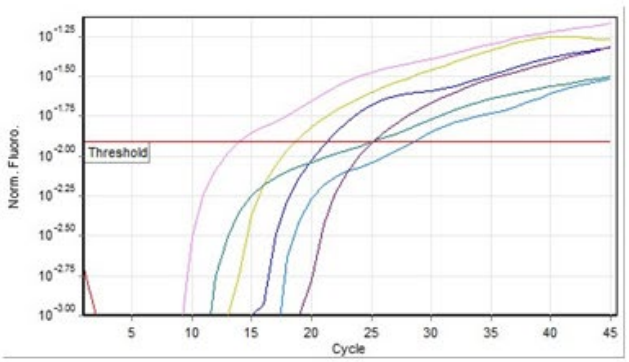

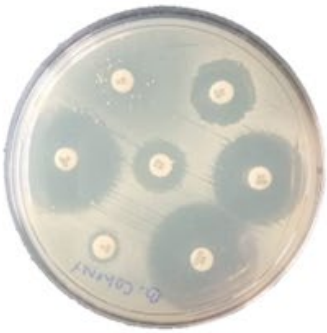

Annual Progress Report for FY 2021 – 2022

Laboratory Services Unit

National Centre for Animal Health

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1.0 Summary

The Laboratory Services Unit (LSU), one of the technical units under the National Centre for Animal Health, Serbithang; functions as the national veterinary referral laboratory in the country. It is mandated with providing referral laboratory services besides its routine diagnostic services. The unit has the capacity for advanced diagnostic tests such as Enzyme-linked immunosorbent assay (ELISA), Fluorescent antibody test (FAT) and molecular assays for emerging and re-emerging infectious disease like Foot and Mouth Disease (FMD), Highly Pathogenic Avian Influenza (HPAI), Classical Swine Fever (CSF), African Swine Fever (ASF), Brucella, Porcine Respiratory & Reproductive Syndrome (PRRS), Rabies, Porcine Circovirus Type 2, Capripox, Lumpy Skin Disease (LSD).

The laboratory is also equipped with real time Polymerase chain reaction (PCR) technology. The unit has Bio-safety level 2 plus facilities for safe handling of high-risk pathogens. In addition, the unit is also responsible for monitoring and evaluating Bio-safety activities in the veterinary laboratories in the country. The unit is also responsible for coordinating collaboration of advance level diagnostic research with international reference laboratories and institutes. It is also mandated to carry out laboratory-based surveillances/research. The lab is also the national referral lab for the Antimicrobial Resistance (AMR) in animal health.

During the financial year 2021 – 2022, the National Centre for Animal Health (NCAH), Serbithang, Thimphu, collected and processed a total of 4275 samples and 11897 different laboratory tests were performed for routine diagnosis, disease outbreaks, disease screening, surveillance and research activities.

New laboratory diagnostic techniques were introduced in the Centre for diagnosis of important emerging animal diseases such as Marek's disease, Canine adenovirus rapid test for African Swine Fever (ASF), PCV2 (RT_PCR and ELISA) and Brucella ELISA for sheep and goats etc. The lab participated in External Quality Assurance (EQAS) through online survey for Laboratory Quality Management System with International Vaccine institute, South Korea. The national external quality assurance system (NEQAS) was also coordinated and conducted for the four Regional Livestock Development Centres (RLDCs), forBrucella Rose Bengal Test (RBT).

2.0 Introduction

The Laboratory Services Unit (LSU), one of the technical units under the National Centre for Animal Health, Serbithang is the only veterinary laboratory at the national level. It is mandated to cater the referral services in the field of animal disease diagnostics in the country and also carries out laboratory-based research and disease surveillances.

The unit has facilities of bio-safety level 1 to 2 plus laboratories for safe handling and processing of high-risk pathogens in the laboratory. In addition, the unit is also responsible for implementing, monitoring and evaluating Bio-safety activities in the veterinary laboratories in the country. The lab also serves as the national referral lab for the Antimicrobial Resistance (AMR) in animal health.

3.0 Mandates

The main mandates of the Laboratory Services Unit (LSU) are:

1. Caters referral laboratory diagnostic services for animal diseases in the country
    - Provide quality laboratory diagnostic services to support clinical services, animal health programs and One-Health activities in the country;
    - Conduct advanced/confirmative diagnostic tests for the referred samples from the field

2. Conduct laboratory-based research/ Disease Surveillance/Survey
    - To lead/coordinate and conduct laboratory-based animal health research activities in the country

3. Laboratory Quality Management – The laboratory ensures efficient laboratory quality management system through the following:
    - Coordination and implementation of Biosafety and Bio-security programs in all the animal health laboratories in the country.
    - Implement and monitor bio-safety measures and good laboratory practices in the animal health laboratories in the country
    - Strengthening and enhancement of laboratory diagnostic capacities
    - Serve as referral laboratory for antimicrobial resistance monitoring in animals in the country
    - Participate in regional proficiency testing for specific diagnostic methods
    - Conduct proficiency testing for specific diagnostic methods for the RLDCs
    - Technically backstop regional, satellite and district laboratories in the country
    - Introduction and validation of new diagnostic tests/upgradation of diagnostic tests for the emerging and re-emerging diseases in the country
    - Coordinate in developing and establishing the electronic record system of laboratory activities e.g., LIMS.

4. Laboratory networking
    - Liaise, collaborate and establish laboratory networks with the outside agencies national laboratories like National Food Testing Laboratory (NFTL), Bhutan Agriculture and Food Regulatory Authority (BAFRA); Clinical Laboratory, Jigme Dorji Wangchuck National Referral Hospital (JDWNRH); Royal Centre for Disease Control (RCDC), Department of Public Health (DoPH); and Wildlife Clinic, Nature Conservation Division (NCD), Department of Forests and Park Services (DoFPS);
    - Establish laboratory networks with the international reference laboratories such as WOAH and WHO Referral Laboratories and also other institutes (NIID Tokyo, NIAD, Bangkok, AAHL Geelong, FMD Laboratory, Pirbright etc.)

5. Human resource capacity development in the field of Laboratory technology
    - Conducting the diploma course in laboratory technology in collaboration with other relevant institutions like RUB.
    - Enhancement of laboratory skills by conducting refresher course and up-gradation courses for laboratory technicians
    - Conduct training on laboratory quality management

4.0 Organizational Structure of LSU

The organogram of the Laboratory Services Unit (LSU) is appended below (Figure 1).

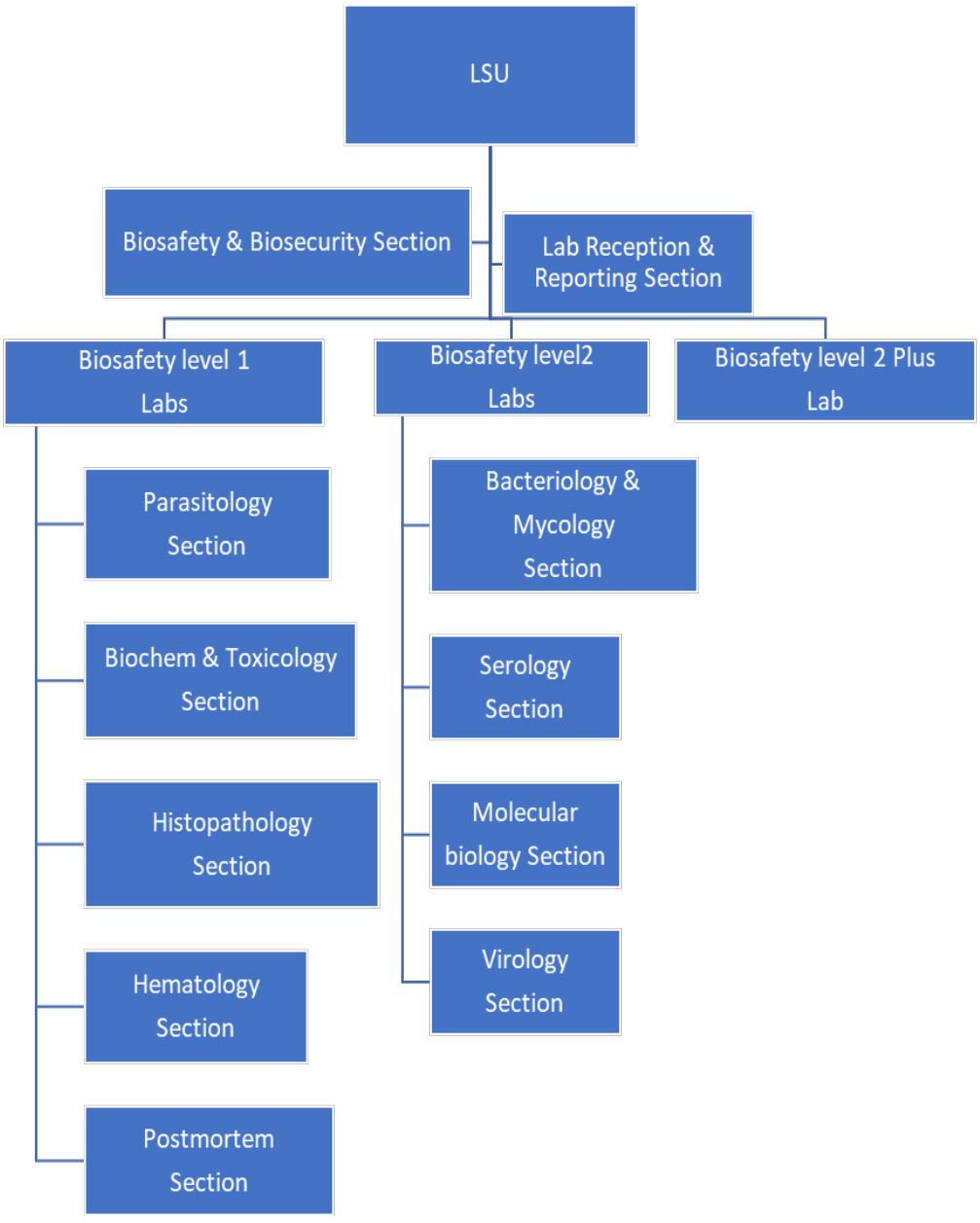

Figure 1: Organogram of Laboratory Services Unit

5.0 Functional Structure of LSU

The functional structure unit of the LSU is appended below (Figure 2).

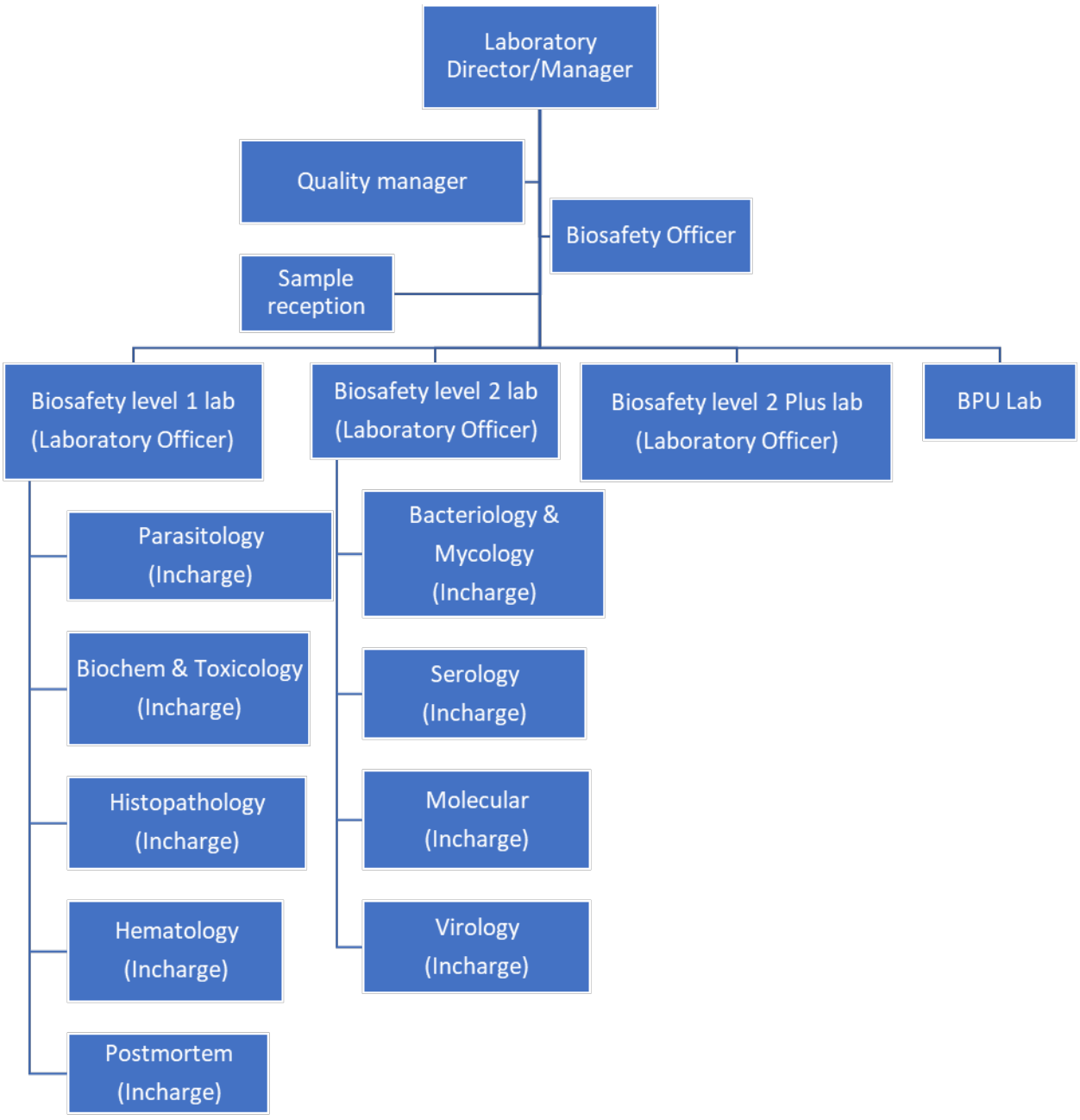

Figure 2: Organogram of laboratory functional unit

6.0 Human Resource

The human resource capacity for the LSU is tabulated below.

