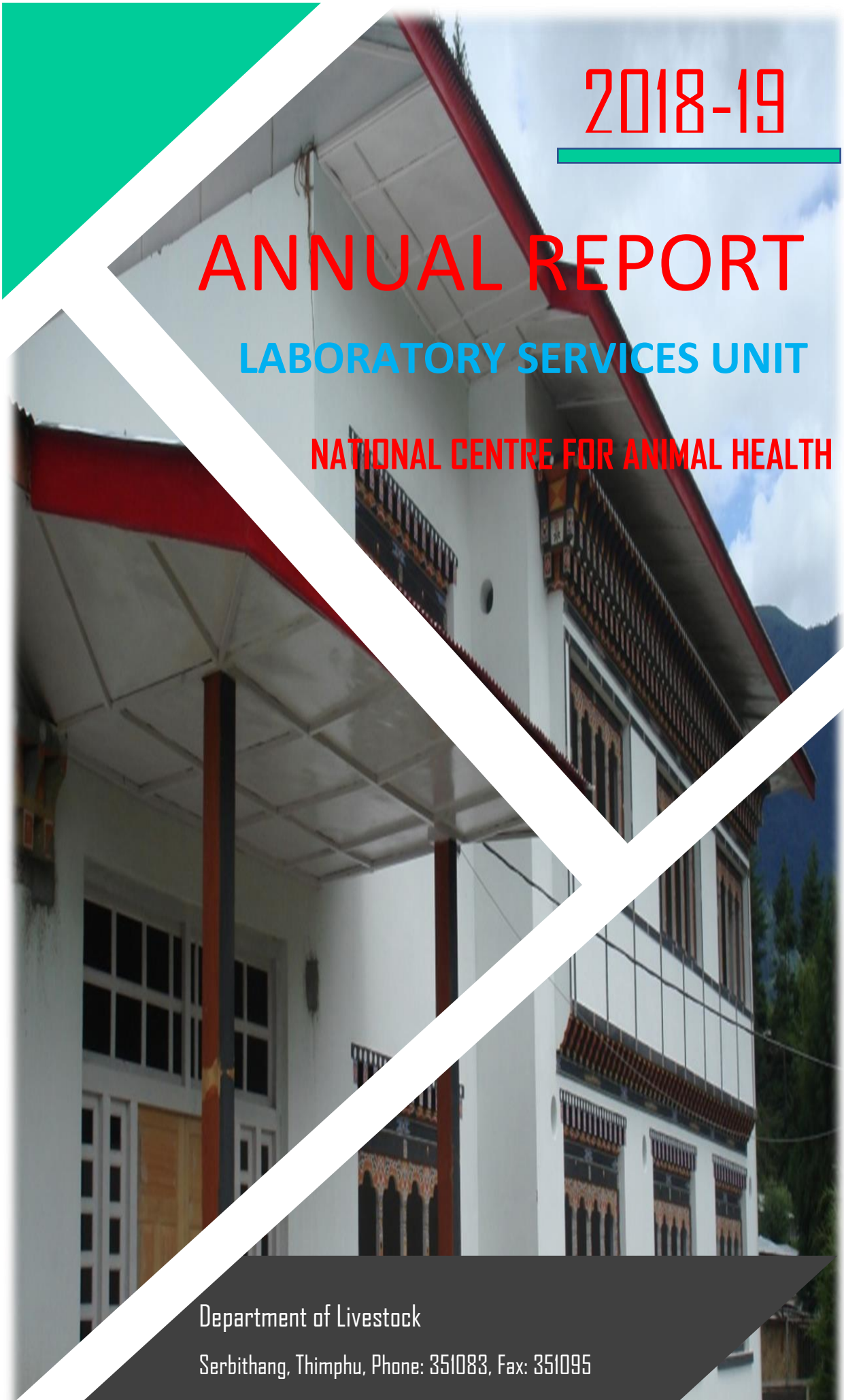
2018-19

ANNUAL REPORT

LABORATORY SERVICES UNIT

NATIONAL CENTRE FOR ANIMAL HEALTH



Department of Livestock

Serbithang, Thimphu, Phone: 351083, Fax: 351095

Contents

1. Background

The Laboratory Services Unit (LSU) is one of the four functional technical units under the National Centre for Animal Health, Serbithang. The unit has the capacity to undertake rapid diagnosis and also advanced diagnostic tests like ELISA, FAT and molecular tests for emerging and re-emerging infectious disease like FMD, highly pathogenic avian influenza (HPAI), classical swine fever (CSF), rabies Brucellosis and Anthrax etc.

The lab is also equipped with high-tech diagnostic equipment like real time PCR machine. Besides the facilities for routine laboratory diagnosis, the unit also has Biosafety level 2 plus facilities for diagnosis of highly infectious diseases including zoonosis. The unit also functions as the National Referral Veterinary Laboratory for the country. In addition, the LSU is also involved in monitoring and evaluating Biosafety in the veterinary laboratories in the country.

2. Mandates of the LSU

The main mandates of the Laboratory Services Unit are:

1. Providing laboratory diagnostic services to the clients
    a. Provide routine 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 and implementation 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 focal laboratory for antimicrobial resistance monitoring in animals in the country
    b. To participate in regional proficiency testing for specific diagnostic methods
    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 Food Testing Laboratory, Bhutan Agriculture and Food Regulatory Authority; Clinical Laboratory, Jigme Dorji Wangchuck National Referral Hospital; Royal Centre for Disease Control, Department of Public Health; and Wildlife Clinic, Nature Conservation Division, Department of Forests and Park Services;
    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 resources in LSU

The followings are the available human resource in the Laboratory Services Unit as on 30th June 2019 (Table 1).

Table 1: Over all human resource capacity of LSU during 2018-19

Specialization	Sections	Numbers
Animal Health Specialist-I (Parasitologist)	Parasitology	1
Animal Health Specialist-III (Pathologist)	Pathology/Bio-chemistry/Toxicology	1
Animal Health Specialist-III (Microbiology)	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 & PM	6
Laboratory Attendant	General	1
Total		**15**

4. Diagnostic capacities in LSU

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

and private livestock agencies. 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 Parasitology section is currently manned by the following staff.

1. Dr. Phuntsho Wangdi, Animal Health Specialist I
2. Ms. Tshewang Dema, Assistant Laboratory Technician

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

- 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;
- Faecal culture (simple tube method, culture tube method, Baermann's method);
- Tick identification (stereo-zoom method);
- Recovery of nematode larvae from soil, herbage and identification;
- Cryptosporidium staining and identification (modified acid fast);
- Microfilaria identification from blood (modified Knott's method);
- Worm staining & preservation;
- ELISA for Fasciola;

4.2 Bacteriology Section

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

The bacteriology section is manned by the following staffs:

1. Dr. RB Gurung, Specialist III, LSU
2. Ms. Puspa Maya Sharma, Laboratory Officer
3. Mr. Tenzinla, Sr. 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 spp., E. coli, Staphylococcus spp., Bacillus anthracis, Clostridium species and Streptococcus species;*
- 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 test (SAT, TAT, Micro-titre plate agglutination test);
- Detection of mastitis in milk samples through CMT, Cell count and WST;
- Antimicrobial susceptibility test;
- Intradermal test for TB using PPD

4.3 Haematology Section

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

The Haematology section is manned by the following staffs:

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

The haematological 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 Bio-chemistry and Toxicology section has the following staff:

1. Dr. NK Thapa, Animal Health Specialist 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 Molecular biology, Serology and Virology sections are manned by:

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

The diagnostic capacities available in this section are:

- Rapid antigen detection tests for AI type A, H5, NDV, IBD, FMD, Rabies and Canine distemper;
- FAT for Rabies;
- Antibody ELISA for FMD, Brucellosis, Rabies, NCD, IBD, CSF, IBR, Leptospirosis, CBPP, CCPP, PRRS, JD and 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, H5N1, ASF, CSF, PPR and NCD
- Agglutination tests - HA/HI for ND and H7N9;
- Slide agglutination test for *Salmonella* and *Mycoplasma*;
- 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 section has the man power as follows:

1. Dr. NK Thapa, Animal Health Specialist III
2. Ms. Pasang Bida, Assistant Laboratory Technician
3. Ms. Ugyen Pema, Assistant 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 human resource in this section is 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 Support on Biosafety and Biosecurity measures
- In House Training
- Reporting and Monitoring
- Samples referral to collaborating laboratories
- Routine and research laboratory test kits, reagents, consumables procurement

5. Key achievements of FY 2018-19

The total samples received/collected and test performed for each section during this financial year is tabulated below in Table 2.

Table 1: Summary of sample received and test performed during 2018-19

Sections	No. of sample processed	No. of Tests conducted
Parasitology	514	1777
Hematology	496	1593
Bio-chemistry &Toxicology	170	725
Bacteriology	634	3388
Serology/Virology /Molecular	2884	4094
Post-mortem	250	250
Histo-Pathology	810	868
International referral		450
Total	**5,758**	**13145**

A total of 5,758 various laboratory samples were processed and 13,145 various laboratory tests were performed for disease screening, surveillance and researches during the year.

5.1 Achievements of individual sections

5.1.1 Pathology section

A total of 250 animal carcasses were necropsied and 810 tissue samples were processed and examined during the year in the pathology section as shown below in Table 3.

Table 3: Samples and test performed in Pathology section during 2018-19

Sample type	Number	Test type	Number
Tissue, organs	810	Histopathology- H & E Staining	868
Carcass	250	Post-mortem/Necropsy	250
Total	**1060**		**1118**

Significant findings

Histopathology: Common cases diagnosed were IBD, ALC, Respiratory infection, Pneumonia and septicaemic infection

Post-mortem: Swine carcasses were received in highest numbers (206) followed by avian (34). Common findings were IBD, HPAI, IBD, ALC and trauma.

5.1.2 Parasitology section

In total of about 514 samples were processed and 1777 tests were performed by parasitology section. The details of tests performed by these sections are shown in Table 4.

Table 4: Samples processed and test performed in Parasitology section 2018-19

Sample type	Number	Test type	Number
Faecal samples	514	Direct examination, Sedimentation, Stolls dilution, Floatation	1777
Total	**514**		**1777**

Parasitology: During the year, the section commonly detected parasitic infestations through the microscopic detection of eggs of Strongyles, Coccidia and Ascaris. All the detected cases were advised for deworming with appropriate anthelmintic. Wherever the parasitism was found at higher level of endemicity, a periodical prophylaxis was also recommended. Besides, the routine work, research on Taeniid infection in dogs is also being conducted.

5.1.3 Bacteriology Section

During the year, the section processed about 634 samples and performed 3388 tests. The details of each type of samples, test types performed in the section are as shown in Table 5.

Table 5: Sample and test performed in Bacteriology section during 2018-19

Types of specimen	Number	Types of tests	Number
Organs	12	Culture	655
Cloacal swab	299	Sub-culture	1310
Whole blood	15	Gram stain	78
Swab	47	Motility	74
Skin scraping	34	Biochemical test	1087
Caeca	36	Antimicrobial sensitivity test	16
Feed	2	LCB Stain	34
Semen	5	Glycerol stock	80
Soil	16	Bacterial revival	54
Fermented milk	114		
Bacterial isolates	54		
Total	**634**	**Total**	**3388**

Significant findings

During the year, *Salmonella* from poultry and piglets and *Erysipelothrix rhusiopathiae* from pigs were isolated and identified from the outbreaks.

Antimicrobial susceptibility tests (AST) for important bacteria like *Salmonella*, *Staphylococcous* were also conducted.

5.1.4 Bio-Chemistry & Toxicology section

In the Bio-Chemistry & Toxicology section, mineral estimation of calcium, magnesium and phosphorous were conducted in 22 serum samples by performing 30 tests. About 145 feed samples were screened against various mycotoxins.
Details of samples and tests conducted in these sections are presented in Table 6.

