

# NATIONAL CENTRE for ANIMAL HEALTH

## Annual Progress Report 2017-2018



**National Centre for Animal Health**

Department of Livestock

Ministry of Agriculture and Forests

Serbithang, Thimphu | Tel No: 00975-2-351083/351093

Fax No: 00975-2-351095 | Toll Free No: 124 | Post Box: 155

Email: [ncah2013@gmail.com](mailto:ncah2013@gmail.com) | [www.ncah.gov.bt](http://www.ncah.gov.bt)

**Compiled & edited by Dr Tenzin**



July 31, 2018

## FOREWORD

The National Centre for Animal Health, Department of Livestock, Ministry of Agriculture and Forests is pleased to release the Centre's Annual Progress Report for the Financial Year 2017-2018. The report highlights the progress, achievements, and experiences of the centre while undertaking the mandate of animal health services in the country. The report also cover the key achievements of the centre during 11<sup>th</sup> FYP (July 2013-June 2018). As the national competent centre for animal health, the centre has a very crucial role to play in supporting the various commodity programs under the livestock sector with the ultimate objective of enhancing livestock production in the country.

I, on behalf of the NCAH management, would like to thank all the Heads of Units and the staff at NCAH for their invaluable contribution in achieving the centre's mandates and more importantly, for documenting all activities undertaken by each unit. I acknowledge their contribution and support in producing this annual report.

I would also like to express my sincere appreciation to all the Regional Directors of RLDCs, the Program Managers, District Livestock Officers, Veterinarians, Farm Managers, and Livestock Extension Officers for their continued support and successful implementation of their animal health programs in their respective Geogs farms/Dzongkhags/Regions. I extend my sincere appreciations to the relevant international partners for their technical and financial support provided in strengthening implementation of animal health activities in the country. I also extend appreciations to Department of Public Health and BAFRA for their continued support and cooperation in prevention and control of diseases.

I also thank the Director General and the Chiefs of various Divisions under DoL for their continuous guidance and support to NCAH.

Lastly, I extend my appreciation to Dr Tenzin, Disease Prevention and Control Unit, NCAH for coordinating publication of this document as an annual event.

Tashi Delek

Dr. RB Gurung  
**Offtg. Program Director**



## TABLE OF CONTENTS

<b>1. Background .....</b>	<b>6</b>
1.1. Main Mandates .....	6
1.2. Functions .....	6
1.3. Four major functional units of NCAH.....	6
<b>2. Key Achievements of NCAH during 11<sup>th</sup>FYP (July 2013-June 2017).....</b>	<b>7</b>
2.1 Establishment and strengthening of Laboratory diagnostic capacity .....	7
2.2 Strengthened Disease Prevention and Control Program in the country .....	9
2.3 Strengthened vaccine procurement and distribution system for disease prevention & control program .....	13
2.4 Strengthened Veterinary Drugs, medicine and equipment procurement and distribution system.....	14
2.5 National Dog population management and rabies control project .....	15
2.6 Conducted Animal Health Survey & Researches .....	15
2.7 Capacity building.....	18
2.8 Establishment of national & international collaboration .....	19
2.9 Financial achievement during 11 <sup>th</sup> FYP.....	20
<b>Key achievement of NCAH during FY 2017-2018 .....</b>	<b>21</b>
<b>3. Disease Prevention and Control Unit.....</b>	<b>21</b>
3.1 The main roles of the DPCU .....	21
3.2 Human resources of DPCU .....	21
<b>3.3. Key achievements of DPCU for the FY 2017-2018 .....</b>	<b>21</b>
3.3.1 Develop disease control plan.....	21
3.3.2 Strengthening of Database Management and Disease Reporting System.....	21
3.3.3 Disease outbreak investigation and containment.....	22
3.3.4 Coordination of World Rabies Day events .....	30
3.3.5 Animal Health information system.....	30
3.3.6 Coordination of One Health activities.....	30
3.3.7 Policy and Research activities .....	31
3.3.8 Capacity building.....	31
3.3.9 Other works.....	31
<b>4. Laboratory Services Unit .....</b>	<b>32</b>
4.1 Mandates of the LSU .....	32
4.2 Human resources in LSU.....	33
4.3 Diagnostic capacities in LSU.....	33
4.3.1 Parasitology Section.....	33
4.3.2 Bacteriology Section .....	34
4.3.3 Haematology Section .....	35
4.3.4 Bio-chemistry & Toxicology Section .....	35
4.3.5 Molecular biology, Serology & Virology Section .....	35
4.3.6 Post-mortem & Pathology Section.....	36
4.3.7 Bio-safety and Bio-security section.....	36
<b>4.4. Key achievements of LSU during FY 2017-2018 .....</b>	<b>37</b>
4.4.1 Achievements of individual sections under LSU .....	37

4.4.1.1 Histopathology, Postmortem and Parasitology sections .....	37
4.4.1.2 Bacteriology Section .....	38
4.4.1.3 Bio-Chemistry/ Toxicology and Haematology sections .....	39
4.4.1.4 Molecular Biology, Serology and Virology Section .....	40
4.4.1.5 Bio-safety and Bio-security section .....	42
4.4.1.6 Clinical services provided .....	42
4.4.2 Introduction of new test .....	43
4.4.3 Samples referred to international laboratories .....	43
4.5. Others activities .....	44
4.5.1 Laboratory assessment, quality assurance audit, proficiency testing and backstopping .....	44
4.5.2 Laboratory Information Management System (LIMS) .....	48
4.5.3 Report on National Stakeholder meeting on Developing Stepwise Control of Brucellosis in Bhutan .....	48
4.5.4 Fleming Fund Project for strengthening AMR surveillance .....	55
4.5.5 Laboratory investigation on mortality on Tiger at WCD Taba .....	57
4.5.6 Laboratory investigation on death of takin .....	57
4.5.7 Investigation of mortality of a common carp in Ludrong Garden .....	59
<b>5. Drugs Vaccine and Equipment Unit .....</b>	<b>61</b>
5.1 Main mandates of DVEU .....	61
5.2 Human Resources in DVEU .....	61
<b>5.3. Key achievements of DVEU for FY 2017-2018 .....</b>	<b>61</b>
5.3.1 Regularization of stock of medicines, equipment and non-drug items in LCS, Phuntsholing .....	61
5.3.2 Write-off and disposal of expired and damaged drugs in LCS, Phuntsholing ..	62
5.3.4 Training and meeting .....	62
5.3.5 Procurement of medicines, vaccines and equipment during FY 2017-18 .....	62
5.3.6 Verification and inspection of veterinary drugs and equipment .....	62
5.3.7 Distribution of Veterinary Medicines, Equipment and Non-drug Items .....	63
5.4. Key Issues and Way Forward .....	66
<b>6. Biological Production Unit .....</b>	<b>67</b>
6.1 Main mandates of BPU .....	67
6.2 Human resource of BPU .....	67
<b>6.3. Key achievements of BPU during FY 2017-18 .....</b>	<b>67</b>
6.3.1 Vaccines production and procurement .....	67
6.3.2 Vaccine distribution .....	68
6.3.3 Other activities .....	71
<b>7. National Dog population Management and Rabies Control Project .....</b>	<b>73</b>
7.1. Key achievements of the NDPM & RCP during FY 2017-2018 .....	73
<b>8. Animal Disease Surveillance and Researches .....</b>	<b>76</b>
8.1 A Review on Antimicrobial Resistance (AMR) in Livestock in Bhutan .....	76
8.2 Identification of Echinococcus spp. in definitive and intermediate hosts in Bhutan .....	77
8.3 A national serosurvey to determine the prevalence of paratuberculosis in cattle in Bhutan following detection of clinical cases .....	78