Table 1: Human resource capacity of LSU, 2021-22

S.N.	Name	Position Title	Position Level	Responsibilities
1	Dr N.K. Thapa	Specialist II (Head)	ES II	Head, Postmortem, Histopathology
2	Ms Puspa Maya Sharma	Sr. Laboratory Officer	P3A	Molecular biology, Bacteriology
3	Ms Dechen Wangmo	Sr. Laboratory Officer	P4A	Biosafety & Biosecurity, Biochemistry & Toxicology
4	Mr Purna Bdr. Rai	Sr. Laboratory Technician II	SS3A	Virology
5	Mr Tenzinla	Sr. Laboratory Technician II	SS3A	Bacteriology, Mycology, Postmortem
6	Mr Dawa Tshering	Sr. Laboratory Technician II	SS3A	Serology
7	Ms Ugyen Pema	Asstt. Laboratory Technician I	S1 A	Parasitology
8	MS Kelzang Lhamo	Asstt. Laboratory Technician I	S1A	Molecular biology, serology
9	Ms Tshewang Dema	Asstt. Laboratory Technician I	S1 A	Hematology
10	Ms Pasang Bida	Asstt. Laboratory Technician I	S1 A	Histopathology
11	Mr Phub Namgay	Laboratory Attendant	ESP	Attendant

7.0 Summary of samples processed, and tests performed

During the fiscal year 2021-2022, a total of 4263 numbers of various diagnostic samples were received/collected and processed; about 11,834 different laboratory tests were performed for routine tests, disease outbreaks, disease screening, surveillance and research. The details are outlined in table 2.

Table 2: Summary of sample received, and test performed during FY 2021-2022

Section	Samples Processed	Tests conducted
Biochemistry &Toxicology	157	168
Parasitology	439	1141
Clinical pathology/Haematology	230	790
Bacteriology/Mycology	868	6361
Postmortem	279	279
Histopathology	332	664
Serology/virology	1921	2338
Molecular biology	49	156
Total	**4275**	**11,898**

Since the lab do not have capacity for all the tests required, some of the samples were referred to international laboratories/institutes for further testing for various purposes (table 3).

Table 3: Samples referred to international institutes/ laboratories

Sl. No.	Specimen	Nos.	Testing Institute	Remarks
1	Taeniid eggs from canines	76	University of Zurich, Switzerland	Surveillance
2	Taeniid eggs from soil	5	University of Zurich, Switzerland	
3	Canine Serum	1	National Institute of Animal Health, Department of Livestock, Thailand	Export
4	Canine Feces in 70% alcohol	67	Veterinary Preclinical Science Building (Bldg) The university of Melbourne Parkville, VIC3052 Australia	Research
5	Serum	92		
6	Eye swab	90		
7	Whole blood	95		
	Total	**426**		

Some samples were also referred to laboratories outside the agencies in the country like Ministry of Health for diagnostic support (table 4).

Table 4: In-country referrals

Sl. No	Species	Specimen	Nos.	Testing Institute	Remarks
1	Swine	Liver & stomach contents	2	Toxicology Section, JDWNRH, Thimphu	Outbreak
2		Poultry Feed	21	Toxicology Section, RCDC, Serbithang	Outbreak
3	Dog	COVID samples	13	RCDC	COVID Quarantine
	Total		**36**		

8.0 Section Achievements

8.1 Bacteriology & Mycology Section

The section provides routine diagnostic test services for microbial diseases (bacteria & fungi) in the animals through culture, identification and antimicrobial susceptibility test (AST).

During the year, about 868 different types of samples were received/collected and 6361 different tests were conducted. The detail of the samples tested in the bacteriology section and about 83 samples for Mycology were processed and 83 tests were conducted during the year (Table 5).

Table 5: Achievement of bacteriology and Mycology sections for 2020-21

Type of specimen	Specimen Processed	Types of tests	Tests Conducted
a. Bacteriology			
Aspirate Swab	1	Culture	2686
Blood	6	Gram stain	175
Blood smear	2	Motility	84
Blood swab	4	BC test	2046
Caeca	590	Sensitivity test	527
E. coli vaccine	1	Leishman stain	19
Ear swab	14	Methylene Blue Stain	4
PT samples	23	Isolate archival	736
Eye swab	4	Acid Fast Stain	1
Feed samples	4	*Sub-total*	*6278*
Froth swab	1		
Heart	2		

Heart swab	1		
Impression smear	2		
Intestine	1		
Intestine contents	2		
Intestine scraping	2		
Isolates	14		
Lung swab	1		
Mouth swab Nasal swab	1 32		
NEQAS	6		
Oro-pharyngeal swab	2		
Pus swab	1		
Rectal swab	58		
Soil	2		
Throat swab	2		
Tongue swab	1		
Tracheal swab	1		
TSB Culture	1		
Upper palate swab	1		
Urine	1		
Total	*785*		
b. Mycology			
Skin scraping	59	Culture	59
Fermented cheese	24	Lactophenol Cotton Blue Stain	**24**
Total	*83*		*83*
Grand Total	**868**		**6361**

The section participated in proficiency testing for NEQAS from RCDC, Serbithang and IEQAS for EQASIA from Thailand.

Significant findings

Significant findings include *Pasteurella multocida* in bovine, *Salmonella* in swine & avian, *Clostridium perfringens* in avian and swine, *Bordetella bronchoseptica* in canine from clinical samples.

In Mycology, common findings were *Trichophyton verrucosum* and *Rhizopous* in canine.

8.2 Biochemistry and Toxicology Section

The section conducts basic tests for clinical biochemistry 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 like in the animal feeds.

During the year, 157 feed samples were processed and 168 tests were conducted basically screened against Aflatoxins. Details of samples and tests conducted in this section are presented in Table 6.

Table 6: Achievements of Biochemistry & Toxicology section 2021-22

Type of specimen	Number	Test type	Number
Feed	157	Aflatoxin	168
		Mineral biochemistry	0
		Urine biochemistry	0
Total	**157**		**168**

Significant findings

11 samples were detected with aflatoxin above the acceptable limit.

8.3 Clinical pathology/Hematology Section

The section conducts basic hematological tests to support clinical diagnosis in the animals. In addition, the section also carries out examination of blood parasites like microfilaria and *Trypanosomes* etc.

About 230 samples were processed and 790 different tests were conducted during the year. Details of samples and tests conducted in these sections are presented in Table 7.

Table 7: Achievements of Clinical pathology/Hematology section for 2021-22

Type of specimen	Number	Test type	Number
Blood smear	9	PCV	205
Whole blood	221	Hb	205
		DLC	185
		TRCC	49
		TWCC	50
		Knott's test	45

		Direct smear examination	51
Total	**230**	**Total**	**790**

Significant findings

During the year, the section commonly detected parasitic infestations through the microscopic detection of *Dirofilaria*, also known as heartworm larva. Out of 45 samples, 7 samples were positive to Heartworm.

8.5 Molecular biology Section

The section performs confirmatory tests on both routine basis and also on the samples referred by the Regional/District/Satellite Laboratories in the country. The section, conducts test for both emerging and re-emerging diseases in the country like, Classical swine fever (CSF), African swine fever (ASF), Capripox, Porcine Respiratory and Reproductive Syndrome (PRRS), NCD, HPAI etc.

The section received/collected 59 samples and carried out 146 different types of tests as described below in Table 8.

Table 8: Achievements of the molecular biology section for 2021-22.

Type of specimen	**Numbers Received**	**Type of PCR tests**	**Tests conducted**
Bone marrow	8	PRRS-Eu & NA, LSDV, PPR, CaPV, HS, MCCP.	18
Ocular/nasal	10	PCV-2, CaPV, PPR, HS, MCCP	30
Bacterial Colony	5	HS	7
Tonsil	10	PCV-2	19
Tracheal/Cloacal swab	5	AI Type A, NDV	14
Organ (Lungs, Spleen, Lymph node)	9	ASF, CSFV, PRRS- EU-NA	25
Epithelial/Secretions	5	FMDV	11
CSF Vaccine	1	CSF	3
Cloacal swab	6	AI, PPR, HS, MCCP	19
Sub Total	**59**		**146**

Significant findings

Important findings in molecular biology include *Capripox* in Goats from Samtse, PPR in Takin from Paro, FMDV from Thimphu, ASF in pigs from Sampheling Chukha, CSF from Wangdue and PCV-2 in NNPBC Yusipang and RPBC Lingmethang.

8.5 Post-mortem and Histopathology Section

The section has Postmortem and Histopathology section which provides necropsy and histopathological diagnosis.

A total of 279 animal carcasses were necropsied during the year. About 332 tissue samples were processed and 661 types of tests were conducted in histopathology section in Table 9.

Table 9: Achievement of Pathology section, 2021-22

Type of specimen	Number	Test type	Number
Tissue, organs	332	Histopathology- H and E Staining	664
Carcass	279	Post-mortem/Necropsy	279
Total	**611**		**943**

Significant findings

Significant findings include IBD, Coccidiosis, Enterotoxaemia, canine Distemper etc.

8.6 Parasitology Section

The section carries out basic parasitological tests to support diagnosis for parasitic diseases in the animals. 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 section also provides other professional backstopping to RLDCs, SVLs and DVHs/DVLs.

A total of about 439 samples were received/processed and 1141 different tests were performed by the section. The details of tests performed by this section are shown in Table.10.

Table 10: Achievement of Parasitology section for 2021-22

Type of specimen	Number	Test type	Number
Faecal samples	244	Direct examination, Sedimentation, Stoll's	976

		dilution, Floatation	
Dog environmental samples	114	Floatation/Sieving technique by using 1:1 sugar solution.	114
Soil Samples	4	Floatation/Sieving technique by using zinc sulphate (1:1)	4
Intestinal content	47	Direct smear	47
Total	**439**		**1141**

Significant findings

During the year, the section commonly detected parasitic infestations through the microscopic detection of eggs of *Strongyles, & Coccidea in* swine and *Taeniid* in stray dogs, *Ascarids* and Tape worm in poultry.

8.7 Serology Section

This section is equipped with advanced diagnostic facilities such as ELISA, SAT etc.

The section received/collected 1021 samples and carried out 2338 different types of tests as described below in table 11.

Table 11: Achievement of Serology section 2021-22

Type of specimen	Numbers Received	Type of tests conducted	Number of tests conducted
Serum	1021	PPR ELISA	16
		SAT Mycoplasma	7
		SAT Salmonella	7
		Rapid test FMD NSP	14
		FMD Serotype O ELISA	31
		FMD Serotype A ELISA	31
		FMD Serotype Asia I-ELISA	31
		RBT Brucella	846
		Brucella ELISA	1028
		Porcine Circovirus Type-2	45
		IBR ELISA	66

		BVD ELISA	55
		RAPINA Porcine Mycoplasma Hyopnuemonae PRRS	38 58 58
Total	**1021**		**2338**

Significant findings

Important findings in serology includes detection of *Brucella* antibody from cattle in NJBC, Samtse, RCBC, Wangkha and Lhuntsi, PCV2 antibody from pigs at RPBC Lingmithang and NNPBC Yusipang. Rabies in dogs from Samtse and Chukha. IBD in poultry from Sarpang. The section also conducted proficiency testing for RBT for Brucellosis for four RLDCs.

8.8 Virology Section

This section is equipped with basic rapid tests and also advanced diagnostic facilities such as Fluoresce Antibody test (FAT) etc.

The section received/collected 50 samples and carried out 110 different types of tests as described below in table 12.

Table 12: Achievements of Virology section

Type of specimen	**Numbers Received**	**Type of tests**	**Tests conducted**
Brain	4	FAT	4
Serum	14	FMD NSP(Rapid)	14
Swab	32	Rapid AI	30
		Rapid NDV	30
		Rapid IBD	30
		CPV	1
		CDV	1
Total	**50**		**110**

Significant findings

Important findings in virology section were Rabies, FMD and Canine Parvovirus.

8.9 Biosafety and Biosecurity Section

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

9.0 Laboratory Quality Management

9.1 Internal auditing of the laboratories

Internal auditing of the laboratories was carried out at LSU and BPU labs. The main objectives of the auditing were reviewing the past auditing findings and follow up for Correction and prevention action (CAPA). The main goal of the internal laboratory auditing is to ensure that the laboratory has good quality systems in place, follows good laboratory practices, and generates data of integrity and quality

A. Findings for BPU:

The main findings for the BPU were as follows:

1. *Follow up of earlier auditing*: The main findings of the earlier auditing were no ***color-coded bins*** available and no ***spill kit*** available. However, upon auditing, the recommendations were not followed up.
2. Main Findings:
   a. Administrative control
      i. Emergency response plan including spill containment was not available.
      ii. Documented biosafety training not available
   b. Laboratory facilities
      i. Both the labs do not have hand washing sinks
      ii. No emergency shower and eye wash available
      iii. Windows towards outdoor do not have screens
   c. Safety equipment -primary barriers-Biological safety cabinets not used while handling with infectious agents

B. Findings for LSU:

The findings for the LSU were as follows:

1. *Follow up of earlier auditing*: The main findings of the earlier auditing were no ***color-coded bins*** available. Not rectified
2. Work benches and floor found to be dusty especially Biochemistry & Toxicology section
3. Temperature record erratic
4. Waste management plan not included in auditing

5. Overstocking bacteriology section with equipment.
6. Main Findings:
   a. Administrative control
      i. Emergency response plan not available.
      ii. Trainings not documented
   b. Laboratory facilities
      i. First aid kit not available
   c. Safety equipment -primary barriers
      - Biological safety cabinets in molecular biology are not located away from the door.
      - Bacteriology section congested with equipment
   d. Auto clave
      - Quality control log not available in biochemistry
   e. Standard Microbiological Practices
      - Lab personnel do not receive appropriate immunizations or test for agents handled e.g., Brucella, Anthrax organisms
   f. Disposal of sharps in appropriate containers-
      - Used needles, glass slides were observed unattended in Histopathology and biochemistry sections
      - Biowaste disposal-there is not proper protocol for disposal of faecal samples
   g. BSL-2 Practices and Facilities
      - Personnel receive training on biosafety sometimes only
      - Exposure control plan not available
      - Entrance glass door in biochemistry section broken
      - Emergency exit passage crowded with obsolete equipment and machines
   h. BSL-3 (BSL 2 Plus at LSU)
      - Adequate PAPR not available
      - CCTV is not operational
      - Protocol on decontamination only partially available

9.2 International External Quality Assurance System (IEQAS)

A. Participation in Assessment of Laboratory Quality Management System (LQMS

During the year, The Laboratory Services Unit (LSU) participated through the online questionnaire on assessment of the Laboratory Quality Management System (LQMS). The Laboratory Services Unit was assessed by international vaccine Institute, South Korea. Based on the online survey, it was recommended for improvement in the quality management system. The main recommendations for improving the laboratory quality management system are as follows:

- Develop laboratory quality manual
- Develop protocol for Proficiency testing

- In addition to equipment maintenance log sheet, to start using environmental record charts. For this need to get hygrometer from LCS
- Maintain sample rejection logbook
- Initiate water quality check for sterility & Glassware cleanliness with detergent check residues in washed glassware.
- Maintain SOP for routine inspection of equipment
- Verification/validation of reagents /kits before use

9.3 Participation in EQAsia External Quality Assurance in Asia

As a part of IEQAS, microbiology laboratory at laboratory services unit participated in the proficiency testing program. The aim of the EQAsia project is to assess and improve the quality of diagnostic services in bacteriology including antimicrobial susceptibility testing (AST) in the Asian region. The EQAsia project is supported by the Fleming Fund (UK Aid Program) in the region.