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

Sample type	Number	Test type	Number
Feed	145	Aflatoxin	417
Serum	22	Fumonisin	108
Urine	3	Ochratoxin	126
		Calcium	44
		Urine Biochemistry	30
Total	**170**	**Total**	**725**

Significant findings

Mycotoxin analysis detected about 46/145 (31.7%) of animal feeds contained aflatoxin above permissible level. Serum chemistry indicated 22/22(100%) of the samples with low calcium in the submitted samples.

5.1.5 Haematology section

Basic haematological tests were also conducted to support the clinical diagnosis in the animals. During the year 496 samples were processed and conducted about 1593 tests.
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

Sample type	Number	Test type	Number
Blood smear	19	PCV	331
Whole blood	477	Hb	331
		DLC	325
		TRCC	300
		TWCC	291
		Knotts test	15
Total	**496**	**Total**	**1593**

5.1.6 Molecular Biology, Serology and Virology Section

During the year, the section processed 2884 nos. of samples and performed about 4094 tests which includes rapid tests: Rose Bengal Test for Brucella abortus in bovine, Influenza A antigen, H5 antigen tests in birds, rabies antigen detection test, IBD, NCD and Fluorescence Antibody Test (FAT) for rabies.

The other screening and confirmatory serological tests include Non-Structural Protein Enzyme Linked Immuno-Sorbent Assay (ELISA) for screening against FMD antibody,

sandwich ELISA for FMD typing, antigen detection ELISA for CSF and Liquid Phase Blocking ELISA for vaccine efficacy studies.

The molecular tests in this section include Multiplex conventional Polymerase Chain Reaction (PCR), real-time reverse transcriptase (RT) PCR for Influenza A, H5 & N1 and Newcastle Disease Virus, Foot and mouth disease, Classical Swine Fever (CSF), PRRS EU, PRRS NA, ASF and Erysipelas diseases. Details of tests performed and samples are shown in Table 8.

Table 8: Sample and test performed in serology, virology and molecular section

Sample Type	Number	Type of Tests	Number
Serum	2361	RBT	847
		Brucella ELISA	842
		FMD ELISA	103
		FMD NSP(Rapid)	63
		FMD-Sero-typing (O, A, Asia-1)	150
		CSF ELISA	92
		PPR ELISA(Ag)	840
		Mycoplasma Synoviae (rapid test)	21
		ALC ELISA	62
		Salmonella pullorum (rapid test)	21
		CDV ELISA	249
		IBR ELISA	96
Tissue/Epithelial/swab	507	PCR (AI, H5, N1)	222
		PCR NDV	32
		Rapid test (AI, H5, NDV, IBD)	56
		PCR FMDV	110
		PCR CSFV	134
		PCR PRRS	94
		PCR ASF	44
Brain	16	FAT	16
Total	**2884**		**4094**

Significant findings

A highly pathogenic avian influenza A (H5N1) virus was confirmed through PCR in the samples of poultry birds received from Dhamdara, Phuentsholing during April 2019. It was also further confirmed at AAHL, Geelong Australia clade Asian H5 clade 2.3.2.1a.

The molecular section also confirmed Classical swine fever and FMD, through real time PCR. Rabies was also confirmed by FAT at serology section from the samples of the outbreak area.

This section conducted international proficiency testing for highly pathogenic avian influenza (H5N1), Newcastle Disease, Classical swine fever, PRRS NA, PRRS EU, ASF and Brucella

5.1.7 Bio-safety and Bio-security section

Bio-safety and bio-security section is mandated to facilitate and regulate strict compliance in performing laboratory activities. Thus, this section is an aide-de-section for all other sections.

Followings are the activities completed by this section:

- Routine Biosafety works
- Developed Laboratory Biosafety manual version 2.
- Maintenance and repairing of equipment
- Developed the incident report form, weekly equipment inspection form and also lab auditing checklist
- Visit to NAH- Motithang for monitoring and evaluation of Lab.
- Visit to CVH SL- Phuentsholing for monitoring and evaluation of Lab.
- Maintained monthly temperature for fridges, incubators and deep freezers.
- Issued spill kits and first aids kits (one in BSL-2plus and one in reception in case of emergency).
- Issued new sharps disposal container for all the sections
- Revised/developed new forms for auditing.
- The section successfully conducted internal auditing for the national laboratory to monitor bio-safety and bio-security measures.
- Visited and technical auditing performed in some National and satellite laboratories.

Biosafety section has successfully evaluated and monitored two laboratories v.i.z City Veterinary Hospital, Satellite Laboratory in Phuentsholing and National Animal Hospital in Thimphu. There were few drawbacks that were addressed which needed to be improved and also some feed backs were given in order to maintain Biosafety in the laboratory. Some brief presentations on Biosafety measures were presented on how to handle samples and also familiarized on how to use proper safety gears and transportation of samples from field to the referral lab. The Biosafety monitoring team has submitted the Biosafety lab report to the head of the centre with recommendation. In the conclusion it was seen that most of the laboratories in country are not able to adhere strictly to Biosafety & Biosecurity protocol. The main reasons being due to budget constraint and also lack of proper awareness and trained lab personnel for Biosafety measures. And not to forget, some lab does not have Biosafety focal person to coordinate and monitor their laboratory, therefore, the team has temporarily appointed Biosafety focal person to monitor their lab on regular basis.

5.1.8 Clinical services provided

Apart from the laboratory diagnostic services provided, the unit also catered clinical service in and around the locality of Serbithang on call basis.

A month wise clinical services such as vaccination, treatment and spaying/neutering of domestic animals attended by LSU is shown in Table 9.

Table 9: Clinical services provided by LSU during the period (July 2018 to June 2019)

Months	Treatment	Deworming	Vaccination Rabies	Sterilization
July	1	5	17	
August	1878	2		
September	6		6	1
October		1	2	
November			4	
December	7	5	6	1
January	151	3	4	3
February			1	3
March		2	2	8
April	3	5	6	8
May	8	3	6	11
June		1	1	
Total	**2042**	**27**	**55**	**35**

Significant findings

Apart from the laboratory diagnostic services provided, the unit also catered clinical service in and around the locality of Serbithang on call basis. Most of the calls were from veterinary hospital, Thimphu. This service is being provided to supplement the services provided by DVH, Thimphu and National Veterinary Hospital, Motithang, Thimphu. Most commonly attended cases were milk fever, manual removal of retained placenta and immunisation against BQ, HS and rabies.

6. Introduction of new tests

During the financial year 2018-19 the following new diagnostic technologies for important diseases were established as follows:

A. molecular techniques

1. Foot & Mouth Disease (FMD)-LAMP test),
2. Porcine Reproductive Respiratory Syndrome (PRRS),
3. Erysipelas
4. African Swine Fever (ASF)

B. Establishment of Cell culture technique; human resource capacity built for cell culture.

C. Bacterial identification for *Erysipelothrix rhusiopathiae*

7. Samples referred to international laboratories

During the year various samples were referred to international laboratories for confirmation. The details are tabulated below in table 10.

Table 10: Sample referred to international laboratories

Sl No.	Referral laboratories	Sample type & tests	Total
1	Armed force research institute of medical science, Bangkok, Thailand	Organs-Scrub Typhus	96
2	Massy university Manawatu campus, New Zealand	Fermented Milk- Bacterial culture	114
3	Pirbright, UK	Epithelial tissue- FMD	33
4	Pirbright, UK	Serum Sample- Vaccine efficacy study, FMD	117
5	Institute for Food safety and Hygiene, University of Zurich, Switzerland	Bacterial isolates-*Salmonella*	54
6	AAHL, Geelong, Australia	Cloacal, tissue, fresh droppings-AIV	7
7	Dept of Veterinary medicine, University of Cambridge, UK	Whole blood, Tissue samples-Canine Transmissible tumour (CTVT)	29
	TOTAL		**450**

8. Laboratory quality assurance

8.1 Laboratory assessment, quality assurance audit and Technical backstopping

Technical Backstopping Missions conducted by Australian Animal Health Laboratory – CSIRO supported through FAO.
Technical Backstopping Missions to Participating Laboratories in Asia under project OSRO/RAP/402/USA
DATE: August, 2018
STAFF: Andrea Certoma, Australian Animal Health laboratory-CSIRO

The visit was aimed to trouble shoot PCR assays and provide technical backstopping to molecular section. The expert reviewed, identified and resolved the underlying issues encountered during Asia Pacific Regional Proficiency Testing for Avian and Swine disease using Quant studio 5 (QS5) real time PCR machine.

New molecular tests for the diagnosis of diseases such as African swine fever (ASF), PRRS was introduced and established at LSU. The section was provided with real time PCR reagents by the team for routine diagnosis and regular participation in PT.

8.2 Asia Pacific Regional Proficiency Testing

The avian diseases and swine disease PCR panel for 2019 proficiency testing consisted of 15 and 18 gamma irradiated samples respectively that were sent to each participating laboratory with instructions to test the samples using their standard diagnostic real-time PCR for Avian influenza A (AIV matrix, MA), Avian influenza A H-type (H5/H7/H9) and Avian paramyxovirus-1 (APMV-1). Some laboratories also tested samples for N-type.

8.2.1 Molecular Diagnostics

The National Centre for Animal Health was provided with the Avian disease PT panel (Influenza A, H-type PCR and Avian paramyxovirus-1) and the swine disease PT panel

(CSF, PRRS, ASF and SIV), for the South Asia and South East Asia 2019 PT programme. LSU reported results for Influenza A matrix, H5, N1, APMV-1, and CSF, PRSS NA, PRSS EU and ASF PCR.

Avian Disease PCR PT panel

Report Date:	**31 May 2019**
Test Name(s):	**Avian influenza virus A and Avian Paramyxovirus-1**
Test Period:	**7th January – 1st February 2019**

Your laboratory reported 15/15 samples correctly. There was no non-specific amplification in the negative control sample (negative allantoic fluid) using the Influenza A type A real time RT-PCR assay. Your laboratory correctly detected representative diagnostic samples at a lower Ct than the consensus median for each of these samples, indicating your assay was overall performing very well.

Table 1. Comparison results for *Influenza A* matrix gene detection

No.	Sample ID	Type	Clade	Bhutan D1	Median	Expected Result
1	P/Chicken/Hoa Binh/A508/2014	APMV-1		UDT		Negative
2	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	31.4	32.2	Positive
3	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	28.8	31.2	Positive
4	A/Environment/Myanmar/SP443/2017	H9N2	Y280	30.8	33.7	Positive
5	A/Duck/Ha Tinh/A338/2014	H5N6	2.3.4(D)	27.9	29.6	Positive
6	Negative allantoic fluid			UDT		Negative
7	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	28.9	31.1	Positive
8	P/Chicken/Lao Cai/A1/2015	APMV-1		UDT		Negative

9	A/chicken/Myanmar/14/2016	H9N2	Y280	27.5	29.8	Positive
10	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	23.4	25.3	Positive
11	A/chicken/Nepal/S-105-TS/2017	H5N8	2.3.4.4icB	30.1	30.1	Positive
12	A/chicken/Lao/NL-1621312/2016	H5N1	2.3.2.1c	27.7	29.2	Positive
13	A/chicken/North Korea/7916/2005	H7N7		26.5	28.7	Positive
14	P/Chicken/Lao Cai/A1/2015	APMV-1		UDT		Negative
15	A/Environment/Myanmar/SP443/2017	H9N2	Y280	31.4	33.8	Positive

UDT- Undetermined/negative. NT- Not tested

Table 2 Comparison based results for *Influenza A* H5 gene detection

No.	Sample ID	Type	Clade	Bhutan D1	Median	Expected Resullt
1	P/Chicken/Hoa Binh/A508/2014	APMV-1		NT		Negative
2	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	32.9	34.6	Positive
3	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	31.8	32.7	Positive
4	A/Environment/Myanmar/SP443/2017	H9N2	Y280	UDT		Negative
5	A/Duck/Ha Tinh/A338/2014	H5N6	2.3.4(D)	29.9	32.5	Positive
6	Negative allantoic fluid	-		NT		Negative
7	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	32.3	33.1	Positive
8	P/Chicken/Lao Cai/A1/2015	APM		NT		Negativ

