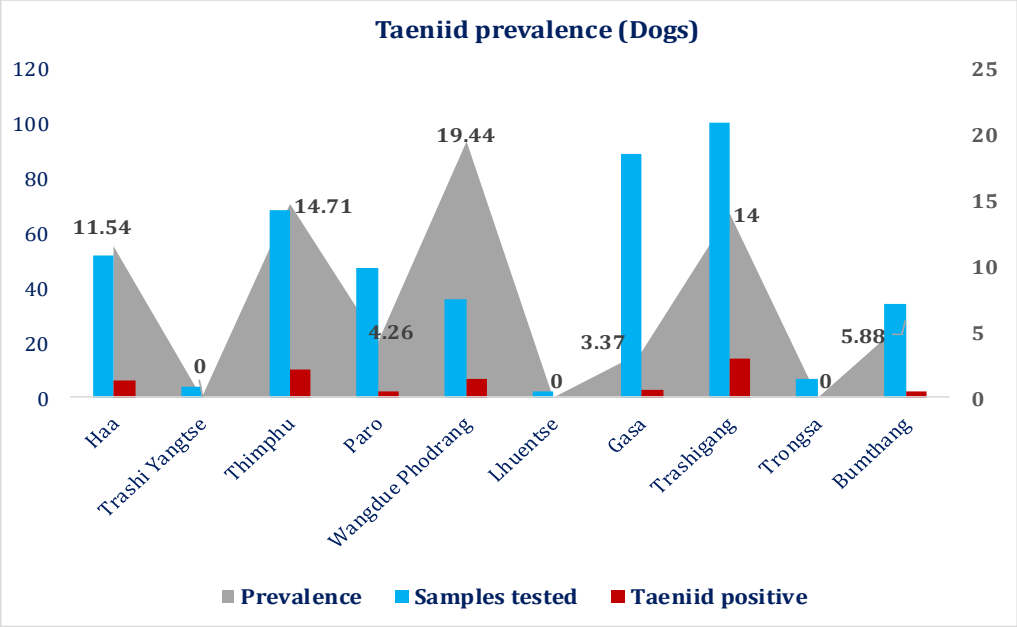



*Figure 2: Dzongkhag-wise Taeniid prevalence*

**Conclusion**

The overall average prevalence of Taeniid in Dogs is 44/439(7.32%) and wild canids 25/121(20.7%). Prevalence of Taeniid is highest in Wangdue Phodrang Dzongkhag 7/36(19.4%) in dogs and also in wild canids 17/34 (50%). Taeniid eggs have been detected in dog faeces in Trashigang (14%), However, the molecular analysis will confirm the presence or absence of *Taenia multiceps.* Controlled deworming needs to be done in the areas.

Further activities include optimization of a method for Taeniid egg isolation from soil samples and then the examination of soil samples for Taeniid eggs and molecular identification of *Taenia multiceps.*

## 17.4 Mortality of native poultry bird at Motithang

*National Centre for Animal Health, Serbithang and National Veterinary Hospital, Motithang*

**1. Introduction**

Native poultry birds are known to be more resistant to some infectious diseases in comparison to commercial strain birds. Studies in the past in Bhutan have shown that native Bhutanese chickens are immunologically tolerant to Infectious bursal disease (IBD)– a highly infectious viral disease in commercial strain birds. In 2016, the source of an outbreak of Avian Leukosis Complex (ALC) and Marek's disease (MD) in Regional Poultry Breeding Centre, Paro was traced back to small flock of native poultry birds temporarily housed at the farm premise. During the outbreak large number of commercial strain birds died of ALC and MD at the farm. The native birds did not show any signs of illness or mortality. Confirmatory diagnosis of samples from these native birds by advance test detected the presence of ALC and MD virus. The investigation confirmed that native birds act as carrier, a potential source of infection to other healthy birds. However, native poultry birds are still susceptible to many other infections. Recently, a continuous mortality of native bird was reported from Motithang. National Veterinary Hospital (NVH), Motithang immediately visited the affected

farm to investigate the sudden death in the poultry birds. The case was further referred to National Centre for Animal Health (NCAH), Serbithang for more detail investigation. Subsequently, a team from NCAH, Serbithang visited the farm and conducted investigation.
The caretaker of the farm was enquired about the history and the clinical signs. The farm compound was inspected to gather any relevant information regarding the mortality. The team conducted postmortem and also collected relevant samples for laboratory investigation.