8.4 Investigation and monitoring of Brucellosis associated abortion in a dairy cattle farm in Bhutan.....	79
8.5 Determinants of health seeking behavior of animal bite victims in rabies endemic south Bhutan: A community-based contact-tracing survey .....	80
8.6 Comparison of antibody responses after vaccination with two inactivated rabies vaccines in dogs in Thimphu, Bhutan .....	81
8.7 Application of geostatistical visualisation and spatial statistics to understand the dispersion of rabies re-emergence in the highland, eastern Bhutan.....	84
8.8 Evaluating rapid immunochromatographic test for diagnosis of rabies in animals in Bhutan.....	86
8.9 Self-assessment of Bhutans' Rabies Prevention and Control programme using SARE tool .....	87
8.10 A Qualitative Risk Assessment for Re-introduction of Rabies into Rabies-free Areas of Bhutan .....	90
8.11 Community-based survey of ecological and epidemiological risks associated with free roaming and feral dog populations in buffer zone of a protected area in Bhutan.....	91
8.12 A Case-Control Study of an outbreak of Newcastle Disease in poultry at the farm level in Pemagatshel district, eastern Bhutan .....	91
8.13 Serological evidence of Rickettsia, Orientia and Coxiella in domestic animals from Bhutan: preliminary findings.....	92
8.14 A large-scale study of a poultry trading network in Bangladesh: implications for control and surveillance of avian influenza Viruses .....	94
8.15 Spatio-temporal epidemiology of Japanese encephalitis in Nepal, 2007-2015 ..	95
8.16 Study on Taenia multiceps infection in yak dogs in Merak-Sakteng, Trashigang .....	95
8.17 First Report of detection and phylogenetic analysis of important zoonotic pathogens from rodents in Gedu, Bhutan.....	96
8.18 Bovine Papillomatosis (wart) treatment in Paro and Trongsa Dzongkhag .....	96
<b>9. Human Resource &amp; Capacity Development .....</b>	<b>99</b>
9.1 Workshop for revision of SOP and development of new Standard Operating Procedure (SOPs) for veterinary laboratory diagnosis towards maintaining GLP.....	99
9.2 Workshop for Development of National Rabies Action Plan .....	99
9.3 International Health Regulation (IHR) Workshop .....	99
9.4 National Stakeholder Meeting on Developing Stepwise Control of Brucellosis in Bhutan .....	100
9.5 Developed G2C datababse for management of veterinary drugs and conducted trainings for focal livestock officials from the Dzongkhags and livestock frams/agencies.....	101
<b>10. Visitors to NCAH, Serbithang during FY 2017-18.....</b>	<b>101</b>
<b>Annexure .....</b>	<b>102</b>
Annexure 1. List of Staff at NCAH as of June 30 2017.....	102
Annexure 2: Staff transferred to and from NCAH, Serbithang.....	104
Annexure 3: Promotion acquired during the FY 2017-2018 .....	104
Annexure 4: Details of Infrastructure .....	104
Annexure 5: Details of vehicle.....	105
Annexure 6: Vehicle Expenditure for maintenance and spare parts .....	105
Annexure 7: Construction & Renovation works carried out.....	105
Annexure 8. Budget expenditure statement as of 30 June 2017 (FY 2017-18) .....	106

## **1. Background**

The National Centre for Animal Health (NCAH) is located about 12 km away from Thimphu, the capital city of Bhutan. Initially the centre started as a laboratory in 1978 at Chubachu, Thimphu. Later under the aegis of UNDP/FAO project, it was named as Royal Veterinary Diagnostic Laboratory (RVDL) and shifted to Serbithang in 1981. It was subsequently strengthened under EU assistance between 1991 and 1999 and was renamed as Royal Veterinary Epidemiology Centre (RVEC). The centre is responsible for animal disease diagnosis and disease prevention and control program, and providing technical backstopping to the Dzongkhags. In 2005, RVEC was renamed as National Centre for Animal Health (NCAH), and is one of the central programs under the Department of Livestock, Ministry of Agriculture & Forests. The centre has a campus area of 8.8259 acres and has 50 staff.

### **1.1. Main Mandates**

1. To function as national referral laboratory and competent centre for animal health
2. To ensure availability of veterinary drugs, vaccines and equipment
3. To function as an institute for capacity development in animal health

### **1.2. Functions**

1. Develop, implement and evaluate disease prevention and emergency response plans for livestock diseases and zoonoses
2. Support development of policies, strategies and plans for animal health
3. Coordinate, monitor and evaluate disease prevention and control programs
4. Prioritize and conduct research on animal health
5. Liaise with national and international agencies for technical collaboration
6. Plan, coordinate and conduct animal health research in liaison with relevant agencies
7. Maintain and disseminate animal health and epidemiological information, reporting on regular basis
8. Provide referral services on laboratory diagnostic services
9. Support development of capacity in animal health programs
10. Implement, monitor and evaluate management of veterinary drugs, vaccines and equipment at national level
11. Conduct disease surveillance and control activities at national level

### **1.3. Four major functional units of NCAH**

The centre coordinates all national level animal health programs in collaboration with the four Regional Livestock Development Centres and the Dzongkhags. The main functional units are (Figure 1):

- Disease Prevention and Control Unit (DPCU)
- Laboratory Services Unit (LSU)
- Drugs Vaccines and Equipment Unit (DVEU)
- Biological Production Unit (BPU)

## 2. Key Achievements of NCAH during 11<sup>th</sup>FYP (July 2013-June 2017)

The National Centre for Animal Health under the guidance of Animal Health Division, Department of Livestock and through support of the Regional Livestock Development Centres, Dzongkhag Livestock Sectors and other commodity programs, achieved the following milestones during 11<sup>th</sup> FYP:

### 2.1 Establishment and strengthening of Laboratory diagnostic capacity

#### Laboratory infrastructure

- Biosafety laboratory level 2 plus (BSL2+) was established at the National Centre for Animal Health, Serbithang

#### Laboratory quality assurance

- Laboratory assessment, quality assurance audit and Technical backstopping conducted
- Technical Backstopping Missions conducted by Australian Animal Health Laboratory – CSIRO supported through FAO.
- Biosafety equipment certification conducted by ESCO supported through FAO

#### Proficiency testing

- Asia Pacific Inter-laboratory proficiency testing scheme for Brucellosis conducted
- Asia Pacific Inter-laboratory proficiency testing scheme for Avian influenza, Classical Swine Fever and Newcastle disease conducted

#### Sample collection and tests

- A total of 41,366 samples were referred or collected by the centre and performed 74,824 tests to confirm the disease.

Year wise sample received and test performed		
<i><b>Year</b></i>	<i><b>Sample collected</b></i>	<i><b>Test performed</b></i>
2013-14	9630	15400
2014-15	9573	15507
2015-16	8851	16133
2016-17	7122	14531
2017-18	6190	13253
<b>Total</b>	<b>41366</b>	<b>74824</b>



- A total of 6358 samples were referred to reference laboratories for disease diagnosis and confirmation

<b>Year wise sample referred to reference laboratory</b>		
<b>Year</b>	<b>Sample referred</b>	<b>Laboratories</b>
2013-14	2388	St Jude Hospital, USA Australian Animal Health Laboratory, Geelong Pirbright Institute, UK
2014-15	63	National Institute of High Security Animal Diseases, Bhopal, India Pirbright Institute, UK
2015-16	173	National Institute of Animal Health, Thailand Pirbright Institute, UK
2016-17	2005	National Institute of Animal Health, Thailand Pirbright Institute, UK
2017-18	1729	Australian Animal Health Laboratory, Geelong National Institute of Animal Health, Thailand Pirbright Institute, UK
<b>Total</b>	<b>6358</b>	National Institute of Animal Health, Thailand Pirbright Institute, UK