		V-1				e
9	A/chicken/Myanmar/14/2016	H9N2	Y280	UDT		Negative
10	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	26	27.1	Positive
11	A/chicken/Nepal/S-105-TS/2017	H5N8	2.3.4.4icB	29.9	31.5	Positive
12	A/chicken/Lao/NL-1621312/2016	H5N1	2.3.2.1c	27.1	29.7	Positive
13	A/chicken/North Korea/7916/2005	H7N7		UDT		Negative
14	P/Chicken/Lao Cai/A1/2015	APMV-1		NT		Negative
15	A/Environment/Myanmar/SP443/2017	H9N2	Y280	UDT		Negative

Table 3 Comparison based results for *Influenza A* N1 gene detection

No.	**Sample ID**	**Type**	**Clade**	**Bhutan D1**	**Expected Result**
1	P/Chicken/Hoa Binh/A508/2014	APMV-1		NT	Negative
2	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	32.2	Positive
3	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	UDT	Negative
4	A/Environment/Myanmar/SP443/2017	H9N2	Y280	NT	Negative
5	A/Duck/Ha Tinh/A338/2014	H5N6	2.3.4(D)	UDT	Negative
6	Negative allantoic fluid	-		NT	Negative
7	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	UDT	Negative
8	P/Chicken/Lao Cai/A1/2015	APMV-1		NT	Negative

9	A/chicken/Myanmar/14/2016	H9N2	Y280	NT	Negative
10	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	24.7	Positive
11	A/chicken/Nepal/S-105-TS/2017	H5N8	2.3.4.4icB	UDT	Negative
12	A/chicken/Lao/NL-1621312/2016	H5N1	2.3.2.1c	28.7	Positive
13	A/chicken/North Korea/7916/2005	H7N7		NT	Negative
14	P/Chicken/Lao Cai/A1/2015	APMV-1		NT	Negative
15	A/Environment/Myanmar/SP443/2017	H9N2	Y280	NT	Negative

Table 4 Comparison based result for APMV-1, matrix, real time PCR results

No.	Sample ID	Type	Clade	D1	Median	Expected results
1	P/Chicken/Hoa Binh/A508/2014	APMV-1		32.9	33.6	Positive
2	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	NT		Negative
3	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	NT		Negative
4	A/Environment/Myanmar/SP443/2017	H9N2	Y280	NT		Negative
5	A/Duck/Ha Tinh/A338/2014	H5N6	2.3.4(D)	NT		Negative
6	Negative allantoic fluid			UDT		Negative
7	A/Chicken/Philippines/0938-1/2017	H5N6	2.3.4.4(C)	NT		Negative
8	P/Chicken/Lao Cai/A1/2015	APMV-1		32.2	34	Positive
9	A/chicken/Myanmar/14/2016	H9N2	Y280	NT		Negative

10	A/Quail/Myanmar/SP232/2015	H5N1	2.3.4.2	NT		Negative
11	A/chicken/Nepal/S-105-TS/2017	H5N8	2.3.4.4icB	NT		Negative
12	A/chicken/Lao/NL-1621312/2016	H5N1	2.3.2.1c	NT		Negative
13	A/chicken/North Korea/7916/2005	H7N7		NT		Negative
14	P/Chicken/Lao Cai/A1/2015	APMV-1		34	35.8	Positive
15	A/Environment/Myanmar/SP443/2017	H9N2	Y280	NT		Negative

Swine Diseases PCR PT panel

Report Date:	**19 June 2019**
Test Name(s):	***Classical swine fever virus, Porcine reproductive and respiratory syndrome virus, African swine fever virus, Influenza A virus from* swine species**
Test Period:	**7th January – 1st February 2019**

Your laboratory reported 18/18 samples correctly for the CSF PCR assay, PRRS EU, NA. Your laboratory showed no cross reaction with differential samples for CSF and PRRS. There was no non-specific amplification in the negative pig serum sample using the real-time PCR. For CSF the sample 2 and 5 showed ct value within (±1-2) the consensus median.

PRRS- For North American Strain PRRS, the sample 6, 14 and 15 showed ct value within (±1-2) the consensus median. For PRSS EU strain the sample 4 and 9 showed ct vaule within the consensus median, indicating your assay was overall performing well.

For ASF sample 1, 10, 12, 13 and 16 were detected with Ct value higher (≥3 Ct) than the median. Sample three proved problematic for many participants with only nine laboratories identifying it as ASF positive, and six laboratories. Sample had a median Ct value of 36.0 and was included in the panel as a representative of a very weak sample that may be observed in processed meat products or from inappropriately sampled samples (i.e. oral swabs) or observed when PCR inhibition may be an issue. The

assays/methods used was unable to detect virus in positive samples and reported higher Cts across most samples reported positive, indicating potential systematic issues with assay sensitivity with Cts (≥3) on average higher than the median

Table 5 Comparison based results for *Classical swine fever* virus real-time PCR

Sample	Virus ID	Isolate	D1	Median	Expected Result
1	ASF	Georgia 2007 (Genotype II)	UDT		Negative
2	CSF	Germany/1964 (Sub-genotype 1.1)	34.4	33.7	Positive
3	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT		Negative
4	PRRS-EU	PRRS European Strain - Lelystad	UDT		Negative
5	CSF	Germany/1964 (Sub-genotype 1.1)	34.6	33.6	Positive
6	PRRS-NA	PRRS - strain NADC-8	UDT		Negative
7	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT		Negative
8	negative	Negative pig sera	UDT		Negative
9	PRRS-EU	PRRS European Strain - Lelystad	UDT		Negative
10	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT		Negative
11	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT		Negative
12	ASF1	BA71V (Genotype 1)	UDT		Negative
13	ASF	Georgia 2007 (Genotype II)	UDT		Negative
14	PRRS-SEA	PRRS-SEA Circulating strain	UDT		Negative
15	PRRS-NA	PRRS - strain NADC-8	UDT		Negative

16	ASF1	BA71V (Genotype 1)	UDT		Negative
17	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT		Negative
18	PEDV	PEDV Colorado	UDT		Negative

Table 6 Comparison based results for North American strains of *Porcine reproductive and respiratory syndrome virus (PRRS-NA)* virus real-time PCR

Sample	Virus ID	Isolate	Bhutan D1	Median	Expected Result
1	ASF	Georgia 2007 (Genotype II)	UDT		Negative
2	CSF	Germany/1964 (Sub-genotype 1.1)	NT		Negative
3	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT		Negative
4	PRRS-EU	PRRS European Strain - Lelystad	UDT		Negative
5	CSF	Germany/1964 (Sub-genotype 1.1)	NT		Negative
6	PRRS-NA	PRRS - strain NADC-8	35	34.09	Positive
7	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT		Negative
8	negative	Negative pig sera	UDT		Negative
9	PRRS-EU	PRRS European Strain - Lelystad	UDT		Negative
10	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT		Negative
11	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT		Negative
12	ASF1	BA71V (Genotype 1)	UDT		Negative
13	ASF	Georgia 2007 (Genotype II)	UDT		Negative

14	PRRS-SEA	PRRS-SEA Circulating strain	30.4	32.06	Positive
15	PRRS-NA	PRRS - strain NADC-8	33.8	34.24	Positive
16	ASF1	BA71V (Genotype 1)	UDT		Negative
17	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT		Negative
18	PEDV	PEDV Colorado	UDT		Negative

Table 7 Comparison based results of *Porcine reproductive and respiratory syndrome virus (PRRS-EU)* real-time

Sample	Virus ID	Isolate	Bhutan D1	Median	Expected Result
1	ASF	Georgia 2007 (Genotype II)	UDT	-	Negative
2	CSF	Germany/1964 (Sub-genotype 1.1)	NT	-	Negative
3	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT	-	Negative
4	PRRS-EU	PRRS European Strain - Lelystad	36.6	35.5	Positive
5	CSF	Germany/1964 (Sub-genotype 1.1)	NT	-	Negative
6	PRRS-NA	PRRS - strain NADC-8	NT	-	Negative
7	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT	-	Negative
8	negative	Negative pig sera	UDT	-	Negative
9	PRRS-EU	PRRS European Strain - Lelystad	36.1	36.1	Positive
10	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT	-	Negative
11	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT	-	Negative
12	ASF1	BA71V (Genotype 1)	UDT	-	Negative
13	ASF	Georgia 2007 (Genotype II)	UDT	-	Negative

14	PRRS-SEA	PRRS-SEA Circulating strain	NT	-	Negative
15	PRRS-NA	PRRS - strain NADC-8	NT	-	Negative
16	ASF1	BA71V (Genotype 1)	UDT	-	Negative
17	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT	-	Negative
18	PEDV	PEDV Colorado	UDT	-	Negative

Table 8. Comparison based result for *African swine fever virus* real-time PCR

Sample	Virus ID	Isolate	Bhutan D1	Median	Expected Result
1	ASF	Georgia 2007 (Genotype II)	33.1	29.97	Positive
2	CSF	Germany/1964 (Sub-genotype 1.1)	NT		Negative
3	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT	35.99	Positive
4	PRRS-EU	PRRS European Strain - Lelystad	NT		Negative
5	CSF	Germany/1964 (Sub-genotype 1.1)	NT		Negative
6	PRRS-NA	PRRS - strain NADC-8	NT		Negative
7	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	UDT	32.92	Positive
8	negative	Negative pig sera	UDT		Negative
9	PRRS-EU	PRRS European Strain - Lelystad			Negative
10	ASF	Malawi Lil-20/1 (1983) (Genotype VIII)	35.2	32.99	Positive
11	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT		Negative
12	ASF1	BA71V (Genotype 1)	31.5	27.99	Positive
13	ASF	Georgia 2007 (Genotype II)	36.9	32.7	Positive

14	PRRS-SEA	PRRS-SEA Circulating strain	NT		Negative
15	PRRS-NA	PRRS - strain NADC-8	NT		Negative
16	ASF1	BA71V (Genotype 1)	35.6	31.2	Positive
17	SIV	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	UDT		Negative
18	PEDV	PEDV Colorado	UDT		Negative

9. Biosafety & Biosecurity Monitoring

9.1 Laboratory Auditing

Under Biosafety monitoring and evaluation program, Biosafety coordinator had done auditing of its centre on monthly basis and submitted the report to head of the Laboratory. The unit had also made visit to City Veterinary Hospital Satellite Laboratory Building in Phuentsholing on December 11th to 12th and National Veterinary Hospital in Thimphu on 7th May, 2018 respectively.

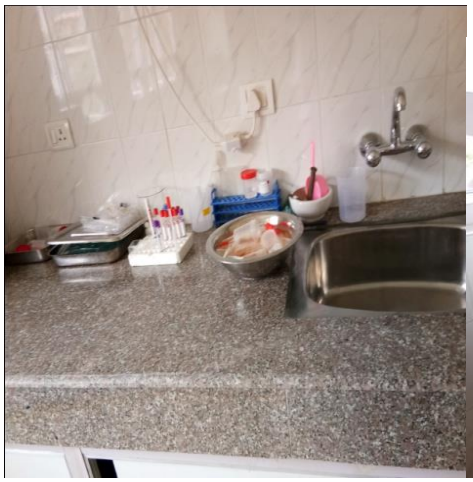
Objective of the visit

The tour was conducted to monitor the bio-safety practices followed at the Regional and city veterinary laboratories in the country. The tour was part of monitoring and evaluation mechanism to be followed by the National Centre in terms of providing necessary feedback and helping in developing guidelines for the Regional levels.