## 2.0 History

*2.1 Stock of poultry*

The farm had about 35 numbers of poultry and two numbers of turkeys in total before the outbreak. Initially only five numbers of birds reported dead. All the birds were native breed reared for egg purpose. The caretaker did not have records of vaccination and deworming.
The farm had one big shed partitioned at one end where only about 10 males were kept and in the bigger compartment housed eight females with two males. There were two small sheds where two turkeys were kept and in another shed the healthy birds were isolated.
As per the history, the female groups along with two males were let out for free ranging. Then the birds in this flock started showing symptoms. The clinical signs observed were droopiness, off feed and sudden death.

*2.2 Biosafety and biosecurity*

There are lot of shrubs and trees around the farm where the wild birds could easily come in contact with the poultry. There is no foot dip at the entrance of the farm. Adjacent to the farm, carpentry works is being carried out where there is frequent movement of the people.

*2.3 Mortality record*

| Date | Nº affected | Nº dead | Remarks |
|---|---|---|---|
| 26/09/2019 | 2 | 5 | • Off feed and droopy |
| 27/09/2019 | - | 2 | • Liver dark and cooked appearance, haemorrhages in trachea, inflamed kidneys<br>• Rapid tests for Newcastle disease (ND) and Avian Influenza (AI) (bird flu) were negative<br>• Screening of faecal samples revealed Cooperia eggs |
| 28/09/2019 | 1 | 1 | • Tetracycline powder and Tylosin antibiotic were provided to be mixed with water for treatment of sick birds |
| 29/09/2019 | 3 | - | • Clinical signs of off feed and droopiness<br>• Advised to isolate the healthy birds to another shed |
| 30/09/2019 | - | 5 | • Necropsy revealed swelling of eye lids, haemorrhages in the proventriculus, ileo-caecal junction and intestinal wall<br>• Rapid tests of sick birds for ND and AI were |

| | | | |
|---|---|---|---|
| | | | negative<br>• Advised for isolation of the healthy birds to another shed and use of foot dips |
| 01/10/2019 | 3 | 1 | • One bird died from the male group<br>• All the sick birds were transferred to National Centre for Animal Health, Serbithang<br>• Disinfection of the surroundings and the shed with 1% Virkon-S<br>• Cleaned the litter materials, disinfected with 1% Virkon-S and advised to burn including the feed<br>• Advised to feed first the healthy and then the sick birds |
| 02/10/2019 | - | 2 | • Depression, off feed and paralysis<br>• Haemorrhages on the mucosal wall of proventriculus, oedema and haemorrhage in trachea |
| 03/10/2019 | - | 2 | • Haemorrhages on the mucosal wall of proventriculus, oedema and haemorrhage in trachea |
| 04/10/2019 | - | 1 | • Haemorrhages on the mucosal wall of proventriculus, oedema and haemorrhage in trachea |
| 05/10/2019 | - | 5 | • Haemorrhages on the mucosal wall of proventriculus and ileo-caecal junction and haemorrhage in trachea |
| 06/10/2019 | - | 3 | • Off feed, white diarrhoea<br>• Haemorrhages on the mucosal wall of proventriculus and ileo-caecal junction<br>• Tracheal swab rapid test: Positive for ND (two birds) |
| 07/09/2019 | - | 3 | • Haemorrhages on the mucosal wall of proventriculus and ileo-caecal junction<br>• Tracheal swab rapid test: Positive for ND (one bird) |

**3.0 Preventive measures undertaken**

*3.1 Action taken by National Veterinary Hospital, Motithang*

- Advise on isolation of the sick birds
- Treatment of the affected birds with antibiotics.
- Transportation of carcasses to NCAH for post mortem examination
- Disinfection of the shed and premises

*3.2 Action taken by Laboratory Service Unit, NCAH, Serbithang*

- Advised for isolation of the affected birds
- Use of foot dips and disinfectants at the farm entrance
- Cleaning and disinfection of the shed
- Transfer of the affected birds to Serbithang since there was no proper isolation shed

**4.0 Prominent clinical signs**

Almost all the affected birds had common signs and symptoms as per the history and observation. They include dullness, depressed, off feed, huddling at the corner, white diarrhoea and paralysis.