### Introduction of new test

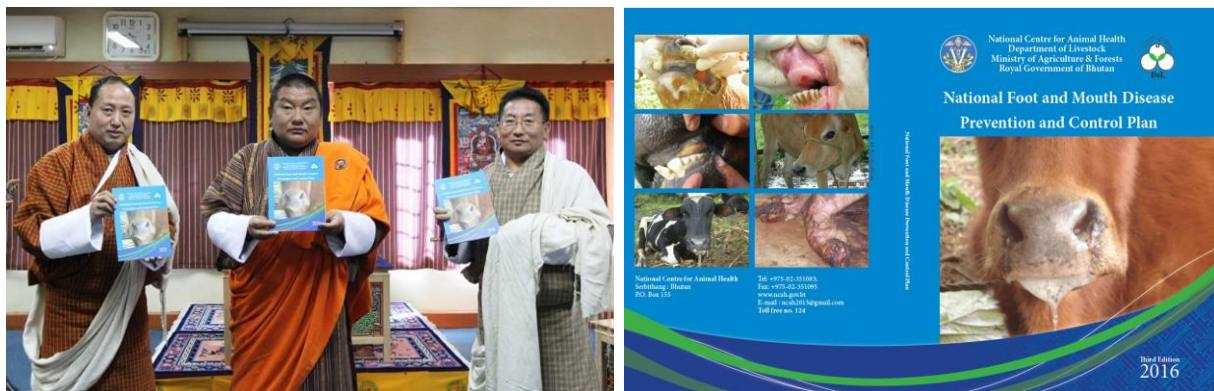
- The following new tests were introduced at NCAH and some of the tests in regional and districts laboratories in the country

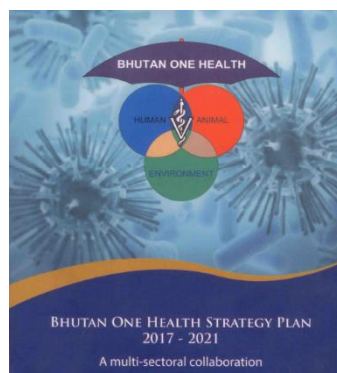
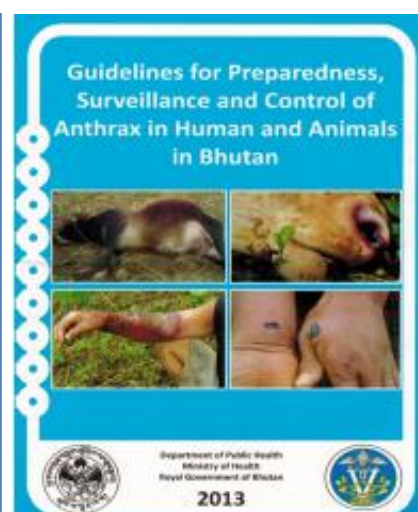
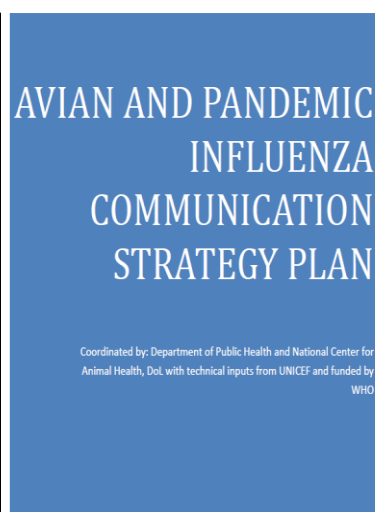
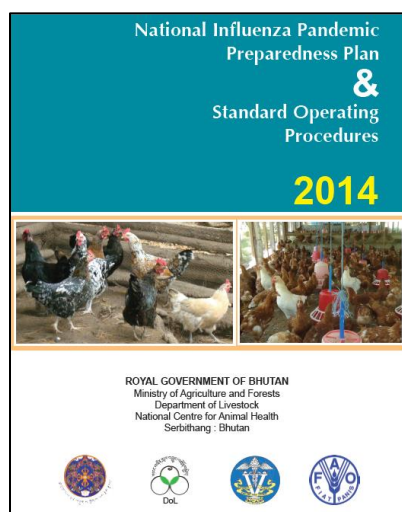
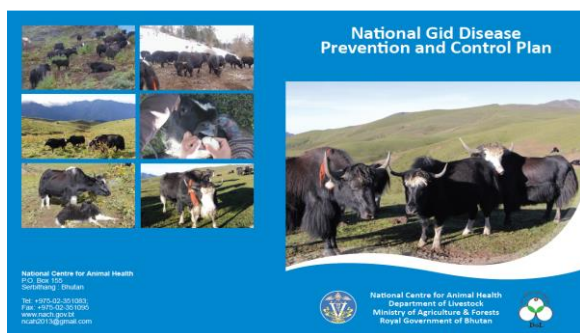
<b>Rapid Tests</b>	<b>Molecular tests</b>	<b>Serology</b>	<b>Toxicology</b>
<ul style="list-style-type: none"> <li>• Canine Distemper</li> <li>• Canine Leptospirosis</li> <li>• Ehrlichia canis</li> <li>• Heartworm</li> <li>• Canine Anaplasma</li> <li>• Feline Pan Leukopaenia</li> <li>• Rabies</li> <li>• FMD</li> <li>• HPAI</li> <li>• EI</li> <li>• E coli o157</li> <li>• PPR</li> <li>• IBD</li> <li>• Lepto</li> </ul>	<ul style="list-style-type: none"> <li>• Real time RT-PCR for FMD</li> <li>• Real time RT-PCR for Pigeon Paramyxovirus</li> <li>• Real time RT-PCR for CSF</li> <li>• Real time RT-PCR for ND</li> <li>• Real time RT-PCR for AI (type A, H5, N1, N8)Conventional PCR for Brucellosis</li> <li>• Conventional PCR for Footrot</li> </ul>	<ul style="list-style-type: none"> <li>• Rapid test for Brucella antibody</li> <li>• Rapid test for combined AI and ND</li> <li>• Antibody ELISA for PPR</li> <li>• Antigen ELISA for PPRAntibody ELISA for CSF</li> <li>• Antigen ELISA for CSF</li> <li>• Antigen ELISA for ALC</li> <li>• Antibody ELISA for JD</li> <li>• HA/HI test for AI</li> <li>• Antibody ELISA for IBD</li> <li>• Antibody ELISA for Lepto</li> </ul>	<ul style="list-style-type: none"> <li>• Rapid test for aflatoxin</li> <li>• ELISA for Fuminosin</li> <li>• ELISA for Ochratoxin</li> </ul>



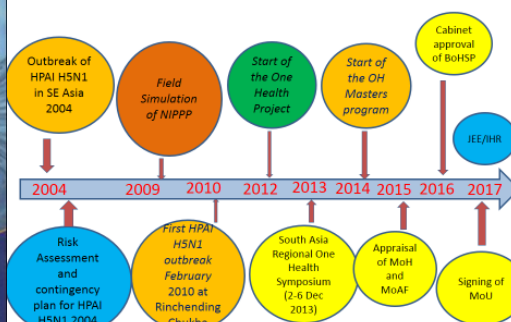
## 2.2 Strengthened Disease Prevention and Control Program in the country

- Developed and operationalized various disease prevention and control plan and animal health standards/guidelines:
- **Disease control plan**
  - National FMD prevention and control plan 2016
  - National Rabies prevention and control plan 2017
  - National Gid disease prevention and control plan 2016
  - National Avian influenza pandemic preparedness plan 2014
  - Anthrax guideline 2013
  - Avian influenza risk communication strategy plan





Timeline of Key Events for Bhutan One Health Initiatives



## Bhutan One Health Strategy Plan

### Vision

"The health and wellbeing of humans and animals are improved through prevention and control of emerging and re-emerging infectious diseases at the human-animal-ecosystem interface through OH approach in Bhutan".