Common observations

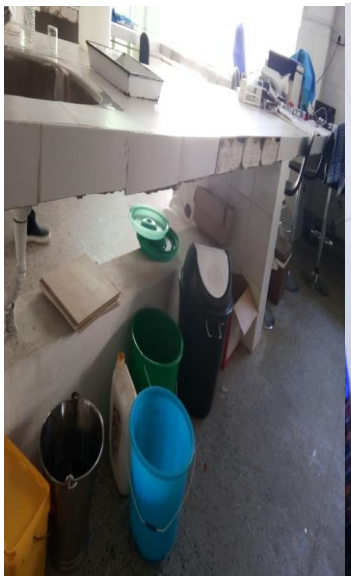
- No proper signage pasted on the main entrance door to the laboratory section (Biohazard sign)
- No proper Guidelines available
- No Proper sorting of hazardous and non-hazardous chemicals with labelling
- No proper record keeping for test done and results
- No close toe shoe/slipper for the lab personnel
- No different lab coats for workers working inside and outside lab
- No proper register maintained for equipment and chemicals to check its functionality
- Temperatures not maintained for any incubator and fridge
- No biohazard signage pasted on fridge where positive samples are kept
- No SOPs for some equipment
- Lab does not have any prevention from insects or rodent
- No eyewash available in laboratory

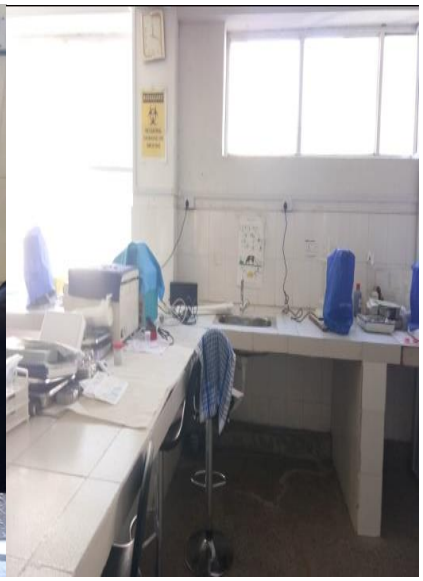
- Not enough Personal protective equipment available (gloves, gowns, lab coat, mouth cover, goggles, etc)
- Need for Biological safety cabinet for those centres which deals with infectious samples
- No proper waste management guidelines available
- No Lab personnel and ESP properly trained on Biosafety measures and protocols
- No spill kits in case of accidents
- No first Aid kits and fire extinguishers



1. No proper arrangement

2. No biohazard signs





Recommendations

- There is a need for the appointment of a Biosafety coordinator/focal person in the respective centres.
- It was observed that the laboratory setup is not up to Biosafety standard protocol but still there can be some adjustment done dividing the sections base on risk factor group handled.
- The biohazard sign should be placed in the entrance door and also sign board displaying that "no eating, drinking and chewing in the lab". Also add contact no. of the in charge or person concern in your biohazard sign in case needed in times of emergency
- Standard operating procedure (SOP) for all equipment are required and also maintain equipment log chart to monitor the functioning and record the user of the equipment.
- There is requirement of visitor form and incident/accident report form, which should be maintained and recorded as and when required.
- Temperature log chart, incubator log chart and autoclave log chart should also be maintained on daily basis.
- Every personnel working in lab should remove their lab coat before leaving the lab area and entering non lab areas (lobby, administration offices, etc,).
- It is also recommended for proper storage of flammables and corrosives inside the closed shelf.
- The work desk should be maintained clean without any stationery or equipment before and after the conduct of laboratory works.
- Thermometer is to be placed inside every fridge so that proper temperature for different test kits can be maintained as a result the quality of test kits will be maintained too.
- Since, there is no proper sites for disposal of laboratory waste, a pit needs to be constructed.
- Waste management guidelines should be followed properly for infectious, sharp and non-infectious etc.
- It is preferable, to use different coloured laboratory coat during working inside lab and outside lab for other purpose which is washable.
- It is recommended to use uniform coding system for the equipment, such as (Region,)/ (equipment code)/section (Number, could be ed with 01) for easy identification and monitoring.
- Awareness training should be provided to the lab personnel and also to the ESPs about proper handling and disposal of different types of wastes.
- It is highly recommended to procure the closed toe shoes, instead of the open toe shoes.
- It is recommended to use of 1-2%Virkon and bleaching (only) as disinfectant and to do away with any other phenol disinfectant.
- There is a need for first aid kits and spill kits and locate them in designated area of the lab.

9.2 Equipment maintenance

During the year, the section co-ordinated and maintained various laboratory equipment as follows:

Sl. No.	Name of equipment	Remarks
1	Two Incubators (In4 &In5 bacteriology section	Issues rectified
2	Freezer(R1-Parasitology)	Issues rectified
3	Freezer (R5, R15 &R16-Bacteriology)	Issues rectified
4	Freezer (R13-Clean room)	Issues rectified
5	Freezer (R10, R11 &R18)	Issues rectified
6	Deep Freezer (Old)	Issues rectified

9.3 Incidence Monitoring & Reporting

The section also maintained the various incidences that occurred in the lab as below:

Date	Incident	Action
14.02.2019	Accidental Spillage of rabies sample inside the eye of one lab personnel occurred while opening the sample package.	• Immediately eye wash and rushed to hospital. • Vaccinated against Rabies • Biosafety officer issued a notification about the incident and advised all the lab personnel to comply by Biosafety measures while dealing with high risk samples.
10.05.2019	While carrying out maintenance of Biosafety Cabinet II, the service providers got injured in his hand as the cabinet fell upon him.	• Reported the incident to the lab head • Washing hand with disinfectant (Dettol) and applying antiseptic cream and bandaging.

10 Laboratory Information Management System (LIMS)

Dr RB Gurung
National Centre for Animal Health, Serbithang

Introduction

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). Once the system is live online it can be remotely accessed by any authorized personnel.

Rationale

Management of laboratory diagnostic activities, associated analysis and reporting is a time-consuming process often riddled with transcription error such as typing and optical character recognition (OCR) errors and more difficult in a manual system. Custom in-house solutions are developed by few individual laboratories, while some enterprising entities at the same time sought to develop a more commercial reporting solution in the form of special instrument-based systems. LIMSs are dynamic because the laboratory's requirements are rapidly evolving and different laboratories often have different needs.

In Bhutan, the veterinary laboratory network covers almost all dzongkhags with one laboratory in each district, four regional laboratories, four satellite laboratories and one national laboratory. On annual basis, these laboratories churn out large number of data from the number of samples submitted and test performed. Until now, all the laboratory networks in the country are maintaining this information on paper-based system. Storage and management of such large volume of data manually is a huge challenge with high degree of vulnerability to loss and damage. A web-based database system will immensely help in electronic storage, enhanced security, easy analysis and control on chain of custody.

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

1. Reception and log in of a sample and its associated customer data
2. Assignment, scheduling, and tracking of the sample and the associated analysis
3. Processing quality controls associated with the sample and equipment
4. Storage of data associated with the sample analysis
5. Inspection, approval and compilation of report or further analysis

Additional functionality

Aside from the key functions of sample management, instrument and application integration, and electronic data exchange, there are numerous additional operations that can be managed in a LIMS. This includes but is not limited to:

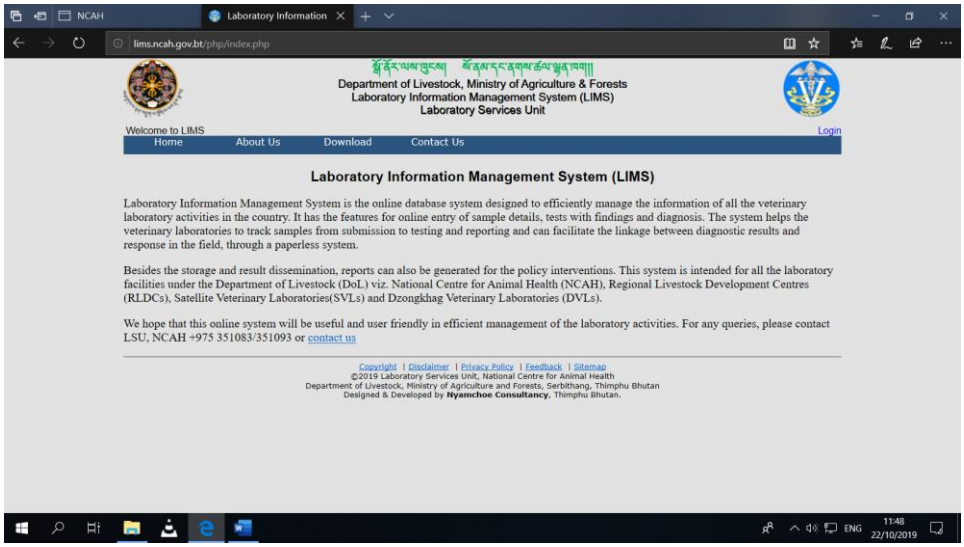
1. Audit management: Fully track and maintain technical audit trail
2. Chain of custody: Assign roles and responsibilities that dictate access to specific data records and management
3. Compliance: Follow regulatory standards that affect the laboratory
4. Customer management: Handle the demographic information and communications for associated clients
5. Document management: Process and convert data to certain formats; manage how documents are distributed and accessed

6. Inventory: Measure and record inventories of vital supplies and laboratory equipment
7. Electronic data entry: Provide fast and reliable interfaces for data to be entered by a human or electronic component
8. Method management: Provide one location for all laboratory process and procedure and methodology to be housed and managed as well as connecting each sample handling step with current instructions for performing the operation
9. Workload management: Organize work schedules, workload assignments, employee demographic information, training, and financial information
10. Quality assurance: Gauge and control sample quality, data entry standards, and workflow
11. Reports: Create and schedule reports in a specific format; schedule and distribute reports to designated parties
12. Time tracking: Calculate and maintain processing and handling times
13. Traceability: Show audit trail and/or chain of custody of a sample
14. Workflows: Track a sample, a batch of samples, or a "lot" of batches through its lifecycle

Features of newly developed LIMS database

1. Nested with NCAH webpage domain
2. LIMS Home page: Log in page of LIMS
   Home page tab
   A. Home
   B. About us
   C. Dashboard
   D. Data management
   E. Report
   F. Admin
   G. Download
   H. Contact us

Details of home page tab



Features of LIMS

A. Home

About the LIMS database

B. About us

About the Centre (NCAH) and the Department (DoL)

C. Dash board

Pending report with

- Registration number
- Interface site (name of laboratory)
- Sender details
- Referral details
- Registration date
- Test status

D. Data management

- Owner registration
- Sample registration
- Sample test and result
- Sample diagnosis and recommendation

E. Report

- Data filter (region, dzongkhags, centre, year, disease etc)
- Analysis
- Data back up and migration

F. Admin

- Password change
- Level of access

G. Download

Reports
Feedbacks
Master files
Configuration parameters
User guide

H. Contact us

Contact information: address, phone, email, fax and hotline

3. Data management interfaces

1. Owner registration interface

a. Owner details: name, ID etc
b. Owner address: village, geog, dzongkhag, country etc
c. Contact number

2. Sample registration interface

a. Sample details: system generated registration number, date sent, date received, sender details, and reference number
b. Owner details (auto connected through ID)
c. Details of animals: ID, age, sex, breed
d. Case history: brief narrative of illness, length of illness, symptoms, PM findings, treatment, vaccination and disease suspected
e. Test requested for: bacteriology, biochemistry, heamatology, histopathology, molecular, mycology, PM, serology, toxicology and virology
f. Specimen: ID and type