The microbiology laboratory at laboratory services unit received proficiency test (PT) panels consisting of 22 unknown samples (11 PT for *Acinetobacter* and 11 PT for *Staphylococcus aureus*). The samples were cultured, and identification were carried out for *Acinetobacter* and *Staphylococcus aureus* respectively. The isolates were then subjected to antimicrobial susceptibility tests as per the CLSI guidelines. The scores were provided on each step of tests and findings compared with the expected findings.

Figure 3: EQAsia QAS Participation Certificate

9.4 Conducting of Internal Quality Assurance System (IQAS) for RLDCs

As a part of IQAS for the RLDCs, the Proficiency testing on Rose Bengal Test for screening of Brucellosis in animals was conducted during the year.

Proficiency testing on Rose Bengal Test for screening of Brucellosis in animals (2021-22)

Conducted by National Veterinary Laboratory, National Centre for Animal Health, Serbithang as a part of National External Quality Assurance System NEQAS) for regional laboratories

Summary
Proficiency testing (PT) is a part of laboratory quality assurance system (QAS) to ensure a test procedure consistently produces quality result. Proficiency testing along with various other components of QAS such as record keeping, quality control, training, evaluation, calibration, monitoring, taking corrective actions and competency assessment will contribute to quality management of a laboratory. PT was conducted on RBT screening of Brucellosis in cattle and four RLDCs participated.

Positive samples: All the four laboratories identified all 8 *true positive* samples as *positive*, thus resulting in overall estimated ***diagnostic sensitivity*** of 1.0. However, only three out of four laboratories diagnosed all 12 *true negative* samples as *negative* resulting into over all estimated ***diagnostic specificity*** of 0.8

An ideal test is the one with diagnostic estimates of 1.0 (sensitivity and specificity). Unfortunately, there is no commercial test available with diagnostic estimate as 1.0. The diagnostic estimates reported here for all participating laboratories and coordinating laboratory are only relative estimates. However, these estimates are useful in recognizing the strength and weakness in the testing capacity of each laboratory and provide directions for improvement.

Introduction

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

individually convey performance of each participating laboratory with details of their strength, weakness and recommendation.

Brucellosis

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

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

The test: Rose Bengal Test (RBT)

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

The disease is being detected in the cattle in the country through regular screening. In order to screen the animals routinely, the laboratory test quality should be ascertained. Hence, the proficiency testing is being conducted for the regional laboratories as a part of NEQAS.

Importance of proficiency testing

From the data collected during proficiency testing, laboratory managers can identify staff that may require further training. This therefore leads to more consistent working practices throughout the laboratory. Regular testing also keeps the team focused on how routine

procedures should be carried out. Proficiency testing can provide an opportunity to further educate staff in the potential areas of testing. Training allows staff to appreciate their contribution to the output quality of their laboratory. Staff can then also appreciate their role in the success of their laboratory. Anyone who is trained and is regularly involved in routine testing needs to be included in proficiency testing.

Brucella RBR-PT for fiscal year 2021-22

During the fiscal year 2021-22, as a part of national external quality assurance (NEQAS) in laboratory test performance, the National Veterinary Laboratory, National Centre for Animal Health, Serbithang organized a round of proficiency testing with regional animal health laboratories at Regional Livestock Development Centres on Rose Bengal Test for screening Brucellosis in cattle. The main objective of this PT was to assess the performance of different regional laboratories in screening of Brucellosis in cattle. Details of coordinating and participating laboratories in PT are as follows:

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

Proficiency testing panel

Twenty serum samples collected and archived at National Veterinary Laboratory, National Centre for Animal Health, Serbithang were identified. These samples were tested with RBT to determine the level of agglutination reactivity in RBT and optical density (OD) values in ELISA. Depending on the level of reactions among positive samples (n =8), they were further classified as + (positive), ++ (positive) and +++ (strong

positive). Panel also included a set of negative samples (n = 12), one positive control and one negative control. Antigen (Rose Bengal-stained *B. abortus* suspension) was also supplied along with serum samples.

Test procedure

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

- Bring antigen and test/panel sera to room temperature (20°C ± 3°C)
- Pipette 25 µl of panel serum and place on agglutination plate (white smooth tile) leaving about 4 cm distance between each serum sample

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

Table 13: Distinction of degrees of reaction

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

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

Collation of RBT results

The participating laboratories were coded as laboratory code 1, 2, 3 and 4 to maintain the anonymity of test results among all participating laboratories. The test results of all participating laboratories were collated and compared with the results of coordinating laboratory. The results were collated as reported by participating laboratories (Table 14).

Table 14: Collated RBT result of coordinating and participating laboratories

Sl.no.	Sample ID	Vial ID	Result NCAH	LAB-01	LAB-02	LAB-03	LAB-04
1	12000680(2)	2022-1	(+++) SP	(+++) SP	(+++) SP	(+++) SP	(++) P
2	12000706(7)	2022-2	(+++) SP	(+++) SP	(+++) SP	(+++) SP	(+++) SP
3	12000694(9)	2022-3	(++) P	(++) P	(++) P	(++) P	(++) P

4	12000583(22)	2022-4	(+++) SP	(+++) SP	(+++) SP	(+++) SP	(+++) SP
5	1209000744(47)	2022-5	(-) N	(-) N	(-) N	(-) N	(-) N
6	1209000749(52)	2022-6	(-) N	(-) N	(-) N	(-) N	(-) N
7	1209000748(51)	2022-7	(-) N	(-) N	(-) N	(-) N	(-) N
8	12000656(19)	2022-8	(-) N	(-) N	(-) N	(-) N	(-) N
9	Sampheling (01)	2022-9	(-) N	(-) N	(-) N	(-) N	(-) N
10	TTF-03	2022-10	(++) P	(++) P	(+++) SP	(++) P	(++) P
11	482 -17	2022-11	(-) N	(-) N	(+) D	(-) N	(-) N
12	19557	2022-12	(+) P	(+++) SP	(++) P	(+) P	(++) P
13	482-16	2022-13	(-) N	(-) N	(-) N	(-) N	(-) N
14	482-11	2022-14	(-) N	(-) N	(-) N	(-) N	(-) N
15	19557	2022-15	(+) P	(+) PD	(++) P	(+) P	(+) D
16	517- 2011183	2022-16	(-) N	(-) N	(-) N	(-) N	(-) N
17	517- 2011210	2022-17	(-) N	(-) N	(++) P	(-) N	(-) N
18	12000694(9)	2022-18	(++) P	(++) P	(+++) SP	(+) P	(++) P
19	482-12	2022-19	(-) N	(-) N	(-) N	(-) N	(-) N
20	482-13	2022-20	(-) N	(-) N	(-) N	(-) N	(-) N
	True positive (TP)			8	8	8	8
	True negative (TN)			12	10	12	12
	False positive (FP)			0	2	0	0
	False negative (FN)			0	0	0	0
	Sensitivity	**TP/TP +FN**		**1**	**1**	**1**	**1**
	Specificity	**TN/TN+FP**		**1**	**0.8**	**1**	**1**

Analysis of test result

The sensitivity and specificity of tests have been calculated as below:

Sensitivity=TP/TP+FN
Specificity=TN/TN+FP
TP= True positive
TN=True Negative
FP= False positive
FN= False negative
Analysis of test results is shown in Table 14.

Positive samples: All the four laboratories identified all 8 *true positive* samples as *positive*, thus resulting in estimated ***diagnostic sensitivity*** of 1.0.

All the three laboratories (1, 3 & 4) diagnosed all 12 *true negative* samples as *negative* resulting into estimated ***diagnostic specificity*** of 1.00. However, lab, No.2 diagnosed 2 true negative as false positive resulting in diagnostic specificity of 0.8.

An ideal test is the one with diagnostic estimates of 1.0 (sensitivity and specificity). Unfortunately, there is no commercial test available with diagnostic estimate as 1.0. The diagnostic estimates reported here for all participating laboratories and coordinating laboratory are only relative estimates. However, these estimates are useful in recognizing the strength and weakness in the testing capacity of each laboratory and provide directions for improvement.

Laboratory 1

a. Did not have issue in identifying true positive samples as positive irrespective of samples having different intensity of agglutination/reactions

b. It also did not have difficulty in identifying true negative as negative

c. Hence, the lab had capacity of diagnosing Brucellosis with 100% sensitivity and 100% specificity with the provided Antigen (Rose Bengal-stained *B. abortus* suspension).

Laboratory 2

a. The lab did not have issue in identifying true positive samples as positive irrespective of samples having different intensity of agglutination/reactions and hence, the lab had capacity of diagnosing Brucellosis with sensitivity of 100% with provided Antigen (Rose Bengal-stained *B. abortus* suspension).

b. However, the lab had difficulties in identifying true negative samples as negative. It identified 2 true negative as false positives hence, the diagnostic specificity of 80%.

c. There is a need to improve on both detecting the true positive and true negative result

Laboratory 3

a. Did not have issue in identifying true positive samples as positive irrespective of samples having different intensity of agglutination/reactions

b. It also did not have difficulty in identifying true negative as negative

c. Hence, the lab had capacity of diagnosing Brucellosis with 100% sensitivity and 100% specificity with the provided Antigen (Rose Bengal-stained *B. abortus* suspension)

Laboratory 4

a. Did not have issue in identifying true positive samples as positive irrespective of samples having different intensity of agglutination/reactions

b. It also did not have difficulty in identifying true negative as negative

c. Hence, the lab had capacity of diagnosing Brucellosis with 100% sensitivity and 100% specificity with the provided Antigen (Rose Bengal-stained *B. abortus* suspension).

Conclusion

The result was collated and analysed for sensitivity and specificity based on the ability of participating laboratory to correctly identify true positives and positive and true negatives as negative. Compared to the past, all the laboratories have improved in diagnosing the Brucellosis using RBT. Hence, the test produced overall sensitivity of 100% and specificity of about 95%. Although RBT is a very sensitive test, it is also a highly subjective test in terms of result interpretation. However, performing and interpreting RBT result requires high level of experience and regular performance of the test. Therefore, performing this test and accurately interpreting result can be gained only regular practice.

Reference

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

Appendix 1: Result recording sheet

TEST RESULT REPORTING FORMAT FOR PT ON BRUCELLA RBT			
	Lab name	Your laboratory name	
	Lab code	Mentioned in the cryo box of PT panel	
		RBT	
		Test performed on:	Test performed date
		Antigen supplier:	NCAH, Serbithang
	Tube ID:	Result	Interpretation (N/D/P/SP)
Positive control		+	P
Negative control		0	N
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			

12			
13			
14			
15			
16			
17			
18			
19			
20			
1: Enter the raw results (0, +, ++, +++) depending on the intensity of the reaction			
2: N = Negative; Positive (P/WP) for + or doubtful (D); Positive (P) for ++ and Strong positive (SP) for +++			
FOR THIS PROFICIENCY TESTING, REPORT ONLY NEGATIVE (N), POSITIVE (P) AND STRONG POSITIVE (SP). IF POSSIBLE "D" FOR DOUBTFUL			

9.5 Drafting of Laboratory Quality Manual

For any diagnosis, surveillance and trade, valid laboratory results are very much essential. Such results can be achieved only by the use of good laboratory management practices, valid test and calibration methods, proper techniques, quality control and quality assurance, all working together within a quality management system. Laboratory quality management includes technical, managerial and operational elements of testing and the interpretation of test results. A quality management system enables the laboratory to demonstrate both competency and an ability to generate consistent technically valid results that meet the needs of its clients.

Currently, the AMR surveillance is being carried out by the National Centre for Animal Health, Regional Livestock Development Centers under DoL and National Food Testing Laboratory, BAFRA.

The laboratory under animal health follows quality assurance system partially only and lack a proper manual for implementation and monitoring of laboratory quality management system (LQMS). Hence, a 5 days' workshop (6 to 10 of June 2022) was conducted at Thimphu to develop the quality management manual to train the laboratory personnel. About 9 participants attended the workshop representing from, DoL HQ, NCAH, RLDC, NVH and BAFRA from MoAF and RCDC from MoH. Through the workshop, the draft quality manual was developed which needs further discussion and revision.