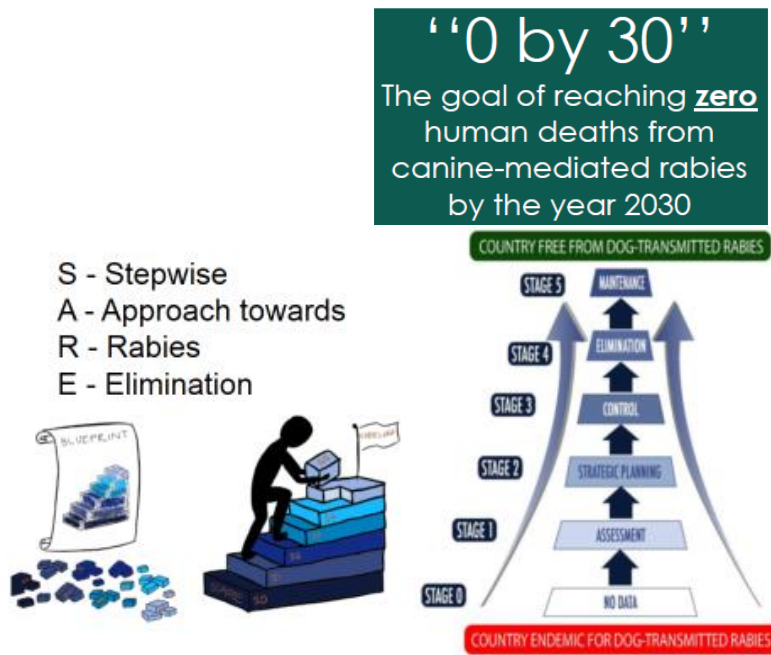


## Guidelines/document/Regulations

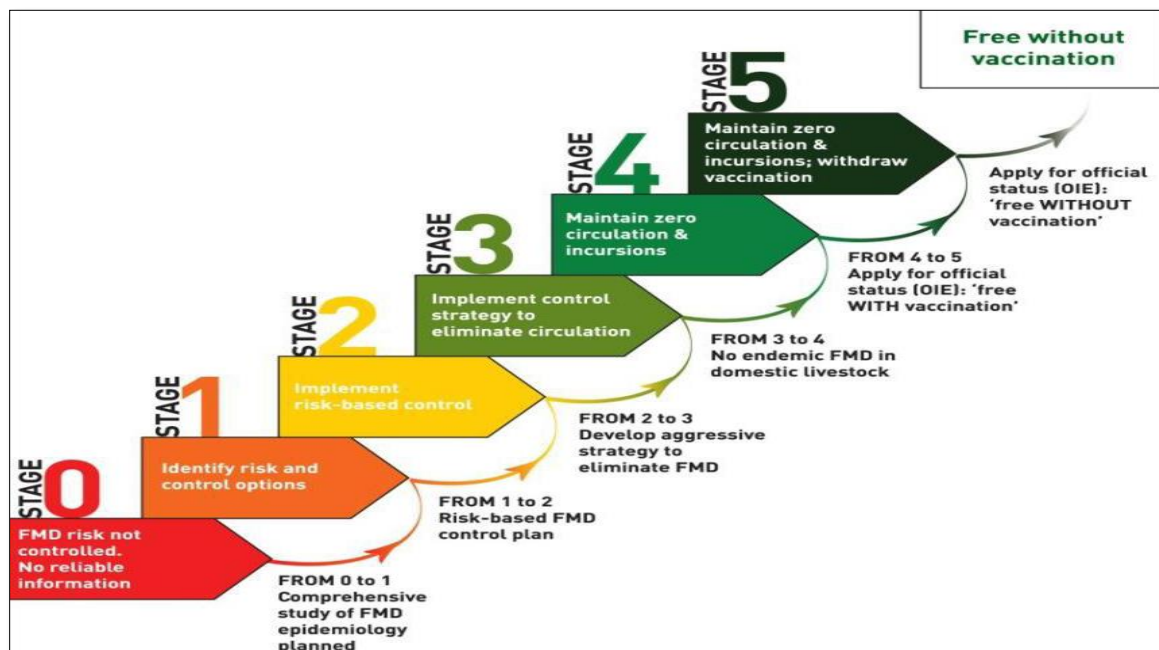
- Bhutan One Health Strategy Plan 2017
- Antibiotic guidelines for Livestock in Bhutan 2016
- AMR National Action Plan 2018-2022 (developed in collaboration with Ministry of Health)
- Livestock Rules and Regulation of Bhutan 2017
- Bhutan Animal Health Code for Import of Animals 2018

- **Developed road map/blue print for important disease prevention and control program**

- National action plan to eliminate dog mediated human rabies by 2023 in line with global target of zero human rabies death by 2030



- **FMD Progressive Control Pathway (PCP) to achieve stage 3 by 2020**



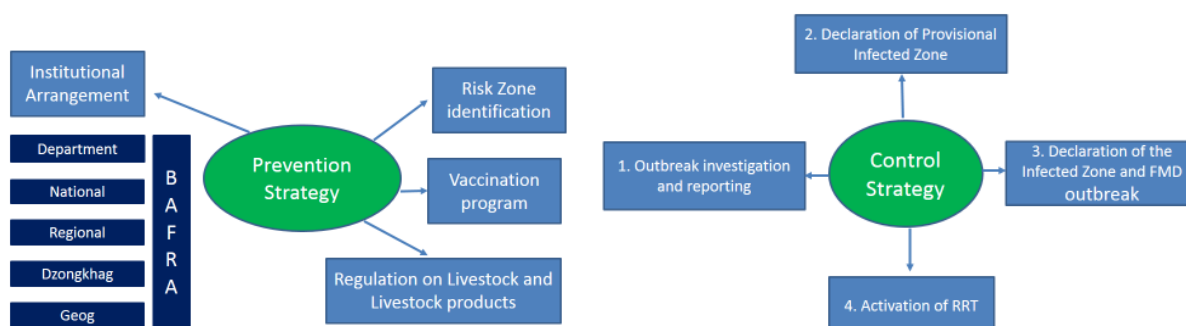


## FMD Roadmap for South Asia

Country	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
Afghanistan	1	1	1	1	1	2*	2	2	2	2	2	3	3	3	4
Bangladesh	1	1	1	1	1	1*									
Bhutan	1	1	1	1	1	1	2	2	2	3	3	3	3	3	3
India	3	3	3	3	OIE endorsed NCP						Zoning 'Free with Vx'				
Nepal	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4
Pakistan	1	1	1	2	2	2	2	2	2	2	2	3	3	4	4
Sri Lanka	1	1	1	1	1	1									

## FMD Roadmap for Bhutan

Year	2012	2013	2014	2015	2016	2017	2018	2019	2020
Stage of PCP	1	1	1	1	2	2	2	2	3



## • Coordination of major disease outbreak investigations and containment:

- Highly Pathogenic Avian Influenza H5N1 outbreaks in Chukha, Lhamoizingkha, Gelephu, Samdrup Jongkhar, Samtse, Mongar, Thimphu
- Rabies outbreak containment in Trashigang, Chukha, Samdrup Jongkhar, Gelephu, Lhamoizingkha and Samtse; (from 2013 to June 2018, only two human deaths due to rabies were reported in the country)
- FMD outbreak containment in the country
- IBD outbreak in Sarpang, Tsirang, Punakha
- Avian Leucosis Complex outbreak containment in Khangkhu Poultry farm
- Mareks disease outbreak containment in Khangkhu Poultry farm
- CSF outbreak containment in the country
- PPR outbreak containment in the country



- **Developed audio visual program and extension materials on disease prevention and control program**
  - Bird flu
  - Rabies
  - FMD
  - Dog population management and dog bite prevention
- **Developed and strengthened animal disease information system**
  - Developed and operationalized online Veterinary Information System (VIS) and operationalized TADInfo database system

## 2.3 Strengthened vaccine procurement and distribution system for disease prevention & control program

- For disease prevention and control program in the country, the centre have distributed 190,885,75 doses of various vaccines to the dzongkhags and central farms in the country