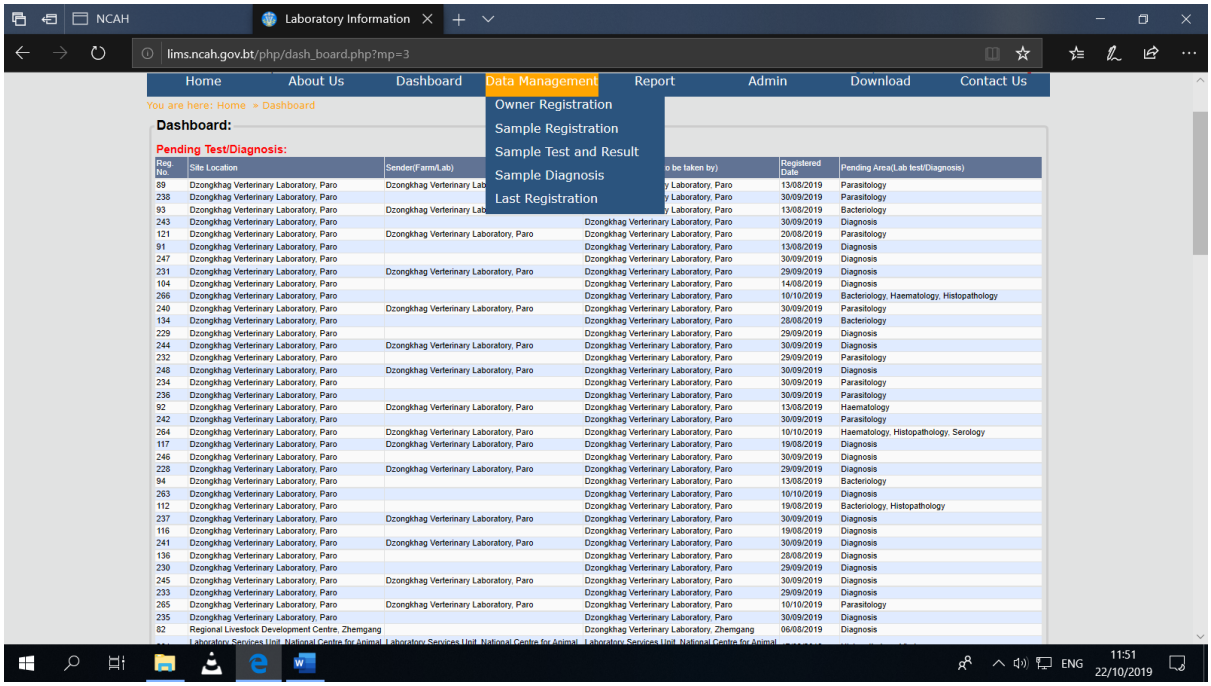
3. Sample test and result interface

a. Range of bacteriology test and result
b. Range of Biochemistry test and result
c. Range of Haematology test and result
d. Range of Histopathology test and result
e. Range of Molecular test and result
f. Range of Mycology test and result
g. Range of PM findings
h. Range of Serology test and result
i. Range of Toxicology test and result
j. Range of Virology test and result

4. Diagnosis and recommendation interface

a. Range of diagnosis based on the test results

b. Authorized and competent veterinarian shall sign off report with appropriate recommendation



Different features in data management

The database (LIMS) was completed with several rounds of discussions. A training of trainers (ToT) was also carried out involving the representatives from RLDCs, SVLs and DVL. A month-long testing was conducted by hosting with the aim of getting feed backs and comments from the end users from regional and dzongkhag levels. The glitches were finally fixed and made ready for launching.

11. Fleming Fund' Country Grant

The UK Government's based Fleming Fund was granted to Ministry of Health (MoH) and Agriculture & Forests (MoAF) to strengthen Bhutan's capacity for dealing with the worldwide problem of antibiotic resistant diseases for strengthening AMR surveillance. The reception was organized to mark the commencement during March 2019 and was hosted by sir Dominic Asquith, KCMG, British high commissioner to India and Michael Rutland, OBE, British honorary consul to Bhutan at hotel Tashi Taj, Thimphu.
The aim of the Fleming Fund is to address critical gaps in surveillance of antibiotic-resistant bacteria in low- and middle-income countries (LMICs) in Asia and Sub-Saharan Africa, which are set to bear the highest burden of antibiotic-resistant infections.

This is the first Fleming Fund Country Grant to be released in Bhutan. In preparation for this grant Mott MacDonald, carried out a Scoping Visit in January 2018 which was followed by positioning activities in March 2018 to refine the design of surveillance systems and conduct laboratory assessments, in order to identify the priority areas to be supported through this RFP.

These activities culminated in identification of the major gaps and needs for strengthening AMR and AMU surveillance in humans and animals, and informed agreement with the Royal Government of Bhutan about grant objectives and outputs.

The focus of AMR surveillance in food animals supported by the first country grant will be on testing for resistance in enteric bacteria in healthy broilers and layer hens in Thimphu and Chukha and Trashigang Regional Livestock Development Centres. It will also include the National Food Testing Laboratory (NFTL) testing for resistance in bacteria on locally grown chicken in the Thimphu area.

The detailed outputs for the animal health component are listed as below:

Objective 3: Strengthen AMR and AMU surveillance in food animals

- Output 3.1: A MOAF AMR and AMU Surveillance TWG is functioning in accordance with a NATC-approved TOR.
- Output 3.2: National Veterinary Laboratory (NVL) is functioning as the AMR reference laboratory.
- Output 3.3: NVL, and Chukha & Trashigang Regional Livestock Development Centre (RLDCs), & NFTL produce reliable quality bacterial culture, identification and Antibiotic Susceptibility Test (AST) results for *E. coli*, *Klebsiella*, *Salmonella* and *Enterococci*.
- Output 3.4: NVL and NFTL have the capacity to culture *Campylobacter*.
- Output 3.5: Biosafety and biosecurity measures are being applied within NVL, the two regional laboratories and the NFTL and to the safe transport of samples and isolates between the laboratories.
- Output 3.6: Good quality samples from healthy layer hens and broilers are regularly sent to NVL, CRLDC and TRLDC for culture and AST, according to the agreed schedule.
- Output 3.7: Good quality samples from locally grown chicken meat are regularly sent from meat shops in Thimphu to NFTL for culture and AST, according to the agreed schedule.
- Output 3.8: A national database of verified AMR results and associated demographic data is maintained in WHONET at the NVL.
- Output 3.9: National Centre for Animal Health (NCAH) shares quarterly and annual reports of AMR and AMU surveillance results with the MOAF AMR and AMU Surveillance TWG, the NATC and the RLDCs.
- Output 3.10: Extended G2C database for electronically recording prescription and antibiotic use data in veterinary districts is recommended.

The grant of about £1-1.5m is expected to be implemented for 18 months. Following the grant, the inception phase of six months (April to September 2019) was initiated with focus on strengthening the governance i.e. office set ups and infrastructure development. The project started with the kick off meeting during April 2019 where the procurement procedures for consumables, reagents, chemicals and media were discussed and finalized.

12. Fleming Fellowship-Chevening Scholarship Awards to Bhutan

One of the components of the Fleming fund is also Fleming fellowship. The aim of the Fleming Fellowship Scheme is to advance the goals of the Fleming Fund by supporting the professional development of key practitioners and change-makers in selected countries.
The objectives are: to enhance investments made through Country and Regional Grants for improved AMR and Antimicrobial use (AMU), surveillance, encouraging peer-to-peer learning and joint problem-solving through participation in One Health communities of practice and contribute to the global dialogue on combatting resistance.

Under this scheme, the management agent has funded three Fleming Fellowships in the animal health in the following areas:

- AMR Surveillance Fellowship
- AMR Laboratory Fellowship
- AMC/U Surveillance Fellowship

The above three fellows were selected through open advertisement during the period.

13. Workshop for developing the SOP for *Salmonella* organism in human health, animal health and food

One of the activities under the AGISAR WHO project is "one health approach to determine antibiotics susceptibility profile of *Salmonella* in human, animal and food products in Bhutan". One of the sub-activities under the project is to develop the SOP for *Salmonella* organism in human health, animal health and food. Accordingly, a one health workshop was conducted to develop and harmonize the test protocol for *Salmonella* in human, animals and food.

The isolation of *Salmonella* from animal faeces may be complicated by several factors. Animals may be sub-clinically infected and shedding small numbers of *Salmonellae* in their faeces. Additionally, the population of *Salmonellae* in faeces is typically much lower than that of other enteric flora. Similarly, *Salmonella* populations in food samples may be stressed due to heat, pH, or salt content, or unevenly distributed through the food matrix.

Considering the, complexities of the isolation methods for *Salmonella*, the protocol is being harmonized from the Standard Operating Procedure (SOP) from the human, animals and the food laboratories. The protocol will serve as record and reference for carrying out the surveillance of *Salmonella* in all the three sectors. The main objective of the harmonization of the test protocol is to obtain uniform result and set an exemplary of one health activity.

This harmonized protocol is for the identification, confirmation and ABST profiling of *Salmonella* organism isolated from human, animals and food. This document covers the protocols for human, animal and food laboratories for carrying out the one health studies on *Salmonella* organism.

The protocol will remain as reference guide for carrying out the surveillance of *Salmonella*.

14. Animal Disease Surveillance and Researches

14.1 Bhutan at the forefront of antimicrobial resistance prevention activities

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1. Abstract
The global concern of AMR is addressed by World Organisation for Animal Health, World Health Organization and the Food and Agriculture Organization. In line with the global strategy of AMR containment, the Department of Livestock under the Ministry of Agriculture & Forests in Bhutan has initiated prevention and containment activities for AMR in the country. We conducted a review to understand the status on containment activities in Bhutan with an aim to align/realign the activities with National Action Plan. Multi drug resistant (MDR) *Salmonella* is common pathogen isolated in both imported and home-produced chicken and were found to be resistant to antibiotics like Gentamicin, Streptomycin, Ampicillin, Trimethoprim, Cephalexin, Ciprofloxacin Nalidixic acid & Amoxicillin. MDR ESBL producing Escherichia coli was isolated from the pigs, which are found resistant to Ampicillin, Cephalothin, Cefotaxime, Ciprofloxacin, Streptomycin, Chloramphenicol, Nalidixic acid, Sulphamethoxazole, Trimethoprim, Tetracycline and Kanamycin.

The Ministry of Health and Ministry of Agriculture & Forests developed National action plan jointly for prevention and containment of AMR. Public awareness are created by observing antibiotic awareness week and through mass media. The antibiotic uses in both human and animal is being regulated by the Drug Regulatory Authority of Bhutan. Use of antibiotics as growth promoters/additives in animal feeds are also restricted in the country.

2. Introduction
At the global level, the issue of Antimicrobial resistance (AMR) is addressed through the tri-partite approach by World Organization for Animal Health (OIE), World Health Organization (WHO) and the Food and Agriculture Organization (FAO). In line with the global strategy, the Department of Livestock (DoL) under the Ministry of Agriculture & Forests (MoAF) in Bhutan has initiated AMR prevention and containment activities in the country. A review was conducted to understand the status on activities with an objective of monitoring and also aligning and realigning of activities as per the National action plan (NAP).

3. Methods
Literature review on published articles related to AMR in the country were conducted. In addition, policy documents like National Action Plan (NAP) 2018-22 of Bhutan,

Medicine Act of Kingdom of Bhutan 2003 and Bhutan Medicine Rules & Regulation 2012 were also referred.

4. Results

Salmonella, multi drug resistant (MDR) to various antibiotics have been detected in both imported and home-produced chicken and ESBL producing *Escherichia coli* MDR to various antibiotics was detected from the government pig breeding farms (table).
The cabinet has endorsed NAP 2018-22 developed jointly by the Ministry of Health and MoAF (One Health approach). The objectives of NAP are establishment of governance structure, promote rational use of antimicrobials, institute surveillance & monitoring system, create awareness, promote research, strengthen national and international collaboration and strengthen control and regulation on AMR.

The Drug Regulatory Authority (DRA) regulates the antibiotic uses in both human and animal as per the Medicine Act of Kingdome of Bhutan 2003 and Bhutan Medicine Rules & Regulations 2012. Use of antibiotics as growth promoters/additives in animal feeds are also restricted in the country. Antibiotic guideline has been developed to promote it's rational use. Bhutan is regularly participating in antibiotic awareness week as an awareness program. Bhutan is soon receiving grant from UK government-based Fleming fund to strengthen the laboratory capacity and diagnosis in AMR.