**5.0 Necropsy findings**

Of all the necropsies conducted in the dead birds had common findings which include:

- Oedema of eye lids
- Haemorrhages on the trachea, and congestion of lungs
- Haemorrhages on the mucosal wall of proventriculus and ileo-caecal junction

**6.0 Rapid Tests**

Samples such as cloacal and tracheal swabs were collected and tested using rapid test kits for Avian influenza and Newcastle disease. The test results were:

- Negative to Avian influenza (both tracheal and cloacal swabs)
- Positive to Newcastle disease (only tracheal swab)
- Negative to Newcastle disease (cloacal swab)

**7.0 Molecular Tests**

The swab samples were used for RNA extraction and tested using real time RT-PCR test. The test results were:

- Avian Influenza A: Negative
- Newcastle disease: M gene positive

A total of 30 birds died in about a period of two weeks. There are five numbers of birds alive but sick. None of the sick birds are responding to treatment.

**8.0 Findings and diagnosis**

Based on the clinical signs, necropsy findings, rapid test and also confirmatory diagnosis by molecular tests the cause of mortality was found to be *Newcastle disease.* Generally, testing of cloacal and tracheal swab samples are recommended for diagnosis of ND either by rapid of molecular assay since clinically affected birds excrete and secrete loads of virus through cloaca and mouth, respectively. This is also recommended in the international protocols and standards for all the test kits. However, it was learned that native birds do not excrete adequate load of virus which was evident by negative result from cloacal swabs using both rapid and real time RT-PCR test. This investigation found that available test kit is able to detect virus only in tracheal swab.

### 9.0 Recommendations

Newcastle disease is a highly infectious disease of poultry. Although native poultry birds are resistant to some bacterial as well as viral infections, they are still susceptible to ND virus. There are several reports of ND outbreak in Bhutan especially in unvaccinated flocks. The outbreaks are reported in both commercial and native breed. The outbreak cause very high mortality in a naïve flock and associated economic loss. Followings are the immediate and long-term control measures:

1. Isolation shed to be maintained bit farther from the other shed and the affected/sick birds should be removed from the healthy flock.
2. Separate caretakers handling sick and healthy birds is one of the fundamental principles of biosecurity and disease control.
3. Routine vaccination of poultry against important diseases like Newcastle disease.
4. Since the current shed is adjacent to the trees and shrubs, it should be constructed bit further and at isolated location.
5. Since there is carpentry works undergoing adjacent to the farm, the shed should be bit away from the human movement too.
6. Install foot dips at the entrance of each shed and use disinfectant routinely.
7. Implement regular monitoring of the health of the birds.
8. Enhance record keeping for any activities carried out at the farm.
9. The new poultry stock should not be introduced in the shed before two months since the disease virus is known to stay in the surrounding for two months.
10. All new stock to be vaccinated against important poultry diseases

## 18. ON-GOING SURVEILLANCES AND RESEARCHES

### 18.1 Collaborative studies on an important zoonotic disease like Anthrax, Rabies, Crimean-Congo haemorrhagic fever (CCHF), and bat-mediated zoonotic diseases

A collaborative research studies on anthrax, rabies, brucellosis, Crimean-Congo Haemorrhagic Fever (CCHF) and bat derived zoonoses was initiated between three institutions: National Institute of Infectious Diseases, Japan; Royal Centre for Disease Control, Department of Public Health, Ministry of Health and National Centre for Animal Health, Department of Livestock, Ministry of Agriculture and Forests. This collaboration was established between these three institutes to improve the health of the people and animals in the two countries based on the practical implementation of the “One Health Concept”. To initiate this collaborative research and as per planned activity, two batches of Bhutanese laboratory staff were trained at National Institute of Infectious Diseases, Japan, on culture and identification of anthrax organism, culture and identification of *Brucella* organism, the immuno-fluorescence assay for CCHF and serum neutralization test (SNT) for rabies. The SNT technology included hands-on training on cell culture that included maintenance of cell lines, cell passage and virus titration. Similarly, two batches of Japanese team from National Institute of Infectious Diseases and National Institute of Animal Health visited the National Centre for Animal Health, Serbithang to establish a diagnostic facility for anthrax, *Brucella*, CCHF and rabies.