FY	Anthrax	SF	E.Coli	FMD Oil	HSBQ	Gumboro	Fowl Pox	NDB <sub>1</sub>	R <sub>2</sub> B	Marek's	Rabies	DHPPI + L	PPR
2013-14	1500	5900	149	191400	125200	1320500	358000	767600	367800	435000	34350	788	0
2014-15	0	5480	455	206850	81780	1351200	351000	666400	407500	460000	24970	1314	0
2015-16	800	8540	157	197450	73500	1748600	573000	780000	572500	570000	63600	861	12200
2016-17	200	7260	0	232200	102990	1847400	524000	758800	581900	543000	44090	936	4900
2017-18	200	8770	0	171600	40260	1092200	224500	543800	359000	218000	16140	85	0
<b>Total doses</b>	<b>2700</b>	<b>35950</b>	<b>761</b>	<b>999500</b>	<b>423730</b>	<b>7359900</b>	<b>2030500</b>	<b>3516600</b>	<b>2288700</b>	<b>2226000</b>	<b>183150</b>	<b>3984</b>	<b>17100</b>

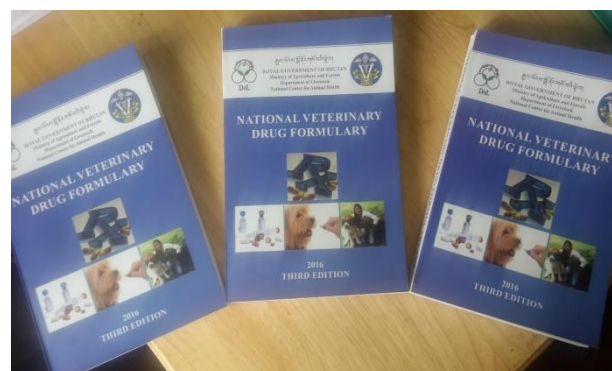
## Reduction of incidence of pandemic and epidemic diseases

- The incidences of pandemic and epidemic disease outbreaks have been reduced during the 11<sup>th</sup> FYP

Indicators	Baselines	Base Year	11th Plan Targets	11th plan Progress Achieved (by Fiscal Year)					Percent Achieved Against 11th Plan Target	Reasons for Any Deviations from Targets (Over/under Achievements)**
				FY 2013-14	FY 2014-15	FY 2015-16	FY 2016-17	FY 2017-18		
Incidences of pandemic and epidemics diseases (Nos.)	26	2012	15	24	21	18	16	14	110	-

## 2.4 Strengthened Veterinary Drugs, medicine and equipment procurement and distribution system

- Strengthened and enhanced veterinary drugs, medicine and equipment procurement and distribution system in the country as per the management cycle
- Developed a Veterinary Drug Formulary 2016 to guide the usage of veterinary drugs and medicine in the country
- Developed G2C database for EVDP management in the country in collaboration with the financial support of G2C Office, Cabinet Secretariat and conducted trainings for focal livestock officials from the Dzongkhags and livestock farms/agencies
- Antimicrobial agents used in veterinary practice in Bhutan categorized as per the **OIE List of Veterinary Important Antimicrobial Agents for Food Producing Animals**



Sl. No.	Class of Antimicrobials	VCIA	VHIA	VIA
A	Aminoglycosides	4		
B	Amphenicols	1		
C	Cephalosporins	5	3	
D	Macrolides	2		

E	Penicillins	9		
F	Quinolones	4		
G	Sulfonamides/Sulfonamides+Diaminopyrimidines/Diaminopyrimidines	10		
H	Tetracyclines	4		
	TOTAL	39	3	

\*\*VCIA=Veterinary **Critically Important Antimicrobial Agents**, VHIA=Veterinary **Highly Important Antimicrobial Agents**, VIA=Veterinary **Important Antimicrobial Agents**

## 2.5 National Dog population management and rabies control project

- Coordinated CNVR program and processed about 85,000 dogs and cats between 2009 and 2018
- Received an award for “Institutionalizing Compassion” by HSI during the 10<sup>th</sup> Asia for Animal conference in Kathmandu, Nepal in 2017 for taking exemplary leadership in animal welfare activities
- Received the “Outstanding Animal Protection Award” by HSI during the Animal Care Expo 2015 in New Orleans, Louisiana USA for its excellent work on dog population management and animal welfare
- Coordinated mass dog vaccination program in high risk zone and supported rabies outbreak containment
- Conducted Street dog count in 8 Dzongkhags and conducted KAP survey in Thimphu and Paro covering 960 HH to gauge the success of the 3<sup>rd</sup> phase of project

## 2.6 Conducted Animal Health Survey & Researches

The following animal health research and surveys were conducted during the 11<sup>th</sup> FYP

### Cattle, Yak, Mithun

- A national serosurvey to determine the prevalence of paratuberculosis in cattle in Bhutan following detection of clinical cases
- Mycobacterium avium sub-species paratuberculosis stressome and diagnostic significance: a review and meta-analysis
- Immunoreactivity of protein tyrosine phosphatase A (PtpA) in sera from sheep infected with Mycobacterium avium subspecies paratuberculosis
- Lymphoproliferative and Gamma Interferon Responses to Stress-Regulated Mycobacterium avium subsp. paratuberculosis Recombinant Proteins



- Immunopathological changes and apparent recovery from infection revealed in cattle in an experimental model of Johne's disease using a lyophilized culture of *Mycobacterium avium* subspecies *paratuberculosis*
- Comparative immunological and coprological screening of Fasciolosis in cattle
- Antimicrobial Susceptibility Testing (AST) profile of bovine milk isolates in Bhutan
- Bovine Mastitis in Bhutan: An Overview
- Microbiological quality of raw milk in Bhutan
- Effect of Megacid® suspension on the lowered rumen Ph in cattle
- Sero-surveillance of cattle Contagious Bovine Pleuropneumonia in Bhutan; a retrospective study using Contagious Bovine Pleuropneumonia Antibody Test, a competitive ELISA based on a monoclonal anti- *Mycoplasma mycoides* subsp. *mycoides* small colony antibody
- A Case-Control Study of an outbreak of Foot and mouth disease at the herd level in cattle in Samdrup Jongkhar district, south eastern Bhutan.
- Successful control of four-years-old lameness in animals at Calf Rearing Centre, Wangkha
- Genetic diversity and bottleneck analysis of Mithun (*Bos frontalis*) population in Bhutan using microsatellite markers
- Prevalence of hydatidosis in slaughtered cattle, yaks and imported meat in Bhutan
- Prevalence of Hypodermosis in Laya Yaks
- Accidental urea poisoning in cattle- Case report
- Abomasal displacement leading to rupture: a case report

### **Sheep and goats**

- Cellular and humoral immune responses in sheep vaccinated with candidate antigens MAP2698c and MAP3567 from *Mycobacterium avium* subspecies *paratuberculosis*
- Development of 316v antibody enzyme linked immunosorbent assay for detection of paratuberculosis in sheep
- Prevalence of PPR in goats in Bhutan

### **Dog**

- Community-based survey during rabies outbreaks in Rangjung town, Trashigang, eastern Bhutan, 2016
- Epidemiological Analysis of Dog bites and Human Rabies post exposure prophylaxis in Bhutan, 2009-2012
- Comparison of mark-resight methods to estimate abundance and rabies vaccination coverage of free-roaming dogs in two urban areas of south Bhutan
- Rabies in East and Southeast Asia - a mirror of the global situation
- Animal Birth Control program and the health status of street dogs in Bhutan
- Comparison of antibody responses after vaccination with two inactivated rabies vaccines in dogs in Thimphu, Bhutan
- Evaluating rapid immunochromatographic test for diagnosis of rabies in animals in Bhutan

- Community-based survey of ecological and epidemiological risks associated with free roaming and feral dog populations in buffer zone of a protected area in Bhutan
- Study on *Taenia multiceps* infection in yak dogs in Bhutan