Table: Important isolates

Isolates	***Source***	***Resistance to Antimicrobials***
Salmonella entiridis	Imported chicken	NA, AMO, CE, CIP, TMP
Salmonella typhymurium	Imported chicken	NA, CE
	Locally produced chicken	TET, TMP, AMO, AMP, GENT, S
Extended-Spectrum ß-Lactamase (ESBL) producing *Escherichia coli*	Pigs (govt. farms)	AMP, CF, CTX, CIP, S, C, NA, SMZ, TMP, TET, K

NA-Nalidixic acid, AMP- Ampicillin, TET- Tet racycline, AMOX-Amoxicillin, CIP-Ciprofloxacin, S- Streptomycin, TMP-Trimethoprim, GENT-Gentamycin, CF-Cephalothin, CTX-Cefotaxime, C-Chloramphenicol, SMZ-Sulphamethoxazole, K-Kanamycin, CE Cephalexin

7. Conclusions

The country has NAP, which is endorsed by the cabinet reflecting the political commitment to combat AMR in Bhutan. In addition, the Medicine Act of Kingdom of Bhutan 2003 and Bhutan Medicine Rules & Regulations 2012 supports the regulation. With the detection of MDR *Salmonella* an AGSIAR WHO supported one-health studies are underway. Use of antibiotics as growth promoters/additives in animal feeds are also restricted in the country. Awareness is being created with regular participation on Antibiotic awareness weeks. Laboratory surveillance will be strengthened with the help of Fleming fund.

Poster Presented at Second oie global conference on antimicrobial resistance and prudent use of antimicrobial agents in animals: putting standards into practice, Marrakesh, Morocco, 29th to 31st October 2019

14.2 Prevalence of Taeniid parasites and molecular characterization of *E. granulosus sensu stricto* in dogs, human and cattle in Bhutan

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1. Introduction

Dogs belong to the first species to be domesticated contributing to hunting activities but also providing companion. In recent years an increase of the free-roaming community dog population has been observed with an estimate of 71,245 owned dogs and 48,379 free roaming community dogs from which 22,772 are in urban areas while 25,607 are in rural areas. A diverse range of zoonotic infections, including parasitic, bacterial, viral and fungal diseases, can be transmitted from dogs to human. Furthermore, dogs are known since decades as definitive host for *Taenia multiceps* causing coenurosis, commonly called as gid disease in yaks in Bhutan. Among various parasitic diseases, eggs of Taeniids *(Taenia* and *Echinococcus)* and *Toxocara spp*. have been found in dogs during routine faecal microscopy examination in Bhutan.

Although cystic echinococcosis (CE) was documented in humans and livestock in Bhutan there is very little information regarding the molecular diversity of *E. granulosus* s.l. in animals. In this study, we characterized the Taeniid eggs from community, private owned, free-roaming dogs and yak dogs from all districts in Bhutan and also conducted a molecular characterization of the *Echinococcus* cysts collected from human patients over a period of two years (2015-2017).

2. Material and methods

A total of 953 faecal samples were collected from all the 20 districts of the country between May 2016 and April 2018. 670 community dog fecal samples and 283 yak dogs and field carnivore samples. 13 human cysts of varying sizes were collected between 2015 and 2017 from patients undergoing surgery at the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Thimphu. Furthermore, bovine cysts from lungs and spleen were collected from a carcass of a mithun [*Bos frontalis*] breeding bull.

3. Molecular analysis

Genomic DNA extraction was carried at the Institute for Parasitology. The positive samples were subjected to multiplex polymerase chain reaction [PCR] using mitochondrial DNA targets for identification of the morphologically indistinguishable

eggs of Taeniid tapeworms. For human and bovine cyst samples, confirmation of the parasite species was accomplished by PCR of the cox1 gene (366bp).

4. Result

A total of 72 out of 953 (7.5%) dog faecal samples were positive for at least one cestode species. 3.15% [30/953] were positive to *E. granulosus s.s.,* 0.84% [8/953] to *E. ortleppi* and 3.46% [33/953] to various other *cestode* species. The highest prevalence of *E. granulosus s.s* was observed in Paro (10.17%) followed by Wangdue Phodrang (9.79%) and Trashigang (5.19%) districts. *T. multiceps* were recovered in dog fecal samples from three yak rearing districts in western Bhutan (Gasa, Thimphu and Haa).

5. Conclusion

This is the first nationwide detailed study conducted to understand the prevalence of various Taeniid, particularly *E. granulosus* s.l. in dogs, livestock and humans and also *T. multiceps*, the causing agent of coenurosis (gid disease) in yaks in Bhutan. Our study documents the presence of *E. granulosus* s.s. in dog faeces and also in human cysts in the same area/districts indicating transmission of *E. granulosus* s.s. from dogs to humans. Hence, regular deworming of community and private dogs that have access to viscera of potentially infected intermediate hosts and especially yak dogs should be implemented to control both, cystic echinococcosis in humans and gid disease in yaks.

14.3 Report on Erysipelas outbreak at Regional Pig Breeding Centre, Yusipang

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[2]Regional Pig Breeding Centre, Yusipang

1. Summary

The outbreak of disease at the GGP shed at RPBC Yusipang with the clinical signs of skin rashes, high fever, inappetance and swollen joints has been diagnosed as Erysipelas also known as "Diamond skin". The disease is caused by a bacterium, Erysipelothrix rhusiopathiae (syn, insidiosa) that is found in most of the pig farms. The bacteria was isolated from nasal swab/rectal swab and pus from joint of the affected animals, including the dead due to disease in the farm.

The bacteria is always present either in the pig or in the environment because it is excreted via saliva, faeces or urine. It is also found in many other species, including birds and sheep and can survive outside the pig for a few weeks and longer in light soils. The disease is confirmed by isolation and identification of the organism in the laboratory. The clinical signs of the affected animals and the history of other events in the farm is also in line with that of Erysipelas.

For the treatment and control of the disease, the animals should be treated with quick acting penicillin twice daily for three days. Alternatively, a long-acting penicillin, given as a single dose to cover 48 hours of treatment, could be given and then repeated. Treat by intramuscular injection 1ml/10kg (300,000 IU/ml) should be done. Medication of the feed with 200g/tonne of phenoxymethyl penicillin for 10–14 days offers a very effective method of

prevention, and can be used in major outbreaks of disease. Amoxycillin, phenoxymethyl penicillin or tetracyclines in the drinking water are also effective. Where large numbers of pigs are involved, it may be necessary to inject all the pigs in the groups at risk. Antibiotic guideline should be referred in carrying out the antibiotic therapy. If there is regular outbreak, vaccination of the animals might have to be implemented.

The disease also has a zoonotic potential and can cause severe to mild form of skin lesions in human too.

In addition, Salmonella organism has also been isolated from the piglets with signs of diarrhoea. The bacteria is also of zoonotic importance. Hence, proper hygiene practices need to be implemented in the farm.

2. Farm Background

The farm was started during 2016 at Yusipang for maintaining the great grandparents (GGP), grandparents (GP) and parent stock (PS) to supply the 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 on 18th July 2016 via chartered aircraft. Complying the 15 days of quarantine observation at Paro International Airport, the pigs were then housed at the newly constructed Nucleus GGP shed on 2nd August 2016 located at Yusipang (Fig. 1). The animals imported were as follows:

Table.1 Different breeds of pigs imported

Species	***Breed***	***Sex***	***Number***
Swine	Landrace	Male	12 heads
Swine	Landrace	Female	80 heads
Swine	Yorkshire	Male	8 heads
Swine	Yorkshire	Female	43 heads
Total			**143**

The centre has started operating GP unit and is yet to complete its first production cycle and have started selecting breeding parent stock for PS unit. The centre has already produced huge number of piglets and has supplied already more than four hundred weaners to pig farmers for fattening purpose after selecting the replacement stock of GGP and GP.

3. History of outbreak

National Centre for Animal Health (NCAH) was informed about the suspected outbreak of Erysipelas on 21/05/2019. The farm was visited and the investigation of disease was carried out on in the farm followed by monitoring visits. Following staff of NCAH, Serbithang:

1. Dr. NK Thapa, AHS III
2. Ms. Puspa Maya Sharma, LO
3. Ms. Tshewang Dema, LT

Fig.1 Location GGP unit at Yusipang- below the highway

As per the discussion with the Farm management, the following information could be gathered:

- One the day of the visit, one animal had signs of reddish skin lesion on the body- such lesions initially appears pinkish and later turns into bluish in colour; the animals had high fever, swelling of jaw region since 3 days back.
- No mortalities reported so far
- 1 adult boar, 1 young boar, 1 gilt affected
- In the females, it was also reported that the animals were discharging cheesy exudates from vagina and often becomes sterile.
- Disinfection of the sheds is being carried out once to twice in a week

4. General observation of the farm

i. Observation on the shed:

- Foot dips are well maintained at the entrance of the farm and each sheds however, disinfectants were not used regularly.
- The sheds appeared cleaned however; the washing of the shed needs to be done.
- There is no isolation shed currently to isolate the sick animals,
- No adequate water supply for cleaning and disinfection of the shed.

ii. Observation of affected animals

On investigation, the animals had varied clinical signs (Table. 2). It could be due to various infections. The details are as follows:

Table. 2 Affected animals with clinical signs

Sl. No.	Tag No. sex	Signs and Symptoms	Samples collected	Remarks
1	700242,	Swelling in the jaw region,	Nasal swab	Located in

	Male	reddish lesion on the rump erythematous lesion on the neck cyanotic and a fresh region (Fig. 2 & 3) Animal treated with Ampicillin	and Blood smear	Replacement shed, Total stock in shed - 73
2.	701060, Female	Animal was recumbent, swelling of both hock joints (Fig. 4)	Whole blood, rectal swab	
3	5000999, Male	Left elbow joint swollen	Nasal swab, rectal, blood smear	Location-Nursery shed, total stock-100
4	304398, Female	Weak, emaciation, sick since 2 months back (Fig. 5)	Rectal swab, nasal swab	Located in Dry sow shed
5	557, Male	Debility, weakness, swollen abdomen, deformed snout, cyanotic ear tips (Fig. 6) since 4 months back	Rectal swab, nasal swab. Whole blood	Located in Farrowing shed
6	304417, Male	Swelling at the jaw region, swelling of joint, since one month back	Nasal swab, rectal swab	Located in dry sow and boar shed Total stock-65
7	500372 Male young	Slightly weak in condition, history of skin lesion	Rectal swab, nasal swab	
8	700084, Female	Cheesy discharge from the vagina.	Vaginal swab	
9	700005, Female	Cheesy discharge from the vagina.		

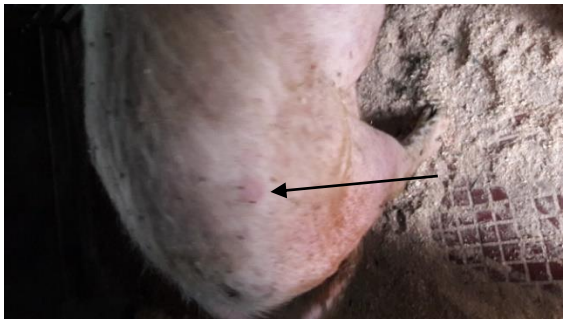

Fig.2 Fresh lesion on rump region

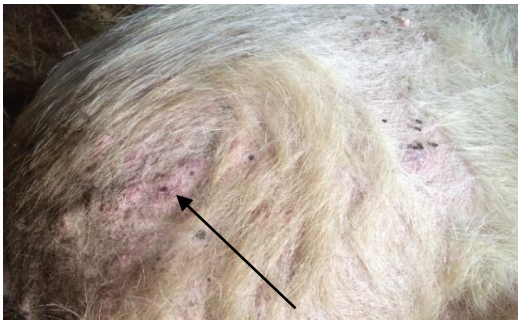

Fig.3 Old lesion

Fig.4 Swollen stifle joint (701060)

Fig.5 Chronic debility with thick skin

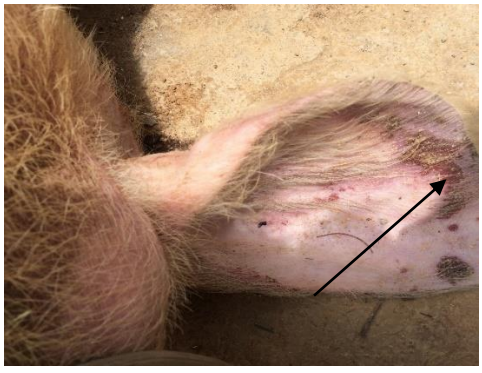

Fig. 6 Lesion on the ear tip

Fig. 7 Pus in the joint

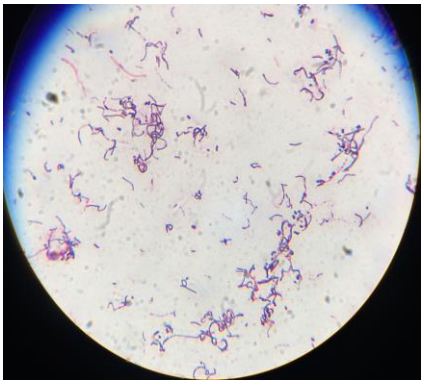

Fig. 8 Gram positive rods

Fig. 9 H_2S production in TSI

Iii Follow up visit

Since, the bacterial culture requires substantial amount of time, new cases were monitored by follow up visits.

a. Follow up visit on 22/05/2019

A follow up visit was done on 22nd May 2019 at the farm, to monitor the cases and sample collection for molecular tests.