Methodology

The method used was basically a write shop. Initially the structure for the quality manual was discussed at length and agreed. Then the template was also discussed and agreed upon. The development of the manual was assignment through the group work as follows:

Introduction	**Groups**	**Supporting documents**
1 Scope	**Group A** Mr. Leto Dr. NK Thapa	Minutes of meeting (file no.)/id
2 Normative references		Conflict of interest form
3 Terms and definitions		Forms
4 General requirements		Organizational structure
4.1 Impartiality		Test lists
4.2 Confidentiality		Laboratory design
5 Structural requirements		
Bibliography		
6 Resource requirements	**Group B** Dr. N Dahal Mr. Durga Sharma	Biosafety manual
6.1 General		Visitor logbook
6.2 Personnel		Incident reporting form
6.3 Facilities and environmental conditions		Security system
6.4 Equipment		Equipment installation
6.5 Metrological traceability		Equipment repair
6.6 Externally provided products and services		Equipment validation
Annex A Metrological traceability A.1 General		Equipment disposal form., log, decontamination, certification etc. Performance appraisal, training,

A.2 Establishing metrological traceability A.3 Demonstrating metrological traceability Bibliography		recruitment, competency assessment, waste disposal, receipt of supplies, tenders, stock management, inventory logs,
7 Process requirements	**Group C** Dr. RB Gurung Mr. Tenzinla Ms. Sonam Pelden	Sample collection, transport, process, storage, SOP for tests
7.1 Review of requests, tenders and contracts		Method validation, quality control,
7.2 Selection, verification and validation of methods		Result validation
7.3 Sampling		Test result form
7.4 Handling of test or calibration items		
7.5 Technical records		
7.6 Evaluation of measurement uncertainty		
7.7 Ensuring the validity of results		
7.8 Reporting of results		
7.9 Complaints		
7.10 Nonconforming work		
7.11 Control of data and information management Bibliography		Information security, result transmission, back ups
8 Management system requirements	**Group D** Dr. Basant Sharma Ms. Punya Mata	
8.1 Options 8.2 Management system documentation (Option A) 8.3 Control of management system documents (Option A) 8.4 Control of records (Option A) 8.5 Actions to address risks and opportunities (Option A) 8.6 Improvement (Option A)		Procedures SOP management, Archival Document control logbook Audit form

8.7 Corrective actions (Option A) 8.8 Internal audits (Option A) 8.9 Management reviews (Option A) Annex B Management system options Bibliography		

The activities carried out daily was presented in the following morning for the comments/ feedbacks from the members of the other groups.

Conclusion

The manual is to be compiled and shared first among the TWG members for comment and feedbacks. The same will be shared further to the RLDCs for the final comments and feedbacks. Some of the annexures required needed to be developed which shall be done as required.

9.6 Enhancement of Laboratory Information Management System (LIMS)

Summary

LIMS, the online database system designed to efficiently manage information of all the veterinary laboratory activities in the country has been in use since few years back. The data base has the features for online entry of owner's details, animal details, sample details, test result, diagnosis and recommendation. The system helps the veterinary laboratories to track samples from submission to testing and reporting. This database also enables real time tracking of sample testing status through a paperless system. Besides data storage and test result dissemination, it is aimed for customized analysis to provide decisions required in policy interventions. Further, this database also immensely reduces turn-around-time for diagnostic service delivery as a whole. The system is intended for all the laboratory facilities under the Department of Livestock (DoL) viz. National Veterinary Laboratory under National Centre for Animal Health (NCAH), National Veterinary Hospital (NVH), Regional Livestock Development Centres (RLDCs), Satellite Veterinary Laboratories (SVLs) and Dzongkhag Veterinary Laboratories (DVLs). The additional advantage of the system is that it can be remotely accessed by any authorized personnel.

The main feature of the LIMS on the microbiology especially in the Bacteriology part is recording of the antibiotic sensitivity profile of the important bacteria isolated from clinical cases and research including food borne pathogens. In addition, the LIMS is aimed to support data generation and analysis for the ongoing AMR laboratory surveillance in chickens under the Fleming Fund Country grant.

Since, the database is developed about three years back we encountered various bugs or flaws during the course of its usage. There is a need to update the information like name of the places, species of animals and also new emerging and remerging diseases. The feed backs were collected during the during the training and also from the end users on day-to-day basis. Especially for the microbiology part, the features like inclusion of zone diameter for AST and the export features needs to be incorporated. Accordingly, as per the DTIP, the enhancement of the LIMS is under process. The final demo was carried out by the developer. However, UAT exercise needs to be carried out to find out bugs and fix it for further carrying out of TAT. Hence, a three-day workshop 12 to 14 of May 2022 was conducted at Post card dewa, Thimphu to carry out the user acceptance Test. The main aim of face-to-face workshop was to discuss on the issues and fixed on site in order to complete the enhancement on time,

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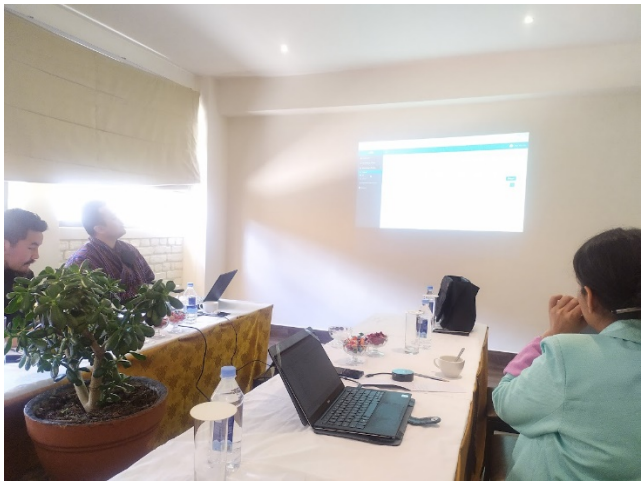
In total of four participants were involved in the exercise representing the user NCAH and the developer, Yeuvan Consultancy.



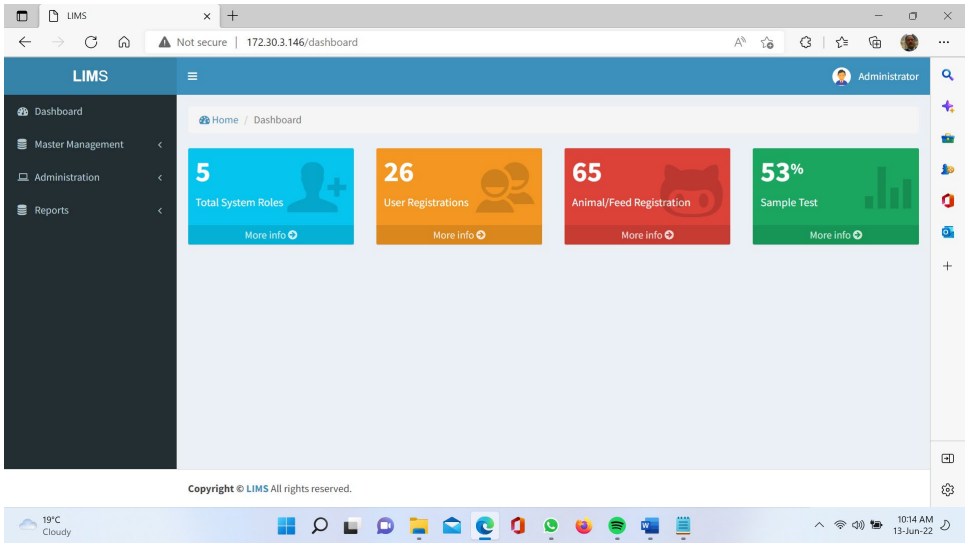
Activities carried out

During the workshop, following issues were discussed and resolved on site. However, whichever was unable to be resolved, the way forward was planned with involvement from ICTD, MoAF. Since, the ICTD officials were involved in other activities, they could not represent the workshop.

Routine Laboratory part

1. Registration of Govt. farm- select in type of owner (CID option not there)- it was clarified that the ARN will be used
2. Owner registration- only individual available – add again from same owner as new
3. View all the information not available- type of feed- This was rectified
4. Result in biochemistry appeared double- it was decided to keep but not make it mandatory
5. Pool sample/individual sample – clarified, as sampling not the owner, include the number of samples
6. For the Lab in charge- only bacteriology was appearing and rest sections appeared in a group of other sections- It was decided and rectified to appear same for all sections. The test for other sections also did not appear. It was resolved.
7. Sample- grouping for feed was missing- it was resolved.

8. Feed sample- cannot be saved after editing or entering- it was rectified
9. Category in registration was decided as: Animal name/feed- individual name/ pool/feed formulated or non-formulated
10. Parasitology- Option for only one parasite was available. It was decided to include **add button**, after genus sps (we get more than one parasite), it was informed that more time will be required.
11. Sample was identified secton wise initially however, it created crowding Hence, it was resolved in a way that (single sample going to different sections) to populate together. There is no need of samples to be identified section wise rather keep it general.
12. Postmortem part was incomplete and hence was decided to complete.
13. In order to count for samples analyzed, it was decided to include **No of samples** before subtest,
14. Value for cell culture not appearing- field to appear where the values will be inserted in.
15. Bacteriology- multiple species with same biochemical tests not appearing- hence it was rectified



Quality Assurance System (QAS)

1. It was discussed and decide that the mapping to be same as regular test in PT tests also

Sample repository

1. Option for sample repo to be kept in -in lab tech interface and also to keep archival for repo
2. Sample repo ref no.? autogenerate or Sample repo- international referral(A), international referral(W)

Referral

1. Sample referral- ex country just end in save, remove lab sections.

2. Sample referral- in country, -lab in charge, include, ***refer*** button in addition to other

Way forward

As per the UAT findings new timeline was framed as required for development

1. Modification/development on PT -one day
2. Sample Repo needed further clarification with ICTD hence, it was decided to confirm on Monday
3. Referral- 1-2 days
4. Parasitology- add button for more than one parasite (1-2 weeks)- it was not mentioned. - will be tried.
5. Report generation to be discussed at the end of testing
6. Some of the Unit and the test not linked- developer will link and insert the mapping by the user.

9.7 Laboratory Equipment Maintenance, certification and Calibration

During the year various maintenance of equipment, certification was carried out with the joining of the new Biomedical Engineer faulty electrification of the laboratory building was also rectified. The detail activities are as follows:

A. Repair & maintenance

- Biosafety cabinets 4 nos.
- Centrifuge 1 no.
- Autoclave 1 no.
- Cold room 1 no.
- Hot air oven 1 no.
- Refrigerator 1 no.
- Weighing scales 1 no.
- Deep freezer 1 no.

B. Certification of Biosafety cabinet 6 nos.

C. Procurement

- Basic tools for biomedical engineering section
- Spare parts for Biosafety cabinets
- UPS for Deep freezers

D. Rectification of earthing inside the laboratory building

10.0 Animal disease surveillance and related activities conducted, FY 2021 – 2022

10.1 Sero-surveillance on of yak priority diseases

As a part of support to Highland Livestock Development Program, the sero-surveillance was carried out at Yak breeding Farm, Haa for priority diseases during May 2022. About 51 sera samples were collected from the farm and were screened against important diseases viz. Brucella, Infectious Bovine Rhinotracheitis (IBR_ and Bovine viral diarrhea (BVD).

Out of 51 sera samples screened, no Brucella antibodies were detected however, 9/51(17.6%) animals were detected with antibodies against IBR and 11/51(21.6%) animals were detected with antibodies against BVDV (Figure 4).

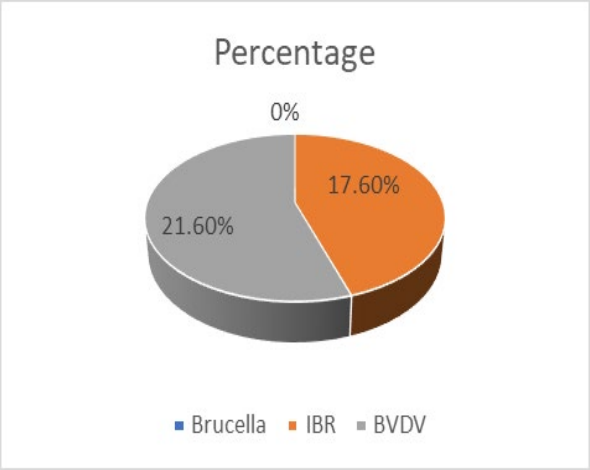


Figure 4: Percentage of seropositivity

This indicated that, no animals had exposure to the Brucella antigen however, 17.6% of the animals sampled had exposure to IBR antigen and 21.6% of the animals had exposure to BVD antigen. Since the animals in the country are not vaccinated against any of the above diseases, it indicates that the animals had infection.The farm should monitor the animals especially while distributing the animals for breeding purposes. Regular screening of the animals are required to monitor the disease.

10.2 Detection of Porcine circovirus-associated disease (PCVAD) in government Pig Breeding Farms in Bhutan

NK Thapa, Dawa Tshering, Puspa M Sharma, Kelzang Lhamo and RB Gurung

Summary

Three of the government pig farms were screened against PCV2 and PRRS. The screening indicated 28/29 (96.5%) pig sera samples from RPPBC Lingmethang, 11/11(100%) samples from NNPBC Yusipang and 5/5 (100%) samples from NPBC Wangchutaba tested positive against PCV2 antibodies. The same serum samples screened for antibodies against PRRSV indicated seropositivity of 7/29(24.1%) at RPBC Lingmethang, 1/11(9%) at NNPBC Yusipang

and no antibody was detected at Wangchutaba farm. More screening of animals in the pig farms will be carried out.

Introduction

Porcine circoviruses (PCV) belong to the genus *Circovirus* in the *Circoviridae* family and is considered as one of the most important pathogens for swine industry worldwide (Segales, 2005; Segales, 2012). Porcine circovirus type 2 (PCV2) is reported to be the primary causative agent of several syndromes collectively known as porcine circovirus-associated disease (PCVAD) and the most significant manifestation of PCVAD the post-weaning multisystemic wasting syndrome (PMWS) (Savic et al. 2012; Segales et al. 2019). Clinical manifestation of wasting, dyspnoea, enlarged lymph nodes, paleness of skin and diarrhoea have been reported earlier (Allan and Ellis, 2000).