## **Pig**

- First Detection of Extended-Spectrum  $\beta$ -Lactamase Producing *Escherichia coli* in Breeder Pigs in Bhutan
- Seroprevalence and associated risk factors of important pig viral diseases in Bhutan
- Evaluation of oral bait vaccine efficacy against classical swine fever in village backyard pig farms in Bhutan

## **Poultry and wild birds**

- Biosecurity survey in relation to the risk of HPAI outbreaks in backyard poultry holdings in Thimphu city area, Bhutan
- Emergency surveillance for novel influenza A(H7N9) virus in domestic poultry, wild birds and feral pigeons in Bhutan
- Second and Third Phase Emergency Surveillance and Response to Avian Influenza A (H7N9) virus in Bhutan
- Feral pigeon disease surveillance following poultry and wild bird mortality event in Bhutan
- A retrospective case-control study of an outbreak of Newcastle Disease in poultry in Pemagatshel district, eastern Bhutan
- An investigation on infectious bursal disease outbreak in vaccinated young poultry flock in southern districts of Bhutan bordering India
- Prevalence of *Salmonella* species in dressed broiler chicken in Chukha Dzongkhag
- Immunological tolerance of Bhutanese native chicken to Infectious Bursal Disease Virus infection
- Application of vaccination takes to confirm fowl pox endemicity in Bhutan
- Investigation of IBD outbreak in vaccinated young poultry flock in Bhutan

## **Fish**

- Investigation of Motile Aeromonas septicemia (MAS) or Haemorrhagic septicemia or Infectious Dropsy in snow trout at Lyngkana: a case report

## **One Health and policy research**

- Investigation and Control of Anthrax Outbreak at the Human–Animal Interface, Bhutan, 2010
- A One Health Approach of Investigation, Prevention & Control of Anthrax: Case Study in Bhutan
- Knowledge, perceptions and practices of rural communities on anthrax in east-central Bhutan

- Crimean-Congo Haemorrhagic Fever Virus IgG in Goats, Bhutan
- Serological evidence of Rickettsia, Orientia and Coxiella in domestic animals from Bhutan: preliminary findings
- Self-assessment of Bhutans' Rabies Prevention and Control programme using SARE tool
- Determinants of health seeking behavior of animal bite victims in rabies endemic south Bhutan: A community-based contact-tracing survey
- Application of geostatistical visualisation and spatial statistics to understand the dispersion of rabies re-emergence in the highland, eastern Bhutan
- Investigation and monitoring of Brucellosis associated abortion in a dairy cattle farm in Bhutan
- A Review on Antimicrobial Resistance (AMR) in Livestock in Bhutan
- Investigation of Leptospirosis outbreak in dairy cattle farm in Bhutan
- Risk-based surveillance of Leptospirosis in domestic animal-human interface in Bhutan
- Identification of Echinococcus spp. in definitive and intermediate hosts in Bhutan
- Molecular characterization of Echinococcus spp in definitive and intermediate hosts in Bhutan

## 2.7 Capacity building

The NCAH have conducted various training/workshop to enhance the skills and expertise of the laboratory and animal health staffs in the country during the 11<sup>th</sup> FYP

- Conducted a Refresher training for Veterinary laboratory technicians (36 participants) in the country;
- Conducted Laboratory co-ordination Workshop to strengthen co-ordination and linkages of the laboratory networks in the country;
- Conducted Laboratory diagnostic workshop to enhance Rabies testing and control in Bhutan in collaboration with AAHL, Geelong, Australia;
- Conducted training and introduced three diagnostic methods for diagnosis of Brucella infection: culture technique, polymerase chain reaction (PCR) and milk enzyme linked immunosorbent assay (ELISA) in collaboration with NIAH, Japan;
- Conducted training on aflatoxin analysis at National Research Centre for Animal Nutrition, Bumthang;
- Conducted training Workshop on Rabies Risk assessment and economic analysis in collaboration with University of Calgary, Canada;
- Conducted training on Geographical Information System & Global Positioning System to Bhutan One Health Fellows
- National Consultative & Awareness Workshop on Antimicrobial Resistance in Livestock Sector
- National Consultative Workshop on Gid Disease Prevention and Control Program
- Two day hands on Training on diagnosis of parasitic diseases
- Organized scientific seminar

- Organized appraisal Meeting on Bhutan One Health Strategy Plan
- Organized Second and National Third One Health Workshop
- Training on Geographical Information System & Global Positioning System to veterinary officials
- Training Workshop on National Animal Health and Production
- Workshop on development of Antibiotic Use Guidelines in animals and Standard Treatment Guidelines
- Training Workshop on Avian Influenza A (H7N9) Surveillance, Sample Collection Technique
- Training on ELISA Techniques for Diagnosis and Control of Avian Influenza A(H7N9) in Bhutan
- Training on Simulation on National Pandemic Preparedness and Response Plan to Avian Influenza A (H7N9 and H5N1) in Bhutan
- Workshop on Risk Assessment and Development of Bio-security Plans
- Training on Geographical Information System & Global Positioning System to livestock officials of western region
- Training on nationwide free-roaming dog population survey
- Training of Trainers (ToT) and System Testing of G2C Database for EVDP
- Training on Clinical Recognition, Case Management and Control of Emerging Infectious and Zoonotic Diseases
- Training on Stata
- Conducted training on Geographical Information System & Global Positioning System to Ministry of Health officials
- Conducted training of Livestock staff on scientific paper writing as resource person in collaboration with Research Extension Division, DoL
- Trained veterinarians of RLDC Wangdue and Livestock staff of Gasa on operation tranquilizing gun for control of feral dog populations at Lunana & provided technical and logistical support for the management of feral dogs in Lunana, Gasa.
- Organized Tick identification training to the laboratory technicians in collaboration with RCDC and AFRIMS, Bangkok.
- Tick identification training to veterinarians and laboratory technicians of RLDC, Wangdue

## **2.8 Establishment of national & international collaboration**

- Established and strengthened international collaboration on animal health with several research institutes around the world including Thailand, India, Japan, Australia, Switzerland, Canada, UK and the USA
- Initiated technical collaboration on protein based Hemorrhagic Septicemia (HS) vaccine production with the University of Calgary, Canada. The goal of this project, is to develop molecular vaccines against Haemorrhagic Septicemia (HS) in ruminants through collaborative research that will improve the strength of the immune response and ability to protect against different strains of HS.
- National collaboration with RCDC, DoPH, WCD, DRA, BAFRA