- No new cases were reported
- Samples were collected for molecular analysis of African swine fever and Classical swine fever

b. Follow up visit on 29/05/2019 for monitoring

- Following the isolation of *Salmonella* from the diarrhoeal cases in piglets, a follow up visit was made at the farm.
- The piglets were treated with Tetracycline
- Resampling of the piglets were carried out including the environmental samples
- The animal with id 701060 & 11401 had died the previous night and the post mortem were conducted.

c. Follow up visit on 31/05/2019 for monitoring

- History of new case with skin lesion.
- 11405 male- Clinical signs of high fever, swollen joint, skin rashes. Nasal swab/ Rectal swab/water swab/feed swab

d. Report of new case on 03/06/2019

A new case was reported from boar pen with the clinical signs of skin rashes, high fever and inappetence.

iv. Laboratory Investigation & Findings:

Relevant samples collected from the affected animals and the sheds were subjected to various tests and the results are summarized as below (table. 3):

Table 3. Summary of Laboratory findings

Sl.	Sample types	Samples (n)	Type of tests	Findings
1	Faecal samples	3	Parasitology	1 Coccidia 300 OPG + Strongyloid 500 EPG 2 Strongyloid 200-500 EPG
2	Faecal samples [Piglets diarrhoea cases)	1	Bacteriology	*Salmonella* isolated
3	Faecal samples [Piglets diarrhoea cases)	2	Bacteriology	No *Salmonella* detected (after antibiotic treatment)
4	Swab from feeding trough	1	Bacteriology	*Pseudomonas & Salmonella* isolated
5	Nasal swabs/rectal swabs	8	Bacteriology	1 *Erysipelothrix rusipathae (Fig. 8 & 9)* *5 Streptococcus* *1. Pseudomonas* *1. Enterococcus*
6	Pus swab	1	Bacteriology	*Streptococcus* isolated
7	Nasal swabs/rectal swabs	3	Molecular test	Negative to African swine fever (ASF) & Classical swine fever (CSF)
8	Whole blood/blood smear	4	Haematology	Neutrophilia with lymphopenia
9	Carcass-Necropsy	2	Pathology	*701060:* Both the stifle joints were swollen and on opening, both the joints had thick pus.

	-entire left lung was adhered on the ribs and entire lobe was abscessed containing thick pus (Fig. 7) joint abscess- *Erysipelothrix rusipathae* was isolated. *11401 female* Entire ventral side of the abdomen were erythematous. Large swelling on the udder region and on opening it contained large volume of pus (liquid) and necrosis of udder noted.

v. Disease Information-Erysipelas

Swine erysipelas is caused by a bacterium, *Erysipelothrix rhusiopathiae* (syn, *insidiosa*) that is found in most of the pig farms. Up to 50 percent of animals may carry it in their tonsils. It is always present in either pig or in the environment because it is excreted via saliva, faeces or urine. It is also found in many other species, including birds and sheep and can survive outside the pig for a few weeks and longer in light soils. Thus, it is impossible to eliminate it from a herd. However, with improved management strategy the impact can be reduced.

Infected faeces is probably the main source of infection, particularly in growing and finishing pens. Contaminated water also aids the spread of infection. Disease is relatively not common in pigs under 8 to 12 weeks of age due to protection provided by maternal antibodies (past infection) from the sow via the colostrum. The most susceptible animals are growing pigs, non-vaccinated gilts and up to fourth parity sows.

The bacterium alone can cause the disease but concurrent virus infections, such as porcine reproductive and respiratory syndrome **(**PRRS) or swine influenza (SI) and Porcine circo virus (PCV), may trigger outbreaks.

Clinical signs in pigs

- Often the only sign is death due to an acute septicaemia or heart failure.

- Restricted blood supply causes small raised areas called diamonds in the skin. These are clearly defined red and finally black, due to dead tissue but no abscesses. Most heal in 7–10 days.
- High fever, inappetence
- Lameness due to joint infection
- Abortion in pregnant sows
- In boars, sperm can be affected for the complete development period of five to six weeks.

Zoonotic implications: It also causes local skin lesions in humans but this is rare. Strains of erysipelas vary in their capacity to produce disease, ranging from very mild to very severe. The incubation period is 24 to 48 hours. Although not fatal, if contacted it can cause severe skin lesion in humans.

vi. Disease diagnosis

1. By correlation of the clinical signs, history and the laboratory findings-isolation of *Erysipelothrix rusipathae* from nasal swab/rectal swab and pus from joint of the dead animal, the disease is Erysipelas in the GGP stock.
2. In the piglets, the cause of diarrhoea is diagnosed as Salmonellosis-isolation of *Salmonella* from fecal sample.
3. Helminthiasis (Strongyles) and Coccidiosis

vii. Recommendations:

a.Treatment & control

1. In general, the erysipelas organism is known to be sensitive to penicillin. During acute infection, animals should be treated with quick acting penicillin twice daily for three days. Alternatively, a long-acting penicillin, given as a single dose to cover 48 hours of treatment, could be given and then repeated. Treat by intramuscular injection 1ml/10kg (300,000 IU/ml).
2. Medication of the feed with 200g/tonne of phenoxymethyl penicillin for 10–14 days offers a very effective method of prevention, and can be used in major outbreaks of disease. However, mixing of antibiotics in feed may be considered as per the animal feed regulation in the country.
3. Or water medication with amoxycillin or phenoxy-methyl penicillin could be carried out. The dose level to be based on the purity of the antibiotic powder used. Amoxycillin, phenoxymethyl penicillin or tetracyclines in the drinking water are also effective.
4. Where large numbers of pigs are involved, it may be necessary to inject all the pigs in the groups at risk.
5. If the disease is acute, treatment should commence immediately via the water and be continued with in-feed medication using phenoxymethyl penicillin (pen. V) 200g/tonne or tetracyclines 500g/tonne. (Pen. V can also be used for prevention in the face of an outbreak). FOLLOW THE ANTIBIOTIC GUIDELINE.
6. In individual outbreaks, pens should be washed and disinfected between batches. If wet feeding is implicated the system must be cleaned out and disinfected.

7. In case of continuous outbreaks in the farm, it might be necessary to vaccinate the pigs at 8 weeks of age, and possibly revaccinate at 10 to 12 weeks of age. Usually, pigs do not get vaccinated before 8 weeks of age because colostrum antibodies reduce the response to the vaccine. Vaccinate replacing gilts. Vaccinate the breeding herd (including boars) every six months.
8. The management may identify one sick shed (sick pen) for use during disease outbreak.
9. Continuous water supply to the farm for drinking and cleaning and disinfection needs to be sourced.

b. Prevention

1. If a boar is ill with a temperature and shows skin lesions, treat immediately and do not use for mating for a minimum period of four weeks. Alternatively, cross mate with boars that have no disease history or use AI. Semen collection from such boars should also be withheld until complete recovery.
2. Sporadic disease is common in sows but if one sow in a group becomes infected, the exposure is high from her urine and faeces and it is advisable to inject all contact animals with penicillin.
3. Birds can also contaminate feed. Assess the levels of the exposure in your herd.
4. In an outbreak remember that water, faeces, dung, nasal secretions, bedding and feed, harbour the organisms. Hence, proper cleaning and disinfection should be practised.

c. Other health parameters

Other bacterial disease conditions like Salmonellosis was also prevalent in the piglets suffering with diarrhoea. In addition, parasites like Strongyles and Coccidia were also detected in screening of faecal samples. Hence, following measures needs to be followed:

1. Improved hygiene practices as mentioned above can also help in preventing salmonellosis in the farm.
2. Routine screening and deworming also needs to be done in the animals.
3. Farm biosecurity and overall health management may need to be enhanced.

The National Centre for Animal Health, Serbithang would like to visit the farm again in future and see what can be done best to avoid disease occurrence.

14.4 One Health approach to determine antimicrobial resistance profile of *Salmonella* organism isolated from human, animal and food samples under WHO AGISAR project

Salmonella has been recognized as an important zoonotic pathogen of economic significance in animals and humans. Salmonellosis is most common and widely distributed food-borne disease and increasing antimicrobial resistance in non-typhoid *Salmonella* species has been a serious concern for public health worldwide. A recent

study identified increasing resistance of *Salmonella* to drugs commonly used to treat severe *Salmonella* infections in adults and children. Outbreaks of multidrug resistant *Salmonella* strains have been recorded in various countries in the Indian subcontinent.

A study on prevalence of *Salmonella* in imported chicken carcasses in Bhutan showed 13% prevalence. *Salmonella enteritidis* dominated with a prevalence of 80.7% and 40 of the 42 isolates harboured two or more resistance determinants. Frequent outbreak of Salmonellosis has been reported in humans, either through the water sources or from the food items. A recent study concluded the prevalence of *Salmonella* at 20.3% and 27.1% in imported and locally produced beef and pork respectively. These isolates were not tested for antimicrobial resistance. Thus, the antibiotic susceptibility profile of these organisms is unknown.

Bhutan proposed a pilot study to develop antibiotic susceptibility profile of *Salmonella* isolates from human, animals and food products of animal origin. The antibiotic susceptibility test (ABST) profiling was a collaborative work between Ministry of Health, Department of Livestock and Bhutan Agriculture & Food Regulatory Authority. The ABST profile data from human samples were generated by Clinical Laboratory, Ministry of Health, while the National Centre for Animal Health generated for animals and National Food Testing Laboratory for all food products of animal origin. The main objectives of this collaborative study was to develop ABST profile for *Salmonella* isolates in Bhutan while enhancing national capacities for laboratory surveillance and antimicrobial resistance monitoring through One Health Approach. The findings are also aimed at rational management of antimicrobial use in human and animals.

A total of 54 isolates were collected from all three sectors: human, animal and food. All these isolates were collected using harmonized culture protocols, basic test and biochemical test. Samples were cultures at respective sector laboratories. All isolates recovered and confirmed as *Salmonella* were referred to Institute of Hygiene and Food Sciences, University of Zurich for serotyping and detection of resistant determinant.

From the 54 isolates only 21 were identified as *Salmonella* of various species. Species level *Salmonella* confirmation was: *S. enteritidis* and *S. newport*- 23.8% each (5/21); *S. typhi*, *S. weltevreder* and *S. virchow* – 14.3% each (3/21); and *S. kentucky* – 5% (1/21). Phenotipically, all isolated of *S. enteritidis* were resistant to nalidixic acid and Nitrofurantoin. Additionally, one each isolate of *S. enteritidis* and *S. virchow* were resistant to TE. A more detail studies at genetic level to determine resistance profile is required. The results of multi locus sequence typing (MLST) is expected to receive soon which may confirm the resistant determinant in these isolates.