As a part of the study on anthrax, about 56 soil samples were collected from the burial sites and the control from the outbreak areas of Samtse, Chhukha, Zhemgang, Dagana, Tsirang, Monggar & Lhuentse Dzongkhags. Culture & isolation of Anthrax will be carried out from these samples.

For the sero-titre study against Rabies, 106 sera samples from stray dogs were collected from Thimphu city. The samples will be analysed for antibody titre against Rabies.

CCHF- A retrospective study with a total of 234 serum samples from goats collected from Samtse and Sarpang districts were tested against CCHFV antibody detection system using recombinant nucleoprotein (NP). A total of 116/234 (49.6 %) serum samples were detected positive for CCHFV antibody. CCHF virus is found to be endemic in Sarpang (50.6%) and Samtse (43.8%) in Bhutan. Since this is an on-going study and more serum samples will be collected from goats and human from the southern parts of the country.

For the bat derived zoonoses component, a study on bat habitat and ecology has been carried out. For further, activities, a team from Japan was expected for sampling, detection of any zoonotic agent in Bhutanese bats.

### 18.2 Study on antimicrobial resistance pattern of Contagious pathogens in milk from mastitis cases at NJBC, Samtse

*Puspa M Sharma[1], Tenzinla[1], Arpana Rai[2], Tshering Dem[2], Kiran gurung[2], Pema Wangchuck[2], Durga Maya Dhakal[2], Purna Bdr. Rai[1], N.K Thapa[1]*

*[1]NCAH, Serbithang; [2]NJBC, Samtse*

**Summary**

Mastitis in dairy cattle is a clinical condition that causes significant economic losses and is being considered one of the largest constraints to the dairy industry worldwide. Contagious pathogens infection occurs from the milk of the other infected animals. This infection can be prevented with proper milking hygiene including post-milking teat disinfection, milking infected animals last and effective management of clinical cases.

The contagious pathogens in milk include gram-positive *Streptococcus agalactiae, Streptococcus uberis, Staphylococcus aureus* and others like *Mycoplasma* sp. and *Protheca* sp. *Staph. aureus* has been reported to be one of the most relevant causative agents of this condition being responsible for contagious intra-mammary infection in dairy herds. The bacterium causes clinical and subclinical mastitis in dairy cattle and is of potential health concern to humans too. Also, the emergence of antimicrobial-resistant bacteria has been a global threat. The objective of this study is to provide insights to the contagious pathogens (*S. agalactiae, S. uberis* and *Staphylococcus aureus*) present in the udder tissues of the cattle and perform antimicrobial resistance susceptibility testing (ABDT) in these isolated pathogens.

**Materials & Methods:**

**Milk sample collection**

A total of 48 udder raw milk samples from all 15 lactating cattle were collected from NJBC farm located in the south-western part of the country. The subclinical and clinical mastitis was pre-examined by Californian Mastitis Test (CMT) (Figure 1). All the milk samples were tested against CMT. Those samples that were positive for CMT were collected for culture and isolation and performing ABST.


Figure A and B: Performing CMT tests in the field, C. Observation of CMT results

**Phenotypic Identification and AST**

A 20ul of milk samples were plated in SBA and MacConkey agar, incubated at $37^0$C up to 24 hours. The bacterial isolates that were gram-positive, cocci shaped were further examined using biochemical tests (Haemolysis, catalase test, oxidase test, coagulase test, CAMP tests, Aesculin tests, mannitol tests). The antimicrobial susceptibility tests will be conducted according to the CLSI guidelines.

**Results**

**Prevalence of Contagious Pathogen**

Out of 48 milk samples, 11 samples were positive for *S. agalactiae* (22.9%), 30 samples were positive for *Staphylococcus species (62.5%)* that includes both coagulase-positive and coagulase-negative *staphylococcus* species. Out of 20 milk samples analyses, 3 were positive for *S. uberis (10%)*. Further tests are still under process.