## 2.9 Financial achievement during 11th FYP

Financial Year	Financing	Revised Budget			Expenditure			Budget Balance			% utilization
		Current	Capital	Total	Current	Capital	Total	Current	Capital	Total	
1.LC Account (NCAH)	RGoB	49.565	11.580	61.145	47.877	10.497	58.374	1.688	1.083	2.771	
	Donor	5.818	3.472	9.290	5.232	2.793	8.025	0.586	0.679	1.265	
2. PLC Account (HSI)	RGoB	5.004	0.000	5.004	5.004	0.000	5.004	-	-	-	
	Donor	14.446	0.000	14.446	11.399	0.000	11.399	3.047	-	3.047	
<b>1st Year (2013-2014)</b>		<b>74.833</b>	<b>15.052</b>	<b>89.885</b>	<b>69.512</b>	<b>13.290</b>	<b>82.802</b>	<b>5.321</b>	<b>1.762</b>	<b>7.083</b>	<b>92.12</b>
1.LC Account (NCAH)	RGoB	51.979	5.850	57.829	50.444	5.179	55.623	1.535	0.671	2.206	
	Donor	5.108	2.410	7.518	4.736	2.305	7.041	0.372	0.105	0.477	
2. PLC Account (HSI)	RGoB	5.000	0.000	5.000	5.000	0.000	5.000	-	-	-	
	Donor	5.115	0.000	5.115	5.001	0.000	5.001	0.114	-	0.114	
<b>2nd Year (2014-2015)</b>		<b>67.202</b>	<b>8.260</b>	<b>75.462</b>	<b>65.181</b>	<b>7.484</b>	<b>72.665</b>	<b>2.021</b>	<b>0.776</b>	<b>2.797</b>	<b>96.29</b>
1.LC Account (NCAH)	RGoB	62.357	6.475	68.832	61.377	5.789	67.166	0.980	0.686	1.666	
	Donor	1.100	0.634	1.734	0.677	0.634	1.311	0.423	-	0.423	
2. PLC Account (HSI)	RGoB	1.950	0.000	1.950	1.924	0.000	1.924	0.026	-	0.026	
	Donor	0.964	0.000	0.964	0.963	0.000	0.963	0.001	-	0.001	
<b>3rd Year (2015-2016)</b>		<b>66.371</b>	<b>7.109</b>	<b>73.480</b>	<b>64.941</b>	<b>6.423</b>	<b>71.364</b>	<b>1.430</b>	<b>0.686</b>	<b>2.116</b>	<b>97.12</b>
1.LC Account (NCAH)	RGoB	57.472	5.840	63.312	55.451	5.471	60.922	2.021	0.369	2.390	
	Donor			0.000	0.000		-	-	-	-	
2. PLC Account (HSI)	RGoB	2.826	0.000	2.826	2.669	0.000	2.669	0.157	-	0.157	
	Donor			0.000			-	-	-	-	
<b>4th Year (2016-2017)</b>		<b>60.298</b>	<b>5.840</b>	<b>66.138</b>	<b>58.120</b>	<b>5.471</b>	<b>63.591</b>	<b>2.178</b>	<b>0.369</b>	<b>2.547</b>	<b>96.15</b>
1.LC Account (NCAH)	RGoB	56.207	4.590	60.797	55.894	4.566	60.460	0.313	0.024	0.337	
	Donor	0.480		0.480	0.480		0.480	-	-	-	
2. PLC Account (HSI)	RGoB	2.520	0.000	2.520	2.447	0.000	2.447	0.073	-	0.073	
	Donor	0.000		0.000			-	-	-	-	
<b>5th Year (2017-2018)</b>		<b>59.207</b>	<b>4.590</b>	<b>63.797</b>	<b>58.821</b>	<b>4.566</b>	<b>63.387</b>	<b>0.386</b>	<b>0.024</b>	<b>0.410</b>	<b>99.36</b>
<b>Grand Total</b>		<b>327.911</b>	<b>40.851</b>	<b>368.762</b>	<b>316.575</b>	<b>37.234</b>	<b>353.809</b>	<b>11.336</b>	<b>3.617</b>	<b>14.953</b>	<b>95.95</b>

## **Key achievement of NCAH during FY 2017-2018**

### **3. Disease Prevention and Control Unit**

The Disease Prevention and Control Unit act as the focal unit for planning, implementation and monitoring of disease prevention and control programs in the country. The unit has three sections: Epidemiology & GIS section; Veterinary Public Health and Clinical section.

#### **3.1 The main roles of the DPCU**

- To formulate, implement and monitor the various nationally coordinated animal disease prevention and control programs in the country
- To formulate animal disease emergency response plans (contingency plans) for trans-boundary emerging animal diseases
- To plan and implement zoonotic disease prevention and control programme through One Health approach in collaboration with the Ministry of Health
- To maintain the livestock diseases information in the country through the online TADInfo database system and analysis and reporting of the data
- It act as the focal agency for contact with international organizations like OIE (World Organization for Animal Health), FAO, WHO, APHCA (Animal Production and Health Commission for Asia Pacific) on all matters of animal health concerns

#### **3.2 Human resources of DPCU**

The DPCU is currently manned by the following staff:

- Dr. Tenzin, PLHO (Head)
- Dr. Yoenten Phuentshok, Sr. Veterinary Officer
- Kinzang Namgay, Sr Livestock Health Supervisor
- Karma Dekar, Data Manager

#### **3.3. Key achievements of DPCU for the FY 2017-2018**

##### **3.3.1 Develop disease control plan**

The expert team have drafted a national rabies action plan using Stepwise Approach towards Rabies Elimination (SARE) tool to guide elimination of dog mediated human rabies in Bhutan by 2023.

##### **3.3.2 Strengthening of Database Management and Disease Reporting System**

- Carried out regular updating and cross checking of TADInfo database
- Two six monthly reports on notifiable animal diseases were compiled and submitted to Office International des Epizootic (OIE) and updated in World Animal Health Information System (WAHIS).

- Immediate notification to WAHIS submitted whenever there was any OIE reportable disease outbreak in the country (e.g. bird flu, PPR)
- E-bulletin: Twenty four number of fortnightly e-bulletin were produced and circulated on important animal disease outbreaks in the country from (July 2017 to June 2018) as readily readable format.
- Carried out regular uploading of news and events, notification, etc. onto the website
- Preparation and submission of centres monthly progress report

### **3.3.3 Disease outbreak investigation and containment**

The DPCU, LSU and other technical units of NCAH including the Regional and Dzongkhag stakeholders coordinated disease outbreak investigation and containment activities in the country.

- Coordinated containment of major FMD disease outbreak in Paro and Chhukha
- Coordinated containment of major rabies outbreak in Trashigang, Panbang, Samtse and Chukha
- Coordinated containment of HPAI outbreak in Samtse and Samdrup Jongkhar
- Coordinated containment of classical swine fever disease outbreak in native Pigs at Wangchutaba Pig Farm, Thimphu

The following report describes CSF and FMD outbreak containment in the country.

#### **Classical Swine Fever disease outbreak in native Pigs at Wangchutaba Pig Farm, Thimphu: Investigation report**

##### **Investigation team**

1. Dr NK Thapa, Animal Health Specialist, NCAH
2. Dr Vijay Raika, Animal Health Specialist, NCAH
3. Dr Tenzin, Veterinary Epidemiologist, NCAH
4. Mr Purna Bdr Rai, Sr Lab Technician, NCAH
4. Mr Dawa Tshering, Sr Lab Technician, NCAH
6. Kelzang Lhamo, Lab Technician, NCAH

##### **1. Background**

The existing pig farm at Wangchutaba was initiated as Regional Pig Breeding Centre under the Department of Livestock. After the relocation of farm at Yusipang, the present farm at Wangchutaba has been maintaining breeding stock of native pigs. As of 18<sup>th</sup> April 2018, the total stock of pigs is 178 in the following categories.

<b>Type</b>	<b>Number</b>
Lactating sow	5
Pregnant sow	14
Dry sow	2



Small boar	6
Culled sow	4
Young boar	5
Gilt	8
Grower	11
Weaner	106
Suckler	20
<b>Total</b>	<b>181</b>

## 2. Mortality history

The NCAH was informed about mortality of pigs by the Farm Management on 17<sup>th</sup> April 2017. Accordingly, team from NCAH visited the farm and carried out investigation and postmortem examinations. The mortality had started since 2<sup>nd</sup> April 2018 exhibiting signs such as off feed, respiratory distress and bleeding from nostril in some cases. The daily pattern of mortality is shown in the table below. Between 2-19 April 2018, 35 pigs have died in the farm of which 16 were adult, 8 suckler, 10 weaner and 1 grower.