National Nucleus Pig Breeding Centre established during 2016 at Yusipang maintains the Great Grand Parents (GGP), Grand Parents (GP) and Parent Stock (PS) to supply genetically improved piglets and PS. The farm was initiated by importing new stock of Landrace and Yorkshire from privately owned farms in Thailand during July 2016. The pigs for parent stock are being supplied to government breeding farms in other parts of the country. However, the pig farm at Wangchutaba rears mainly the local breed in small numbers. Regional Pig & Poultry Breeding Centre in the eastern part (RPPBC), Lingmethang breeds and supplies the piglets for the eastern region of the country

Various forms of clinical signs and even mortalities were reported from the National Nucleus Pig Breeding Centre (NNPBC), Yusipang, Thimphu. During the investigation, the animals were observed with clinical manifestations ranging from weakness, dermatic lesions, lameness, bluish ear, reproductive disorder and anorexia. Two of the animals were detected with antibody against PCV2. This led to further investigation and screening of the animals against the PCV2 in other farms too.

Materials and Methods

Sample site

The screening of sera samples was carried for three of the Government Pig Breeding farms, v.i.z. Regional Pig & Poultry Breeding Centre in the eastern part (RPPBC) Lingmethang, National Nucleus Pig Breeding Centre, Yusipang and Wangchutaba in the western region during November 2021 at National Centre for Animal Health. About 43 sera samples from (RPBC Lingmethang 29, NNPBC Yusipang 11 and Wangchutaba 5) and 10 oropharyngeal swab samples from clinical case at NNPBC Yusipang were collected and analyzed. Due to lack of kits in other labs, the oropharyngeal samples were collected at the NNPBC only for molecular analysis.

ELISA

Sera samples were screened against antibody against PCV2 using ELISA kit (Elab Science, US, Catalogue No: E-AD-E003). The ELISA Microtiter plate pre-coated with recombinant cap protein of porcine circovirus 2 (PCV2), HRP conjugate and other auxiliary reagents applied the principle of enzyme-linked immunoassay (ELISA) to detect porcine circovirus 2 antibody of porcine serum. The test was performed as per the protocol provided by the manufacturer. The absorbance value of each well was measured using a Micro-plate Reader (Heales MB-530) with 450 nm wavelength for the PCV2 antibodies in the samples.

Serum samples were also screened for antibody against porcine reproductive & respiratory syndrome virus (PRRSV) protein using ELISA kit (Elab Science, US, Catalogue No: E-AD-E006). The ELISA Microtiter plate pre-coated with recombinant protein of PRRSV, HRP conjugate and other auxiliary reagents applied the principle of enzyme-linked immunoassay (ELISA) to detect PRRSV antibody of porcine serum. The test was performed as per the protocol provided by the manufacturer. The absorbance value of each well was measured using a Micro-plate Reader (Heales MB-530) with 450 nm wavelength for the PRRSV antibodies in the samples.

PCR

The oropharyngeal swab samples were subjected to DNA extraction using Qiagen DNA Mini Kit extraction (Qiagen, Hilden, Germany) and real time PCR were carried out on a QuantStudio 5 thermocycler (Applied Biosystems) with a final volume of 25 µL against PCV2 and porcine reproductive & respiratory syndrome virus (PRRSV) ThePCR kit from applied biosystem (catalog number: 4387391), primers and probe as described by Zhao et al. 2010 were used for PCV2 reaction with the cycling condition as 95°C for 10 min and 45 cycles of 95°C for 15 s and 60°C for 40 s. The samples were also subjected to RNA extraction using Qiagen RNA Mini Kit extraction (Qiagen, Hilden, Germany) and real-time (TaqMan) reverse transcriptase (RT)–PCR assays were carried out as described by Kleiboeker for PRRS North amercian type (NA) and PRRS European type (EU) (Kleiboeker et al., 2005).

Statistical analysis

Serum samples were collected based on the random sampling and the oropharyngeal samples were targeted towards the animals having clinical signs. Prevalence were estimated for each diseases using excel.

Results and Discussions

28/29 (96.5%) pig sera samples from RPPBC Lingmethang, 11/11(100%) samples from NNPBC Yusipang and 5/5 (100%) samples from NPBC Wangchutaba tested positive against PCV2 antibodies (table 1).

Table 15: Details of screening of serum samples for PCV2 & PRRS by ELISA

Sl. No.	*Farm*	*Serum (n)*	*Positive to PCV2*	*Positive to PRRS*
1	RPPBC Lingmethang	29	28/29(96.5%)	7/29(24.1%)
2	NNPBC Yusipang	11	11/11(100%)	1/11(9%)
3	NPBC Wangchutaba	5	5/5(100%)	0
	Overall	**45**	**44/45(97.7%)**	**17.7%**

The overall seroprevalence of PCV2 appeared very high 44/45(97.7%) in all the three farms ranging from 96.5% to 100%. Similar high prevalence of PCV2 of 85% was reported from Cambodian farms (Tornimbene et al. 2015), 20.3% from Hong Kong Special Administrative Region (Flay et al. 2022). High seropositivity could be due to actual exposure of animals to virus and also assumed to be due to persistence of the antibodies in the host for variable period of time even after elimination of pathogen (Metcalf et al., 2016).

The same serum samples screened for antibodies against PRRSV indicated seropositivity of 7/29(24.1%) at RPBC Lingmethang, 1/11(9%) at NNPBC Yusipang and no antibody was detected at Wangchutaba farm (table 1). Sero-prevalance of 7.6% to 12.2% to PRRS were reported from farmed pig population from Hong Kong Special Administrative Region (Flay et al. 2022).

Table 16: Molecular test for oropharyngeal swab of NNPBC Yusipang

Diseases	*Nos. tested(n)*	*Nos. positive*	*Percentage*
PCV2	10	5	50%
PRRS	10	0	0

5/10 (50%) of the oropharyngeal swab samples from clinical cases at NNPBC Yusipang tested through molecular process were found 5/10 (50%) positive to PCV2(table 3), detection of as high as 28.2% was also reported from the North-eastern India through PCR (Bhattacharjee et al. 2021).

There is possibility of getting the infection in the farm from the source through the importation of these pigs. Further, the farm area of NNPBC Yusipang is also surrounded by orchards and the forest hence, probability of getting infection from the wild boar is also quite high. However, this could be confirmed only after testing the wild boars for PCV2 in the surrounding forest. Detection of PCV2 in the wild boar population serving as the reservoir for infection for the nearby pig farms have been reported in other countries like Romania, Poland and Serbia (Turcitu et al. 2011; Fabisiak et al. 2012, Nisavic et al. 2022).

The PCVAD is due to multifactorial causes and occurs as co-infection of PCV2 with other pathogens and immune inflection of host (Tsai et al., 2019). Co-infection with other viral and bacterial pathogens have known to increase the incidence and also more severe clinical form; among the viral, porcine reproductive & respiratory syndrome virus (PRRSV), porcine parvo virus (PPV) and *Mycoplasma hyopneumonae*. However, at the NNPBC Yusipang, the co-infection with PRRSV and *Mycoplasma hyopneumonae* was not detected earlier during 2021 (Thapa et al. 2021). Due to lack of kit for *Mycoplasma hyopneumonae*, the samples could not be screened against the infection. Co-infection of the farmed pig population with PRRS and PCV2 was also reported earlier at Hong Kong Special Administrative Region (Flay et al. 2022).

During the investigation, the animals were observed with clinical manifestations ranging from weakness, dermatic lesions, lameness, bluish ear, reproductive disorder and anorexia. According to the American Association of Swine Veterinarians (AASV), PCVAD cand be subclinical or include one or more clinical manifestations including multisystemic disease with weight loss and high mortality, respiratory disease, porcine dermatologic and nephropathy syndrome, enteric signs including diarrhea, and reproductive disorders on an individual or herd basis (AASV, 2017). In this case lameness and bluish colour of ear tip could be due to other conditions.

Virus transmission occurs in several ways and out of which most common being the oro-nasal contact through contaminated feces (Seagles et al. 2005). The virus is shed through respiratory secretions, oral secretions, urine and feces in both with clinical signs as well as sub clinically affected pigs. Being viral infection, there is no specific treatment however, symptomatic treatment is usually recommended. Prevention of PCV2 is often difficult and the infection have been detected even in the well managed farms with strict isolation practices (Gillespie et al.2009). However, the trivalent vaccine containing PCV2a/b and *M. hyopneumoniae* evaluated in the field trials elicited protective immunity against PCV2d and *M. hyopneumoniae* (Um et al. 2021). Routine disinfection and also disinfection before addition of every batch of pigs in the herd and regular monitoring needs to be carried out in the farm.

Conclusion

PCV2 has been implicated for economic impact in the pig farms. PCV2 is considered as the primary agent for PCVAD many of the syndromes are associated with other infectious agents like *Mycoplasma* and Porcine Reproductive and Respiratory Syndrome (PRRS). The seropositivity of PRRS was detected at both RRPBC Lingmethang and NNPBC Yusipang. Strict biosecurity measures at the farms should be taken to stop further spread of the virus. Vaccination of pigs like in other countries may have to be adopted in these government farms.

References

Allan, G. M. & Ellis, J. A. (2000). Porcine circoviruses: a review. *J Vet Diagn Invest* 12, 3–14.

American Association of Swine Veterinarians (AASV). (2017). Porcine circovirus associated disease (PCVAD) case definition, Available at:

http://www.aasp.org/aasv/position-PCVAD. htm. Accessed March 20, 2022.

Bhattacharjee U, Sen A and Sharma I. (2021). A retrospective study reveals the Porcine circovirus-2f genotype predominant in the indigenous pig population of North-eastern India. Infection, Genetics and Evolution 96:105100.

Fabisiak M, Szczotka A, Podgorska K, Stadejek T. (2012). Prevalence of infection and genetic diversity of porcine circovirus type 2 (PCV2) in wild boar (Sus scrofa) in Poland. J Wildl Dis. 48(3):612-8.

Grau AF, Jorgensen J, Thacker B, et al. Field performance of a conditionally licensed vaccine: The US experience. Proc Am Assoc Swine Vet.: PCV2/PMWS Sem. 2007;38:159–161.

Gillespie J, Opriessnig T, Meng XJ, Pelzer K, and Buechner-Maxwell V. (2009). Porcine Circovirus Type 2 and Porcine Circovirus-Associated Disease. Vet Intern Med 23:1151–1163

Flay KJ, Yang DA, Choi SC, Ip J, Lee SH and Pfeiffer DU. (2022). First Study to Describe the Prevalence of Porcine Reproductive and Respiratory Syndrome Virus and Porcine Circovirus Type 2 among the Farmed Pig Population in the Hong Kong Special Administrative Region.Vet. Sci. 9, 80.

https://doi.org/10.3390/vetsci9020080.

Metcalf C, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, Ferguson NM, Burke DS and Grenfell BT. (2016). Viewpoint Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet 388. https://doi.org/10.1016/S0140-6736(16)30164-7.

Nisavic J, Milic N, Radalj A, Mirilovic M, Vejnovic B, Cosic M, Knezevic A, Veljovic L and Zivulj A. (2022). Detection and characterisation of porcine circoviruses in wild boars in northeastern Serbia. Vet Med-Czech 67, 131–137.

Savic B, Milicevic V, Jakic-Dimic D, Bojkovski J, Prodanovic R, Kureljusic B, Potkonjak A and Savic B. (2012). Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) in Serbia. Arch Virol. 57(1):21-8.

Segales J, Allan GM and Domingo M. (2005). Porcine circovirus diseases. Anim Health Res Rev 6:119–142.

Segal´es, J., 2012. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Res. 164, 10–19. https://doi.org/10.1016/j.virusres.2011.10.007.

Segales J, Allan GM and Domingo M. (2019). Circoviruses. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, editors. Diseases of swine. New Jersey, USA: John Wiley & Sons, Ltd; p. 473-87.

Thapa NK, Rai PB and Pema U. (2021). Report on Investigation of Porcine circovirus-associated disease (PCVAD) at National Nucleus Pig Breeding Centre (NNPBC) Yusepang. Unpublished report.

Tornimbene B, Frossard JP, Chhim V, Sorn S, Guitian J and Drew T. (2015). Emergence of highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) in medium-scale swine farms in southeastern Cambodia. Prev. Vet. Med.118, 93–103.

Tsai GT, Lin YC, Lin WH, Lin JH, Chiou MT, Liu HF and Lin CN. (2019). Phylogeographic and genetic characterization of porcine circovirus type 2 in Taiwan from 2001–2017. Sci. Rep. 9, 1–13. https://doi.org/10.1038/s41598-019-47209-1.

Turcitu MA, Wellenberg GJ, Barboi G, Codreanu MD, Vuta VB, Nicolae S, Barbuceanu F, Coste H and Cioranu R. (2011). Genetic diversity of porcine circovirus type 2 (PCV2) in the Romanian wild boar population. Res Vet Sci.91(3): e103-6.

Um H, Yang S, Oh T, Cho H, Park HK, Suh J, and Chae C. (2021). A field efficacy trial of a trivalent vaccine containing porcine circovirus type 2a and 2b, and *Mycoplasma hyopneumoniae* in three herds. *VetMedSci*.2022;8:578–590.

Zhao K, Han F, Zou Y, Zhu L, Li C, Xu Y, Zhang C, Tan F, Wang J, Tao S, He X, Zhou Z, and Tang X. (2010). Rapid detection of porcine circovirus type 2 using a TaqMan-based real-time PCR. *Virol J* **7,** 374. https://doi.org/10.1186/1743-422X-7-374.