14.5 HPAI H5N1 virus characterization in collaboration with AAHL Geelong

In 2019 April one outbreak of HPAI H5N1 was reported from Dhamdara, Phuentsholing. The swab samples were tested by real time RT-PCR at National Centre for Animal

Health, Serbithang and confirmed the involvement of H5N1 subtype HPAI virus. H5 HA gene sequencing and amino acid motif:
Complete HA gene nucleotide sequences from H5 positive samples showed sequence similarities to A/duck/Bangladesh/34283/2017(H5N1. The H5 HA amino acid sequence alignment" showed the presence of multiple basic amino acid residues (PQKERRRKR*GLF), arginine (R) and lysine (K), indicating highly pathogenic avian influenza (HPAI) viruses. Phylogenetic analysis: Bhutan H5N1 samples had 99% amino acid sequence similarities to A/duck/Bangladesh/34283/2017(H5N1)-like viruses. Phylogenetic analysis based on near complete HA gene sequences confirmed that the H5N1 viruses detected in Dhamdara, Phuentsholing in 2019 belong to Asian H5 clade 2.3.2.1a.

Avian samples – real time PCR results summary: AIV matrix assay was conducted on all samples received. 4/7 samples detected as positive in AIV Type A (matrix) Taqman (TQM) assay. All samples were tested in the H5, H7, H9, N1, N2, N6 and N8 TQM assays. No samples were detected as H7 or H9 positive (H7 using the FLI-CODA H7 real-time PCR method). No samples were detected as N6, N2 or N8 positive. Virus isolation and sequencing have been selected to be undertaken as appropriate on select samples depending on Ct and species.

Sample	Specimen ID	AI_TQM_A	Type A Ct	AI_TQM_H5	H5 Ct	AI_TQM_H9	AI_TQM_H7
19-02209-0001	(Spleen) 642 Avian	Positive	15.7	Positive	18.5	Negative	Negative
19-02209-0002	(Lungs) 642 Avian	Positive	14.4	Positive	17.9	Negative	Negative
19-02209-0003	(Liver) 642 Avian	Positive	15.8	Positive	18.5	Negative	Negative
19-02209-0004	(T/C Swab) 642 Avian	Positive	16.6	Positive	21.6	Negative	Negative
19-02209-0005	(T/C Swab) 666 Duck	Negative	45.0	Negative	45.0	Negative	Negative
19-02209-0006	(F Dropping) 667 Pigeon	Negative	45.0	Negative	45.0	Negative	Negative
19-02209-	(F Dropping) 668 Wild	Negative	45.0	Negative	45.0	Negative	Negative

0007	Bird						

Sample	Specimen ID	AI_TQM_N1	N1 Ct	AI_TQM_N2	AI_TQM_N6	AI_TQM_N8
19-02209-0001	642 (Spleen) Avian	Positive	25.4	Negative	Negative	Negative
19-02209-0002	642 (Lungs) Avian	Positive	23.8	Negative	Negative	Negative
19-02209-0003	642 (Liver) Avian	Positive	24.2	Negative	Negative	Negative
19-02209-0004	(T/C Swab) 642 Avian	Positive	26.7	Negative	Negative	Negative
19-02209-0005	(T/C Swab) 666 Duck	Negative	45.0	Negative	Negative	Negative
19-02209-0006	(F Dropping) 667 Pigeon	Negative	45.0	Negative	Negative	Negative
19-02209-0007	(F Dropping) 668 Wild Bird	Negative	45.0	Negative	Negative	Negative

Section 2. Sequencing Report for SAN 19-02209

This report provides a summary of the sequencing and molecular characterization of avian influenza A positive samples detected in SAN 19-02209.

Avian Influenza A virus (AIV) HA sequences were generated for H5 subtype viruses directly from samples as shown in Table 1.

Table 1: 19-02209 AIV PCR positive samples from which virus sequences were obtained

AAHL SAMPLE No.	Bhutan Sample No.	Host	Subtype	HA cleavage site sequence	Clade
19-02209-01*	642 (Spleen) Avian	Chicken	H5N1	N/A	N/A

19-02209-02	642 (Lungs) Avian	Chicken	H5N1	PQKERRRKR*GLF	2.3.2.1a
19-02209-03	642 (Liver) Avian	Chicken	H5N1	PQKERRRKR*GLF	2.3.2.1a
19-02209-04	642 (T/C Swab) Avian	Chicken	H5N1	PQKERRRKR*GLF	2.3.2.1a

***partial sequence only**

H5-HA gene:

- Complete HA gene nucleotide sequences from H5 positive samples 19-02209 #2 to #4 and partial sequence from #1 were aligned against two H5N1 HA reference sequences, A/duck/Bangladesh/34283/2017(H5N1) (top blast match) and A/chicken/Bhutan/836(B1)/2018/(H5N1) (H5N1 virus from previous Bhutan submission, 18-01391-01). The H5N1 viruses from 19-02209 have 98% HA nucleotide sequence similarities to A/duck/Bangladesh/ 34283/2017(H5N1) (Figure 1a: 19-02209 H5 HA nucleotide sequence similarities)
- The HA nucleotide sequence alignments are shown in Figure 2 "19-02209 H5 HA nucleotide sequence alignment".

- The 19-02209 H5N1 samples had 99% amino acid sequence similarities to A/duck/Bangladesh/34283/2017(H5N1)-like viruses

- Phylogenetic analysis based on near complete HA gene sequences confirmed that the H5N1 viruses detected in Bhutan in 19-02209 belong to Asian **H5 clade 2.3.2.1a**

14.6 FMD vaccine efficacy study conducted at CRC Wangkha

FMD is a highly contagious viral disease that affects all cloven-footed animals including cattle, yaks, sheep, goats, pigs and other wild ruminants. The disease is endemic in many parts of the world, particularly in developing countries of Asia, Africa, the Middle East, and some parts of Europe. FMD is endemic in all countries of South Asia. In FMD-endemic countries, FMD can have serious economic losses through reduced production in terms of milk, meat and draught power and deaths. FMD is the most important disease affecting livestock production in Bhutan and it is a notifiable disease as per the Livestock Rules and Regulations of Bhutan 2017. One of the control measures practiced in Bhutan is vaccination of animals using trivalent – O, A and Asia 1 serotype vaccine manufactured by Indian Immunologicals Ltd. India. Although vaccination is practiced on annual basis either biannual or annual depending on the endemicity of the disease Bhutan experienced sporadic outbreaks even in vaccinated herd. These outbreaks could be attributed to factors such as vaccine mismatch with wild type virus, cold chain failure or poor vaccination coverage. A study was designed to determine the protection level conferred by trivalent vaccine. Study was conducted at Regional Cattle Research

farm/Calf Rearing Centre, Wangkha. The Centre did not report FMD outbreaks for more than 10 years. Three groups of animals were identified: i) Booster group (n = 18), which received vaccine on 0-day, 28 days and 120 days; ii) Non-booster group (n = 18), which received vaccine only on 0 day and; iii) Control group (n = 5), which did not receive vaccine. All animal were bled on 0 day, 28 days and 120 day of the study. The serum samples were used for vaccine matching at Pirbright Institute, UK. The result showed that about 37% of the animals had high proportion of NSP antibodies at 30 days post vaccination. Vaccine matching test was performed by 2dmVNT with two each field isolates of Bhutan for serotype A and O. Sera used for serotype A were from A IRN/2005, A TUR 20/06, A/GVII and A22 IRQ/24/64. The sera used for serotype O were O 3039, O Manisa and OTUR 5/09. The highest 2dmVNR r_1 value for serotype A was against A/GVII and similarly the highest 2dmVNR r_1 for serotype O was against O TUR 5/09. All r_1 values for O3039, O Manisa and O TUR 5/09 were above 0.30 indicating close antigenic relationship between the field isolates and vaccine strain. However, it is not known at what point of time the r_1 value was determined.

15 Animal Health Surveillance/Researches (on-going)

The following animal health surveillance/researches is ongoing the data presented are preliminary.

15.1 Initiated collaborative studies on 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 for the purpose of improving 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, immunofluorescence 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 diagnostic facility for anthrax, Brucella, CCHF and rabies. The bat derived zoonoses component of the collaboration will be initiated soon with the visit of Japanese team to identify bat habitat, collect samples and refer to Japan for detection of any zoonotic agent in Bhutanese bats.

16. Ex-country training/workshop/meeting attended

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

17. Visitors to LSU during FY 2018-19

In the financial year 2017-2018 the following officials has visited the laboratory service unit for various purposes. This list of visitors and the purposes are as follows:

Table 12: Visitors at LSU

SI No.	Date	Visitor's Name	Purpose of visit
1	3.7.2018	Allison Litre John, Tim Seal	Fleming fund scoping study
2	9.7.2018	Ganga & the 9th batch of RNR-PDP	Institutional visit
3	24.7.2018	Martin Gicbert	Tiger Health Research
4	22.8.2018	Dr. Dheerasata Pipatpongsopon	DLD, Thailand
5	4.9.2018	Esther Van Wegen, Dr. Sithar Dorjee	Visit to understand the procurement system of MOAF
6		John Allen & Team	AAHL r AGK-BIO
7	15.9.2018	PK Prusty Vaishro	To know the lab disease diagnosis of equine
8	22.11.2018	Lyonpo (MOAF) and team	Facility visit
9	1.3.2018	Glenn Browning, Helen Crabb, James Gil Kerson	Visit for Fleming fund fellowship
10	10.4.2019	Dr. Shekhar Chettri and team	CNR trainee's

Annexure: Ex-country training/workshop/meeting attended

Table 13: List of NCAH staffs who have attended ex-country training/workshop/meeting during FY 2017-18

Name	EID. No	Position Title	Course title	Institute	Country	Start Date	End Date	Duration (days)	Funding Agency
Dr. Ratna Bdr. Gurung	9603028	Specialist III	Lab-TAG and ISWAVL	Hotel Empress	Thailand	18/06/2019	22/06/2019	5 days	FAO
Dr. Ratna Bdr. Gurung	9603028	Specialist III	AMR and AST	Chulalongkorn University	Thailand	28/05/2019	31/05/2019	4 days	OIE
Dr. Ratna Bdr. Gurung	9603028	Specialist III	Zoonotic infections	NIID	Japan	18/02/2019	22/02/2019	5 days	NVAL and NIAH
Dr. Nirmal Kumar Thapa	9302007	Specialist III	Intercountry meeting to review implementation of National Action Plans on AMR	Bangkok	Thailand	23-07-2018	25-07-2018	3 days	WHO
Dr. Nirmal Kumar Thapa	9302007	Specialist III	Second oie global conference on antimicrobi	Marrakesh	Morocco	29-09-2018	31-07-2018	3 days	OIE

			al resistance and prudent use of antimicrobial agents in animals: *putting standards into practice*						
Dr. Nirmal Kumar Thapa	9302007	Specialist III	Regional Meeting on Fleming Fund regional Grant	Kathmandu	Nepal	17-06-2019	18-06-2019	2 days	International vaccine Institute, S. Korea
Puspa Maya Sharma	2014030185	Laboratory officer	Rabies cell culture and CCHF	NIID	Japan	18-2-2019	22-2-2019	5 days	NIID, Japan
Ms. Dechen Wangmo	20150105019	Laboratory officer	Regional worshop on Biological safety cabinet Technology		Thailand	26 Novembr,2018	28 November,2018	3 days	The department of medical science, the ministry

									of public health in collaboration with the department of Livestock Development
Ms. Dechen Wangmo	20150105019	Laboratory officer	.‘‘Laboratory sustainability at the heart of Biosafety & Biosecurity’’ And ISWALD 2019.	OIE in collaboration with	Thailand	17June, 2019	21 june,2019	5 days	OIE in support from kingdom of Thailand and ministry of Agriculture of the People’s Republic of China
Tshewang Dema	200407360	Laboratory Technician I	Regional worshop on Biological safety cabinet Technology		Thailand	26 Novembr,2018	28 November,2018	3 days	The department of medical science, the ministry of public health in

									collaboration with the department of Livestock Development
Kelzang Lhamo	200310013	Laboratory Technician I	Rabies cell culture and CCHF	NIID	Japan	18-2-2019	22-2-2019	5 days	NIID, Japan
Pasang Bida		Sr. Laboratory Technician III	Laboratory Training in Feeding and Animal Health	Maejo University, Chiangmai	Thailand	15/10/2018	28/10/2018	3 days	EU-TCP