## 18.3 Retrospective sero-surveillance of Crimean-Congo haemorrhagic fever virus (CCHFV) in Bhutan

**Introduction**

Crimean-Congo haemorrhagic fever (CCHF) is a highly infectious disease caused by a tick-borne virus (*Nairovirus*) belonging to the *Bunyaviridae* family. In humans, the overall case-fatality rate of CCHF is ≈30%, but in severe and hospitalized patients, fatalities may be up to 80%. CCHF is widespread in various countries in Africa, Asia, and Europe; the virus had been identified in humans in China, Pakistan, Afghanistan and India. The hosts of the CCHF virus include a wide range of wild and domestic animals such as cattle, sheep and goats. Animals get infected by the bite of infected ticks and the virus remains in their bloodstream for about one week after infection, allowing the tick-animal-tick cycle to continue when

another tick bites. The CCHF virus is transmitted to people either by tick bites or through contact with infected animal blood or tissues during and immediately after slaughter
Bhutan has very little recorded data on the prevalence of CCHFV in the country. However, an earlier study indicated the presence of CCHFV IgG in 31/81 (38.2%) goats from the central-southern part of the country which shares a porous border with India. In this study, the presence of CCHFV antibodies will be determined from the goat's serum as well as from the human serum living from the same locality.

**Material and Methods**

The risk of CCHFV infection and serious human disease will be assessed by surveillance in the region with a risk of CCHFV by detection of CCHFV antibodies in animals, detection of CCHFV antibodies in humans and detection of CCHFV in ticks by RT-PCR.
A retrospective study with a total of 234 serum samples from goats collected from Samtse and Sarpang districts were tested against CCHFV antibody detection system using recombinant nucleoprotein (NP).
No human samples and ticks are collected to date

**Results**

A total of 116/234 (49.6 %) serum samples were detected positive for CCHFV antibody. CCHF virus is found to be endemic in Sarpang (50.6%) and Samtse (43.8%) in Bhutan. Since this is an on-going study and more serum samples will be collected from goats and human from the southern part of the country.

## 17. EX-COUNTRY TRAINING/WORKSHOP/MEETING ATTENDED

The list of various meeting/training/workshop attended by LSU staffs is presented in the annexure table 18.

## 18. VISITORS AT LABORATORY

During the financial year 2019-20 the following officials has visited the laboratory service unit for various purposes (Table 17).

**Table 17: Visitors at LSU**

| Sl. No | Date | Purposes | Place/Country | Total |
|---|---|---|---|---|
| 1 | 28/8/2019 | Demonstration practical on Molecular & Serology Sections for CNR, Students | CNR, Lobesa | 35 students with Module tutor |
| 2 | 21/9/2019 | Fecal samples analysis by Gasa Dzongkhag Staff | DVL, Gasa | 3 staff |
| | | | FAO ATLASS | |

| | | | | |
|---|---|---|---|---|
| 3 | 3/9/2019 | Laboratory Mission | mission Team | 4 |
| 4 | 24/9/2019 | Discuss on Zoonotic disease | WHO Consultant | 3 |
| 5 | 5/12/2019 | Explore of Molecular facility at NVL, Serbithang | University of Arkansas | 3 |

## Annexure: Ex-country training/workshop/meeting attended

**Table 18: List of NCAH staffs who have attended ex-country training/workshop/meeting during FY 2019-20**

| Name | EID. No | Position Title | Course title | Institute | Country | Start Date | End Date | Duration (days) | Funding Agency |
|---|---|---|---|---|---|---|---|---|---|
| **Dr. Nirmal Kumar Thapa** | 9302007 | Specialist III | Southeast Asian regional Symposium on Microbial Ecology | Pokhra | Nepal | 12-2-2020 | 14-02-2020 | 3 days | Fleming Fund & ISME |
| **Puspa Maya Sharma** | 2014030185 | Sr. Laboratory officer | AMR Laboratory | Melbourne university | Australia | 10-2-2020 | 28-2-2020 | 16 days | UK, Government, Fleming Fellowship |
| | | | | | | | | | |