10.3 Seroprevalence of brucellosis in goats in selected high-risk areas of Bhutan

NK Thapa, Dawa Tshering, Sangay Rinchen and RB Gurung

Abstract: Brucellosis in goats is caused by *Brucella melitensis* and *Brucella ovis*. *Brucella melitensis* is also known to cause Brucellosis in human. It causes abortion, retention of placenta, and orchitis in male animals and losses is mainly accounted due to abortions in goats. Goat population is concentrated in the southern districts; free mixing up of animals with those of across the international border occurs in these areas leading to high risk of incursion of disease. Brucellosis in cattle has been reported in the country however, it's prevalence in goats have not

been established before hence, study on seroprevalence in goats were conducted in two of the high-risk districts. Retrospective screening of 949 goat sera samples for Brucella antibody were conducted. The samples were collected during March 2021 from 16 villages of 3 sub-districts under Chukha district and 25 villages of 8 subdistricts under Sarpang district. Sera samples were tested using Rose Bengal Test and Enzyme Linked-immunosorbent Assay. All the samples, tested negative to Brucella antibodies. However, screening of more samples from other districts needs to be conducted.

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10.4 AMR Surveillance on live poultry

NK Thapa, Tenzinla & Puspa M Sharma

Summary

AMR surveillance is aimed for WHO GLASS pathogens (Salmonella, E. coli, Campylobacter & Enterococci) and as per target, about 800 samples are to be collected. As of date, 409 samples were cultured and isolation of pathogens includes 369 isolates of E. coli, 16 isolates of Salmonella, 88 isolates of Campylobacter and 381 isolates of Enterococci were detected and the antibiogram of each isolate. The antibiogram of each isolate is not confirmed at this stage though depicted below. The study is ongoing and further about 400 samples will be tested. Only after completion of the study, the actual antibiogram of the organisms will be known

Introduction

Bhutan is one of the recipients of Fleming Fund Country Grants to improve the diagnosis and surveillance of AMR in both human health and the animal health sector thereby to inform policy and practices at national and international levels. The human health component implemented by Department of Medical Services which benefits five laboratories (surveillance sites) and animal health component is implemented by the Department of Livestock with four laboratories benefitting (National Centre for Animal Health, National Food Testing Laboratory and two Regional Livestock Development Centres Tsimasham and Kanglung). As per the DTiP, one of the activities under animal Health is AMR surveillance in live poultry.

Materials & Method

AMR surveillance is aimed for WHO GLASS pathogens (*Salmonella, E. coli, Campylobacter & Enterococci*) As per target about 800 samples are to be collected. However, due to various factors including lockdowns and other emergency ad hoc programs, the sampling is not completed in all the target dzongkhags. The target areas of sampling are depicted below:

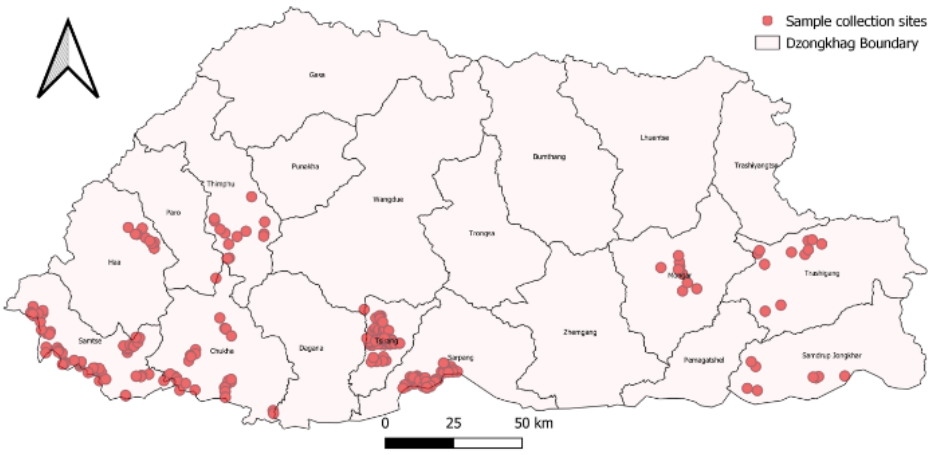


Figure 5: Sampling areas for AMR surveillance

About 11 dzongkhags with high numbers of poultry farms have been selected and till date, the samples collected has been depicted below table 17.

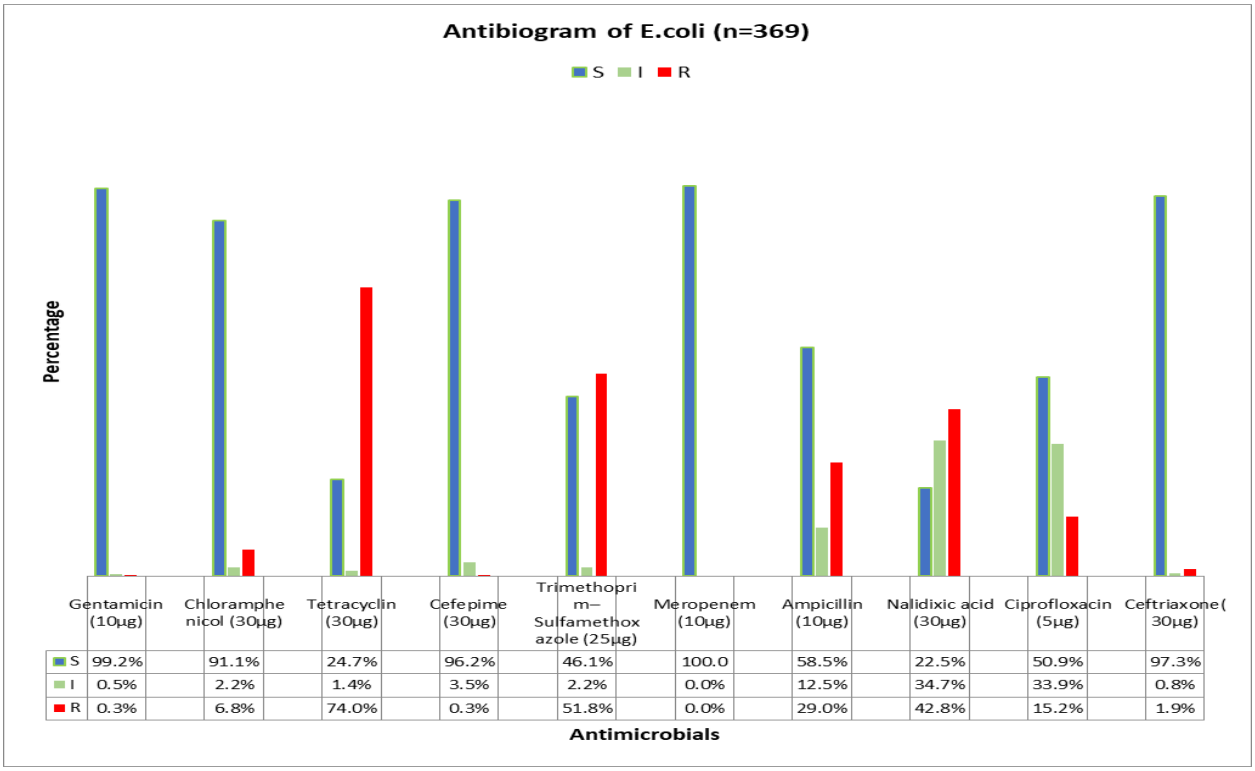
Table 17: Dzongkhag-wise sample collected and processed.

Sampling area	***Samples collected till date***	***Bacteria isolates***			
		E. coli	***Enterococcus***	***Salmonella***	***Campylobacter***
Thimphu	41	14	11		5
Tsirang	104	95	103		31
Sarpang	87	81	87	6	12
Chukha	49	48	49	6	21
Haa	9	7	9	2	2
Samtse	81	77	81	2	21
Mongar	33	29	29		
Tashigang	16	13	10		1
Pema Gatshel	3	2	2		
Samdrup Jongkhar	14	8	5		
Paro	20	20	20		
Total	**409**	**369**	**381**	**16**	**88**

Live chicken was collected randomly from the farms as per the sample size calculated and were sacrificed at the regional laboratories and NCAH. The cultures were made directly from caeca to detect, *E. coli*, *Salmonella, Enterococci* and *Campylobacter*.

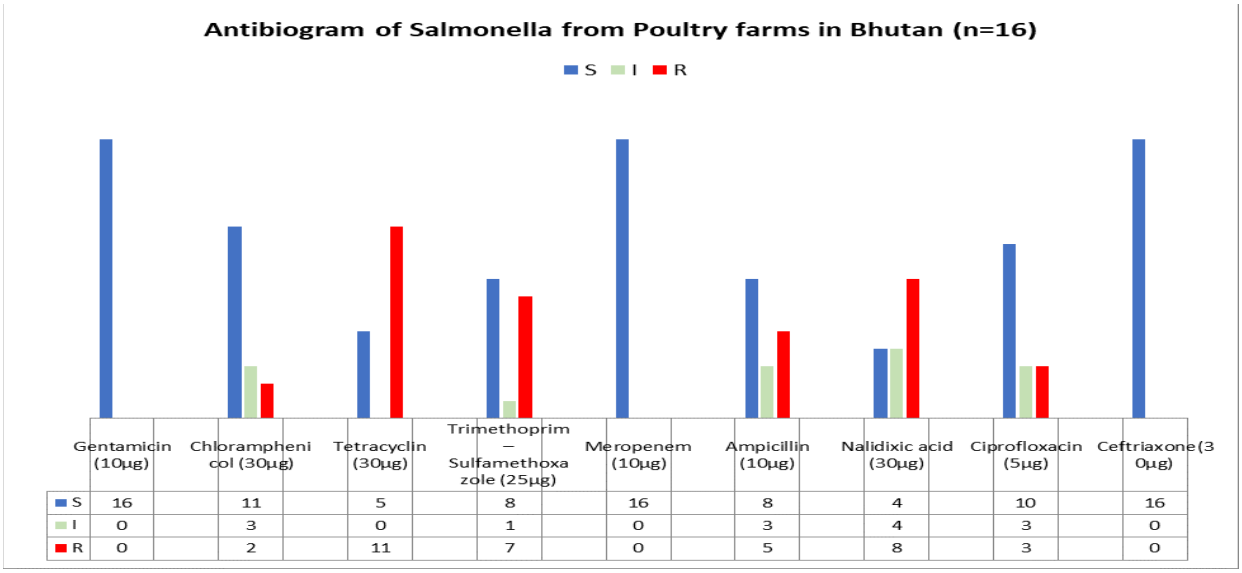
Results

Out of 409 samples culture, 369 isolates of *E. coli*, 16 isolates of *Salmonella*, 88 isolates of *Campylobacter* and 381isolates of *Enterococci* were detected and the antibiogram of each isolate are presented below (Figures 6, 7, 8 & 9).



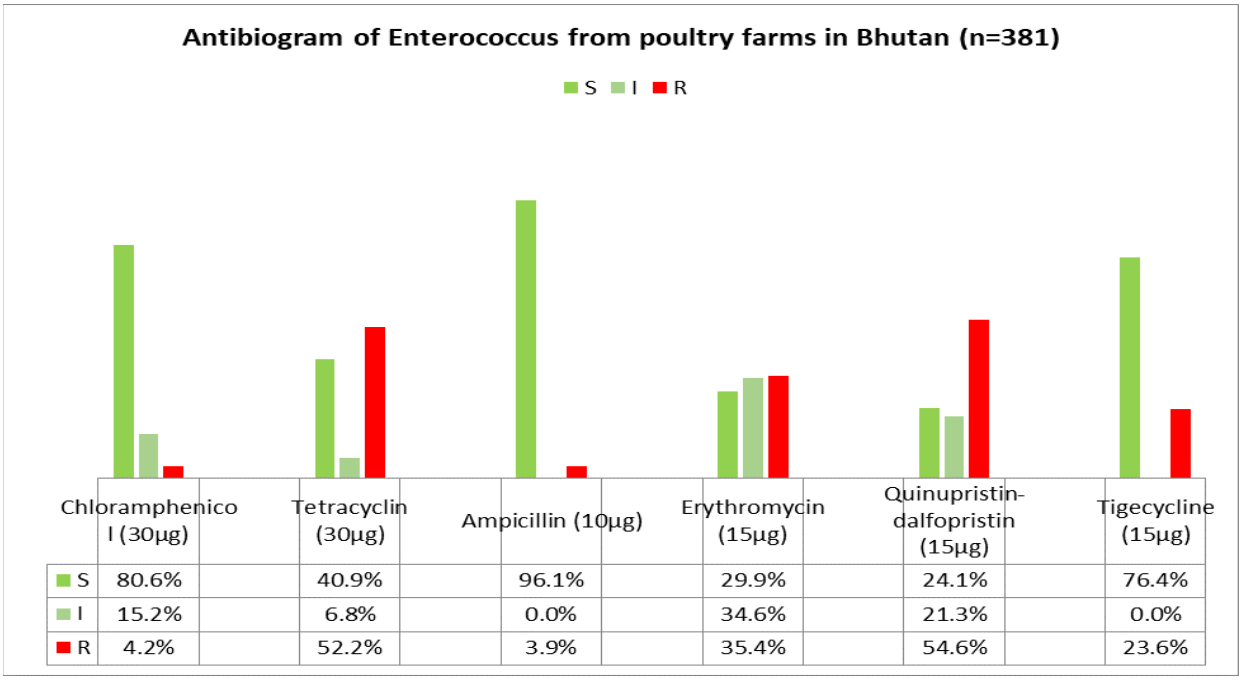
	Gentamicin (10µg)	Chloramphenicol (30µg)	Tetracyclin (30µg)	Cefepime (30µg)	Trimethoprim–Sulfamethoxazole (25µg)	Meropenem (10µg)	Ampicillin (10µg)	Nalidixic acid (30µg)	Ciprofloxacin (5µg)	Ceftriaxone(30µg)
S	99.2%	91.1%	24.7%	96.2%	46.1%	100.0	58.5%	22.5%	50.9%	97.3%
I	0.5%	2.2%	1.4%	3.5%	2.2%	0.0%	12.5%	34.7%	33.9%	0.8%
R	0.3%	6.8%	74.0%	0.3%	51.8%	0.0%	29.0%	42.8%	15.2%	1.9%

Figure 6: Antibiogram of E. coli (%)



	Gentamicin (10µg)	Chloramphenicol (30µg)	Tetracyclin (30µg)	Trimethoprim – Sulfamethoxazole (25µg)	Meropenem (10µg)	Ampicillin (10µg)	Nalidixic acid (30µg)	Ciprofloxacin (5µg)	Ceftriaxone (30µg)
S	16	11	5	8	16	8	4	10	16
I	0	3	0	1	0	3	4	3	0
R	0	2	11	7	0	5	8	3	0

Figure 7: Antibiogram of Salmonella (%)



	Chloramphenicol (30µg)	Tetracyclin (30µg)	Ampicillin (10µg)	Erythromycin (15µg)	Quinupristin-dalfopristin (15µg)	Tigecycline (15µg)
S	80.6%	40.9%	96.1%	29.9%	24.1%	76.4%
I	15.2%	6.8%	0.0%	34.6%	21.3%	0.0%
R	4.2%	52.2%	3.9%	35.4%	54.6%	23.6%

Figure 8: Antibiogram of Enterococci

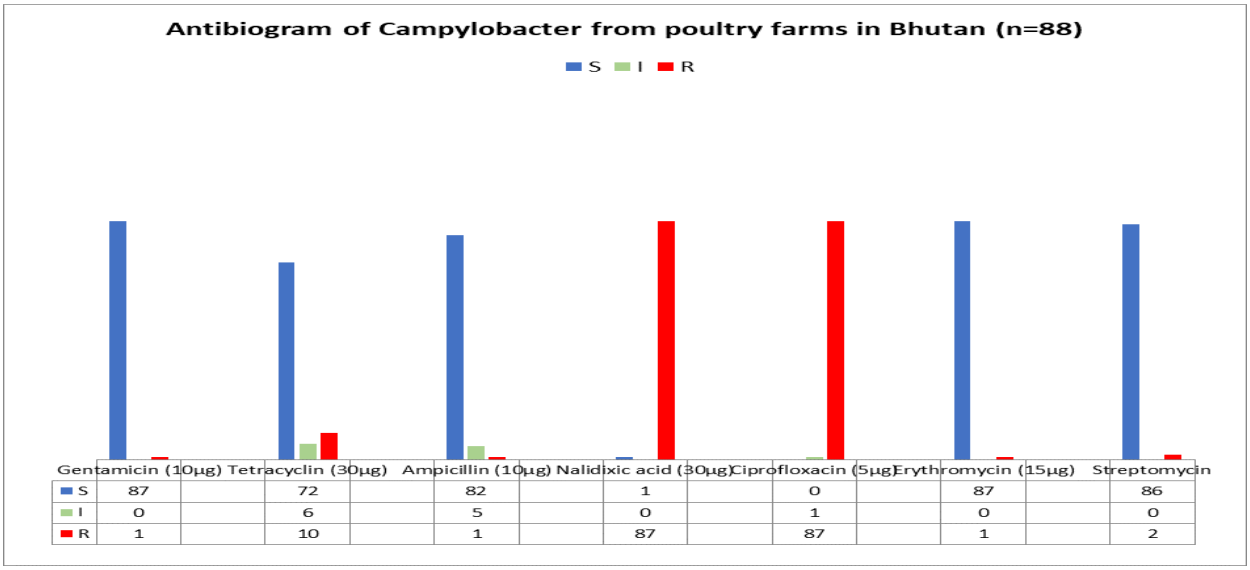


Figure 9: Antibiogram of Campylobacter (%)

Conclusion

The result is based on the partial study only since it is ongoing and the actual proportion of isolate of each bacterium will be known only after the end of surveillance. Further, the antibiogram for each bacterium also will be analyzed after the completion of surveillance.

10.5 Laboratory Surveillance for Taeniid species in yak watch dogs (2021-22)

Gid disease in yaks caused by *Coenurus cerebralis*, the larval stage of *T. multiceps* is prevalent, in the yak-rearing highland areas of Bhutan. It causes considerable economic losses to the highlanders due to loss by mortality. A strategic effort is being put in through the National Highland Development Program for the goal to eliminate Gid (Coenurosis) in yaks in Bhutan by 2025. The goal is being aimed through the intensive implementation of strategic Gid prevention and control measures. Following the elimination of the disease, continued measures are required to prevent the re-establishment of transmission.

The laboratory surveillance of yak watch dogs to screen the *Taeniid eggs* in the feces is aimed to support the goal to eliminate Gid (Coenurosis) in yaks in Bhutan by 2025. The achievement of this goal shall be monitored through verification of the absence of Gid cases in yaks, further supported by the absence of *T. multiceps* in the samples (faecal and soil) collected from the focus areas during risk-based laboratory surveillances.

To support the program, 114 fecal samples of yak dogs and 4 soil samples were collected during the awareness programs from the high-risk yak rearing areas. The samples were screened for *Taeniid* eggs from yak rearing high risk areas of the four dzongkhags during the year 2021-22 out of which, about 7 samples were detected with *Taeniid* eggs. Overall highest prevalence was

observed at Bumthang dzongkhag with 4/23 (17.4%) followed by Thimphu dzongkhag 2/32(6.3%) and Gasa 1/51(2%) and no taeniid was detected at Haa.

The detailed laboratory findings are depicted below table 18:

Table 18: Details of taeniid eggs detected

Dzongkhag	*Geog*	*No. of samples tested*	*Positive to Taeniid eggs*	*Percentage*
Thimphu	Soe	16	2	12.5
	Lingzhi	16	0	0.0
Gasa	Lunana	21	1	4.8
	Laya	30	0	0.0
Bumthang	Cheokhor	23	4	17.4
Haa		8	0	0.0
Total		**114**	**7**	**6.1**

Overall highest prevalence was observed at Bumthang dzongkhag with 4/23 (17.4%) followed by Thimphu dzongkhag 2/32(6.3%) and Gasa 1/51(2%) and no taeniid was detected at Haa. Probably due to the less sample size compared to other dzongkhags.

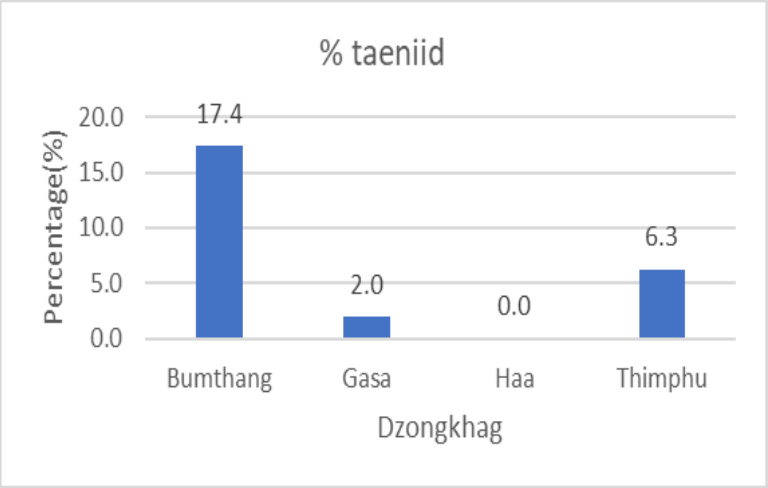


Figure 10: Percentage of taeniid detected

Detection of Taeniid eggs alone by microscopic examination cannot predict the presence *T. multiceps* since, molecular methods can only confirm. Laboratory surveillance will be carried out on annual basis in the high-risk highland areas of the country to monitor the disease for control and prevention.

10.6 Molecular analysis of Taeniid species in Yak dogs

NK Thapa, Puspa M Sharma, Ugyen Pema, Sonam Deki, Tenzinla, Dawa Tshering

The study was conducted in 8 Dzongkhags and 19 Gewogs. The samples include environmental faecal sample, scat samples, soil samples from the yak rearing areas. The samples were processed with using F/S, sequential sieving (100µ, 40µ, 21µ) and examined for Taeniid eggs at NCAH Serbithang. DNA isolation was carried out by Alkaline lysis method and Qiagen kit (Stephanic et. al, 2004). Multiplex PCR was conducted using Trachsel multiplex PCR (Trachsel et al, 2007) and for the sequencing, Sanger sequencing (Microsynth, AG, CH.) was used.

In total of 96 isolates including negatives were analyzed at Institute of Parasitology, University of Zurich which through molecular analysis 11 were detected with T. multiceps. 40/96(41.6%) comprised of E. granulsosus, 25/96(20%) Taenia sp.

T. multiceps is detected from Taneiid eggs in all the high-risk areas except in Bumthang, Tashiyangtse and Trongsa. This indicates the prevalence of Gid in all the dzongkhags except the above three. However, we cannot declare freedom from Gid at this point. More sampling needs to be carried out. Tashigang dzongkhag historically free from clinical disease also has been detected with T. multiceps in the feces of yak dogs. May be the origin of the dog needs to be traced for monitoring.

Introduction

Gid caused by larval stage of *Taenia multiceps*, one of the several genera under Taenidae family is a concern for yak herding community in the highland. Taenia causes neuropathy in young yak calves by lodging cyst (intermediate stage) of parasite. Yaks, cattle, sheep, goat are the intermediate hosts, dogs and wild canids are the definitive host (intestinal adult worm). The disease causes significant economic losses to yak herders due to mortality of young stock. Some studies even suggested to be of zoonotic in nature.

A strategic effort is being put in through the National Highland Development Program for the goal to eliminate Gid (Coenurosis) in yaks in Bhutan by 2025. The goal is being aimed through the intensive implementation of strategic Gid prevention and control measures. A baseline study on Taeniid was conducted since 2019-20 in the high-risk yak rearing areas of the country. Wherein the scat and soil samples were collected and examined microscopically for the Taeniid eggs. The eggs from the positive samples were preserved and sent for molecular studies to Institute of Parasitology, University of Zurich.

Material & Methods

The study was conducted in 8 Dzongkhags and 19 Gewogs. The samples include environmental faecal sample, scat samples, soil samples from the yak rearing areas. The samples were

processed with using F/S, sequential sieving (100µ, 40µ, 21µ) and examined for Taeniid eggs at NCAH Serbithang.

In the study, about 96 samples were processed for Taeniid eggs and the isolates were referred to IPZ for molecular study. Approximately three grams of faeces from each sample were processed using flotation with saturated sugar solution (1:1) and sequential sieving with nylon mesh of decreasing sizes of 105 µm, 40 µm and 21 µm (Lanz-Anliker AG, Allmendstrasse 12, 4938 Rohrbach, Switzerland). This method allowed taeniid eggs to be concentrated in the last sieve, as described by Mathis et al. [1996]. PET bottles, a cost-efficient and readily available material, were used as containers and funnels in a system adapted by Deplazes. The funnel was created by cutting the PET bottles into two uneven parts, the part with the cap serving as a funnel. A round hole was cut into the centre of the caps to allow the nylon meshes' insertion to be used as sieves. The bottom part of each bottle was used as a container to hold the filtrate after passing all filters. Sediments from the 21 µm sieves were then collected and microscopically examined. Those positive for taeniid eggs were stored at −80 ◦C until further referral to the Institute of Parasitology, University of Zurich, Switzerland for molecular analysis. Sieving materials from positive samples were discarded to avoid cross-contamination. To prevent egg and DNA contamination between the samples, all sieving materials used in samples that were negative for cestode eggs were incubated in sodium hypochlorite (1% active chlorine concentration) for at least 30 min and subsequently washed before reuse.

DNA isolation was carried out by Alkaline lysis method and Qiagen kit (Stephanic et. al, 2004). Multiplex PCR was conducted using Trachsel multiplex PCR (Trachsel et al, 2007) and for the sequencing, Sanger sequencing (Microsynth, AG, CH.) was used.

Results & Discussion

In total of 96 isolates including negatives were analyzed at Institute of Parasitology, University of Zurich which through molecular analysis 11 were detected with *T. multiceps*. 40/96(41.6%) comprised of E. granulsosus, 25/96(20%) *Taenia sp*. The analysis result is presented below in the table (Table 19):

Table 19: Sequencing result of Taeniid of yak dogs

	Trachsel multiplex PCR Number positive/Total	**Sequencing results**
Taenia spp. and other large cestodes (267bp)	25/96	*T. multiceps*: 11 *Hydatigera taeniaeformis* : 12 *Taenia* spp.: 2
E. granulosus (117bp)	40/96	*E. granulosus* s.s: 6 *E. ortleppi:* 33 *E. granulosus* (unclassified): 1

Negative (24)		
Co-infections	8/96	*T. hydatigena* and *E. ortleppi:* 7 *T. hydatigena and E. granulosus* s.s*:* 1

The Taeniid isolate from Gasa was detected with 50% *T. multiceps* followed by Thimphu 33.3%, Haa 25%, Paro 20%Tashigang 4.3% and Wangdue 4%. (Figure 11).

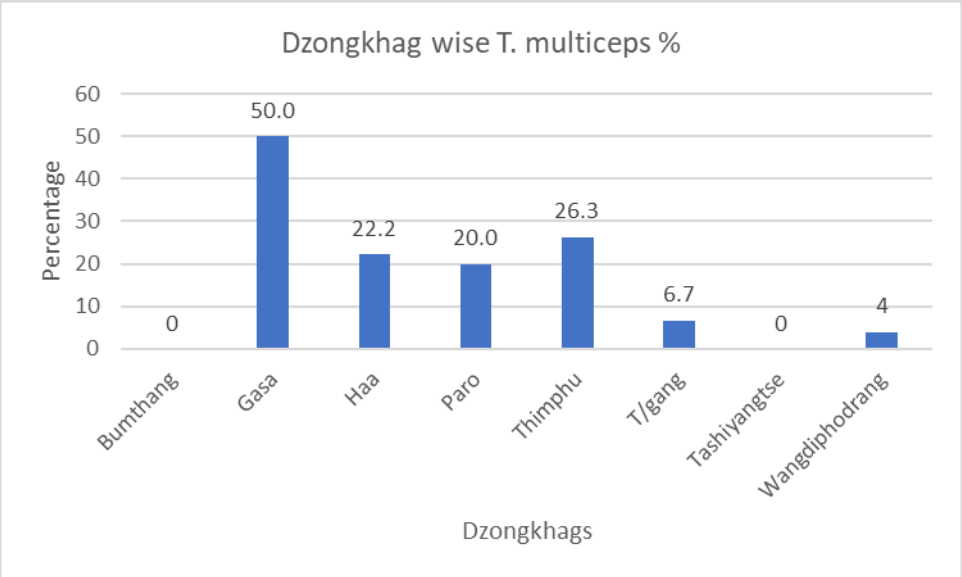


Figure 11: Dzongkhag wise proportion of *T. multiceps*

No. *T. multiceps* were detected from Bumthang, Trongsa and Tashiyangtse dzongkhags (Table. 2). Though not reported clinically, *T. multiceps* was isolated from dog feces of Merak geog. Tashigang Dzongkhag. Hence, at this stage, the Tashigang dzongkhag cannot be declared free from Gid.

Table 20: Table 2: Dzongkhag wise T. multiceps detected

Dzongkhags	***Place/Geog***	***n***	***No. of T. multiceps***	**%**
Bumthang	Chokor	6	0	0.0
Gasa	Laya	1	1	100.0
	Lunana	3	1	33.3
	Sub-total	**4**	**2**	**50.0**
Haa	Bji	6	2	33.3
	Eusu	3	0	0.0
	Sub total	***9***	***2***	***22.2***
Paro	Tshento	***15***	***3***	20.0

Thimphu	Dagala	3	0	0.0
	Dolam	1	0	0.0
	Dochupang	1	0	0.0
	Lingshi	5	1	20.0
	Naro	6	1	16.7
	Soe	3	3	100.0
	Sub-total	***19***	**5**	***26.3***
T/gang	Merak	11	1	9.1
	Sakteng	4	0	0.0
	Subtotal	*15*	*1*	*6.7*
Tashiyangtse	Bumdeling	1	0	0.0
Trongsa	Nubi	2	0	0.0
Wangdiphodrang	Sephu	3	1	33.3
	Gangtey	2	0	0.0
	Kashi	20	0	0.0
	sub-total	**25**	**1**	**4.0**
	TOTAL	**96**	**14**	14.6

Conclusion

T. multiceps is detected from Taneiid eggs in all the high-risk areas except in Bumthang, Tashiyangtse and Trongsa. This indicates the prevalence of Gid in all the dzongkhags except the above three. However, we cannot declare freedom from Gid at this point. More sampling needs to be carried out. Tashigang dzongkhag historically free from clinical disease also has been detected with *T. multiceps* in the feces of yak dogs. May be the origin of the dog needs to be traced for monitoring.

11.0 Third Inter-ministerial Committee on One health (IMCOH)meeting on Antimicrobial Resistance 18th November 2021

November 18th – 24th is observed as *World Antimicrobial Awareness week* (WAAW) globally. The goal of this week was to raise awareness on the antimicrobial resistance (AMR) to the public, health workers and the policy makers and to promote good practice in this area of concern, to limit the emergence and spread of resistant bacteria throughout the world. The theme for WAAW 2021 is: **Spread awareness, stop resistance**. As in previous years, the overall slogan for antimicrobial resistance awareness and WAAW is: **Antimicrobials: Handle with care.**

Figure 12: Participants of IMCOH at Le Meridian Hotel, Thimphu 18th November 2021

To mark the occasion and join the global move, **Third Inter-ministerial Committee on One health (IMCOH)meeting on Antimicrobial Resistance** was held on 18th November 2021 at Le Meridian Hotel, Thimphu. The meeting was organized by the Ministry of Health.

The meeting was chaired by the Hon'ble Minister for Ministry of Health. The participants included Secretary, MoH, MoAF, Former Secretary of MoAF, Director, Department of Livestock, World Health Organization (WHO) representative to Bhutan and representation from the World Organisation for Animal Health (OIE). Other participants included representation from various agencies such as Fleming Fund, National Centre for Animal Health(NCAH), from Department of Livestock (Do); AMR program, Department of medical Services and JDW National Referral Hospital from Ministry of Health (MoH); Drug Regulatory Authority (DRA); Khesar Gyalpo University of Medical Sciences of Bhutan (KGUMB); One Health Secretariat; Nature Conservation Division (NCD) from Department of Forests & Park Services (DoFPs) and also the Fleming Fund fellows.

Representation from Animal Health highlighted the importance of one health approach in combating AMR as antimicrobials are not only used in humans but in veterinary and food production, and the development and spread of AMR can easily be from human to animal and environment and vice versa. He then highlighted the activities that have been carried out in animal health settings which were aligned with the National Action Plan on AMR. He informed that technical working groups have been established to provide technical and leadership, laboratories were renovated in the surveillance sites as part of the infrastructure development, development of guidelines and SoPs and surveillance in poultry is being carried out both in farm

and meat shops as part of the surveillance and systems development. He also highlighted the activities carried out in the education and promotion of awareness and the regulatory activities.

12.0 Sheep shed construction at animal health laboratories for sharing of resources

Summary

As per the discussion held on 14th to 16th of January 2020 at Gelegphu among the decision-making authorities from AH; the Chief Program Officer (DoL), Program Director (NCAH) and Regional Directors (RLDCs Kanglung, Tsimasham, Zhemgang and Wangdi) and the human health facilitated by Felming Fund PMU, the sharing of sheep blood by the Animal Health laboratories with the human hospital surveillance labs were agreed. Further, the protocol was also agreed and endorsed technically by the authorities from DoL and RLDCs, as per the requirement of the HH microbiology laboratories.

As per the agreement, the sheep blood required for AMR surveillance at Regional Hospitals are to be shared from the nearby veterinary laboratories. Accordingly, it was planned that NCAH, RLDC Tshimasham, RLDC Zhemgang and RLDC Kanglung will support the human hospitals/laboratories in providing sheep blood for the surveillance. However, only some of these laboratories' rear sheep and some do not due to lack of infrastructure. Further, those labs rearing sheep also did not have proper shed to house the sheep. Hence, it was agreed to construct new sheds and also add the sheep numbers and in order to arrange continuous supply of blood for AMR surveillance. Hence, as per the recommendation of the third NATC and consequent approval from the Mott Mc Donald, the sheds were constructed at 4 animal health laboratories (NCAH, RLDC Tashimasham, RLDC, Zhemgang, RLDC Kanglung). The construction was based on the number of sheep to be housed. NCAH had target for rearing about 8-10 nos. of sheep whereas RLDCs have target of rearing about 6 to 8 nos. for sheep blood in carrying out AMR surveillance in animal health and human health.

Introduction

It was realized that large amount of budget remained unutilized even after about a year of implementation of DTiPs. The same was discussed during the Fourth NATC meeting held on 28/10/2020 at Tiger Nest Camp, Paro. During the discussion, it was found out that majority of the activities which had no progress were mainly the activities requiring external Technical Assistance (TA), capacity development of the human resource (ex-country and in-country), meetings and workshops involving a large number of participants. These activities were impacted significantly following the COVID-19 pandemics and the ensuing notifications on travel and gathering restrictions issued by the Ministry of Health (MoH). Hence, it was recommended to review the activities and reprioritize the activities to meet the objectives of the project. For the animal health, it was also proposed to utilize these left-over budget for other

activities like the sheep shed construction at strategic Animal Health laboratories v.i.z. NCAH, Serbithang, RLDC Tshimasham, Zhemgang and Kanglung. Hence, as per the approval from the Department of medical services, Ministry of Health vide their letter No. 5(9-15) DMS/HCDD/BSDP/FFPMU/2019-2021/9929 dated 07/05/2021 and upon subsequent approval from the Mott Mac Donald, the construction of sheep sheds was carried out.

Rationale

As per the discussion held on 14th to 16th of January 2020 at Gelegphu among the decision-making authorities from AH; the Chief Program Officer (DoL), Program Director (NCAH) and Regional Directors (RLDCs Kanglung, Tsimasham, Zhemgang and Wangdi) and the human health facilitated by Felming Fund PMU, the sharing of sheep blood by the Animal Health laboratories with the human hospital surveillance labs were agreed. Further, the protocol was also agreed and endorsed technically by the authorities from DoL and RLDCs, as per the requirement of the HH microbiology laboratories.

As per the agreement, the sheep blood required for AMR surveillance at Regional Hospitals are to be shared from the nearby veterinary laboratories. Accordingly, it was planned that NCAH, RLDC Tshimasham, RLDC Zhemgang and RLDC Kanglung will support the human hospitals/laboratories in providing sheep blood for the surveillance. However, only some of these laboratories' rear sheep and some do not due to lack of infrastructure. Further, those labs rearing sheep also did not have proper shed to house the sheep. Hence, it was agreed to construct new sheds and also add the sheep numbers and in order to arrange continuous supply of blood for AMR surveillance. Hence, as per the recommendation of the third NATC and consequent approval from the Mott Mc Donald, the sheds were constructed in the animal health laboratories.

Sheep Sheds

The sheds were constructed following the government norms of award through open tender. The construction process required some time since the Department of Livestock did not have engineer hence, the engineering services had to be requested from other agencies/project which required some time. Besides, the tender rates also escalated due to pandemic in some areas of construction sites. Hence, the sheds were constructed at four sites at the total cost of about **Nu. 4,380,802.50** (Fourty three lakhs eighty thousand eight hundred two).

A. Sheep shed at NCAH Serbithang

Besides its own use, the sheep blood required for AMR surveillance at JDWNRH and RCDC, MOH is also needs to be shared from NCAH Serbithang. Therefore, the current stock of 5 sheep would not be enough and also considering the animal ethics, more sheep are to be added. Hence, the sheep shed at NCAH was targeted for housing about 8 to 10 nos. of sheep.

Through the open bidding the shed was constructed at the little over the estimated cost of Nu. 1708745.00 (Seventeen Lakhs eight thousand seven hundred forty-five) over the estimated cost of Nu. 1596572.41 (Fifteen Lakhs Ninety-Six Thousand five hundred seventy-two).

The shed has two rooms one for the isolation of the sick animals and one for housing the healthy animals and has the flooring with wooden planks with adequate spaces for dropping of feces. The shed is elevated from the ground so that the feces and urine of the animals drop down from the floor easily which are laid into the drain. The shed has facility of feeding trough, trough for fodder/grass and the waterer inside the shed itself with ad lib water supply and also electricity.

Figure 13: Sheep shed at NCAH, Serbithang

Outside the shed there is a feed store, with a changing room for the caretaker and entrance into the shed has to pass through a food dip at (Figure 13).

B. Sheep shed at RLDC Kanglung

The sheep shed at Kanglung is aimed to cater the need of sheep blood for both RLDC Kanglung and the Referral hospital Monger for AMR surveillance. The shed is designed to house about 4-8 nos. of sheep (Figure 14). The contract was awarded through open tender and the construction was carried out at the little above the estimated cost of Nu. 1,136,372.50 (Eleven lakhs thirty-six thousand three hundred seventy-two).

Figure 14: Sheep shed at RLDC Kanglung

C. Sheep Shed at RLDC Zhemgang

The establishment of sheep shed in RLDC premise was carried out with the main objectives to rear sheep and enable collection of sheep blood required for carrying out AMR surveillance in the country. The sheep blood will be shared with regional referral hospital Gelegphu, under Ministry of Health (MoH) in the region and the fund support for the construction of sheep shed was through Fleming Fund Project in MoH.

The construction of sheep shed in RLDC, Zhemgang was carried out as per Department of Livestock (DoL) approval letter no. 9(1) NCAH/FF/2020-21/383 dated 2nd March 2021 and MoH approval letter no. 5(9-15) DMS-HCDD/BSDP/FFMU/2019-2021/9929 dated 7th May 2021.

Figure 15: Sheep shed at RLDC Zhemgang

The construction of sheep shed in RLDC, Zhemgang was completed within the time frame specified in the standard bidding document. Although the initial contract works was awarded at Nu. 5,45,208.40/- as compared to the technical estimated amount of Nu. 910,561.00 the additional works had to be executed unavoidable ground situations. The shed is built with facilities for feeding trough and water.

D. Sheep shed at RLDC Tshimasham

The sheep shed at Tshimasham is aimed to cater the need of sheep blood for both RLDC Tshimasham and the Referral hospital Phuntsholing for AMR surveillance. The shed is designed to house about 4-8 nos. of sheep. The contract was awarded through open tender and the construction was carried out at the little above the estimated cost of Nu. 625125 (Six lakhs twenty- five thousand one hundred and twenty-five).

The shed has basic structure like feeder and waterer and electricity supply.

Figure 16: Sheep shed at RLDC Tsimasham

Table 21: Total Cost of sheep shed construction

Sl.	Sites	Estimates (Nu.)	Tender Amount (Nu.)	Additional requested (Nu.)	Total (tender + additional) (Nu.)
1	National Centre for Animal Health (NCAH), Thimphu	1,589,164.46	1,596,572.41	112,172.00	1,708,744.00
2	RLDC, Tshimasham, Chukha	600,000.00	600000	25125	625125
3	RLDC, Kanglung, Trashigang	998,627.03	1,033,795.50	102,577.00	1,136,372.50
4	RLDC, Zhemgang	743,329.00	545,208.00	365,353.00	910,561.00
	TOTAL	**3,931,120.49**	**3,775,575.91**	**580,102.00**	**4,380,802.50**

13.0 Visitors at Laboratory Services Unit

During the year, visitors from various institute visited the laboratory as below:

Table 22: Visitors list

Sl. No	Date	Purposes	Place/Country	Total
1	5/8/2021	Equipment calibration	Bio-Medical Engineering Division, MoH	03
2	27/8/21	Internship	Serbithang, Thimphu	02
3	17/11/21	Familiarization visit	Dasho Secretary, MoAF	02
4	3/5/2022	Demonstration practical on Molecular & Serology Sections for CNR, Students	CNR, Lobesa	35 students with module tutor