Date	Death					Total death	Mortality rate	Sold/birth	
	Stock	Adult	Suckler	Weaner	Grower			Sold	Birth
1/4/2018	218						0		
2/4/2018	209	8	1			9	4.31		
3/4/2018	208	1				1	0.48		
4/4/2018	202						0.00	6	
5/4/2018	199		1	2		3	1.51		
6/4/2018	198	1				1	0.51		
7/4/2018	201						0.00		3
8/4/2018	201						0.00		
9/4/2018	195	1	1	1		3	1.54	3	
10/4/2018	195						0.00		
11/4/2018	195						0.00		
12/4/2018	191		2	2		4	2.09		
13/4/2018	189	1			1	2	1.06		
14/4/2018	189						0.00		
15/4/2018	189						0.00		
16/4/2018	186		2	1		3	1.61		
17/4/2018	181	2	1	2		5	2.76		
18/4/2018	178	1		2		3	1.69		
19/4/2018	177	1							
<b>Total</b>		<b>16</b>	<b>8</b>	<b>10</b>	<b>1</b>	<b>35</b>		<b>9</b>	<b>3</b>

## 3. Postmortem examination

A PM examination was conducted at NCAH on 17<sup>th</sup> April 2018 with the following findings.

- Inflammation of lungs (Pneumonic lungs)
- Fusion of cortico-medullary junction of kidneys
- Haemorrhagic gastro enteritis
- Haemorrhagic lesions in lymph nodes

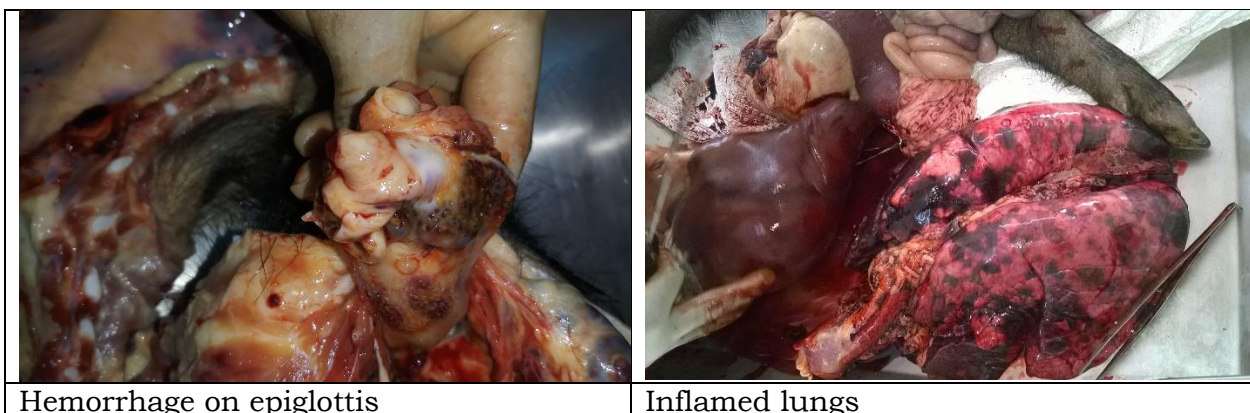
Second PM examination was conducted at NCAH on 18<sup>th</sup> April 2018 with the following findings.

- Haemorrhagic gastro enteritis with typical button ulcer on the large intestine
- Haemorrhagic lesions in lymph nodes (mesenteric and sub-mandibular lymph nodes)
- Pin point hemorrhages on the surface of the kidneys
- Hemorrhage on medulla and pelvis
- Severe congestion of mesentery along with hemorrhagic lymph nodes
- Hemorrhage of tonsil and necrotic lesion on epiglottis
- Hemorrhage on peritoneal wall and diaphragm

Third PM examination was conducted at NCAH on 19<sup>th</sup> April 2018 with the following findings.

- Hemorrhage in the stomach mucosa
- Enlargement and haemorrhagic lesions in lymph nodes (sub-mandibular and inguinal lymph nodes)
- Pin point hemorrhages on the surface of the kidneys
- Hemorrhage on medulla and pelvis
- Pin point hemorrhage lesion on epiglottis
- Enlargement and infarction of spleen
- Myocarditis

The following pictures show typical PM lesions suggestive of Classical Swine Fever





Hemorrhage on peritoneal wall



Severe congestion of mesentery



Button ulcer on the mucosa of large intestine



Enlarged and inflamed lymph nodes

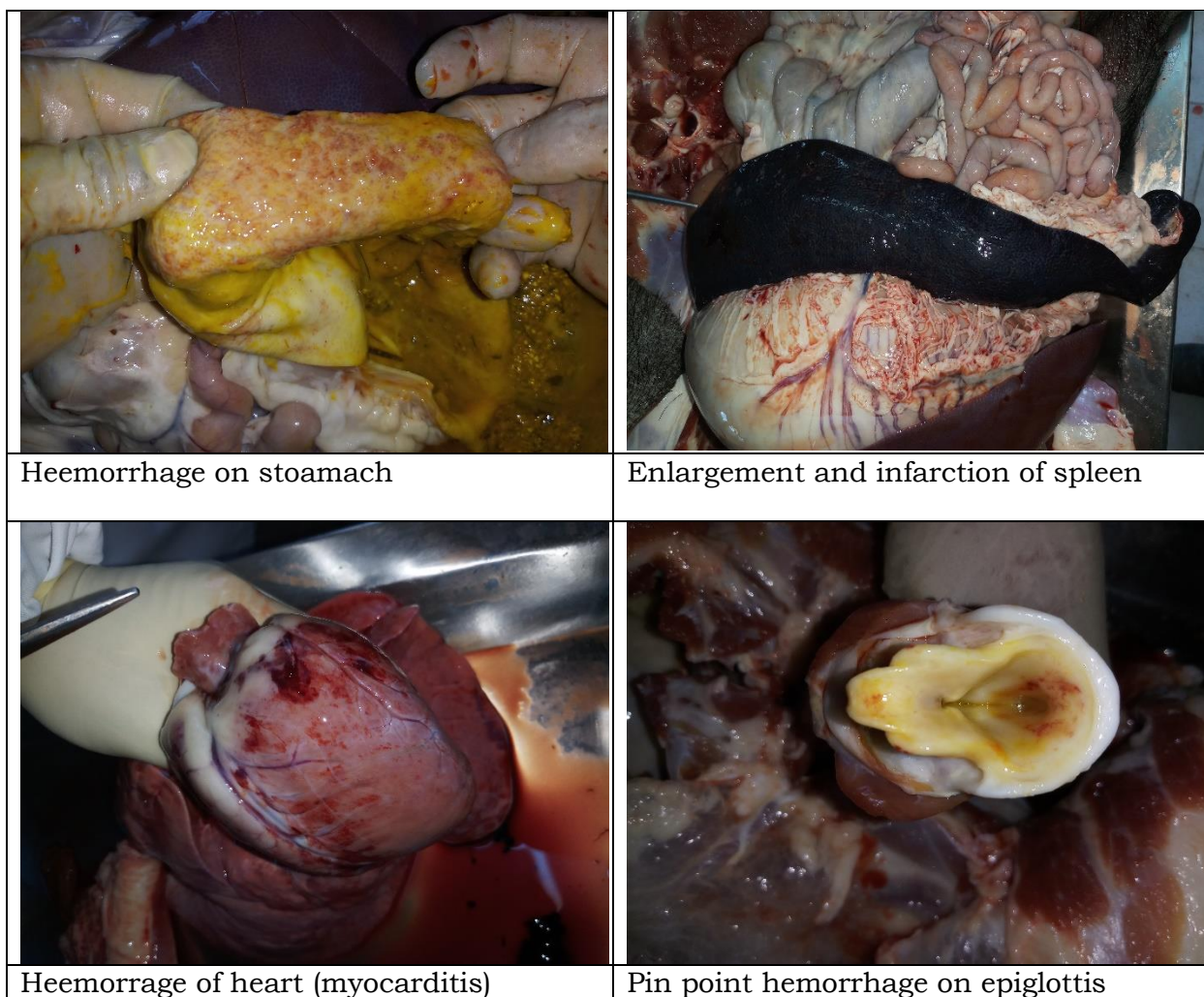


Pin point hemorrhages on the surface of the kidney



Hemorrhage on medulla and pelvis of kidney

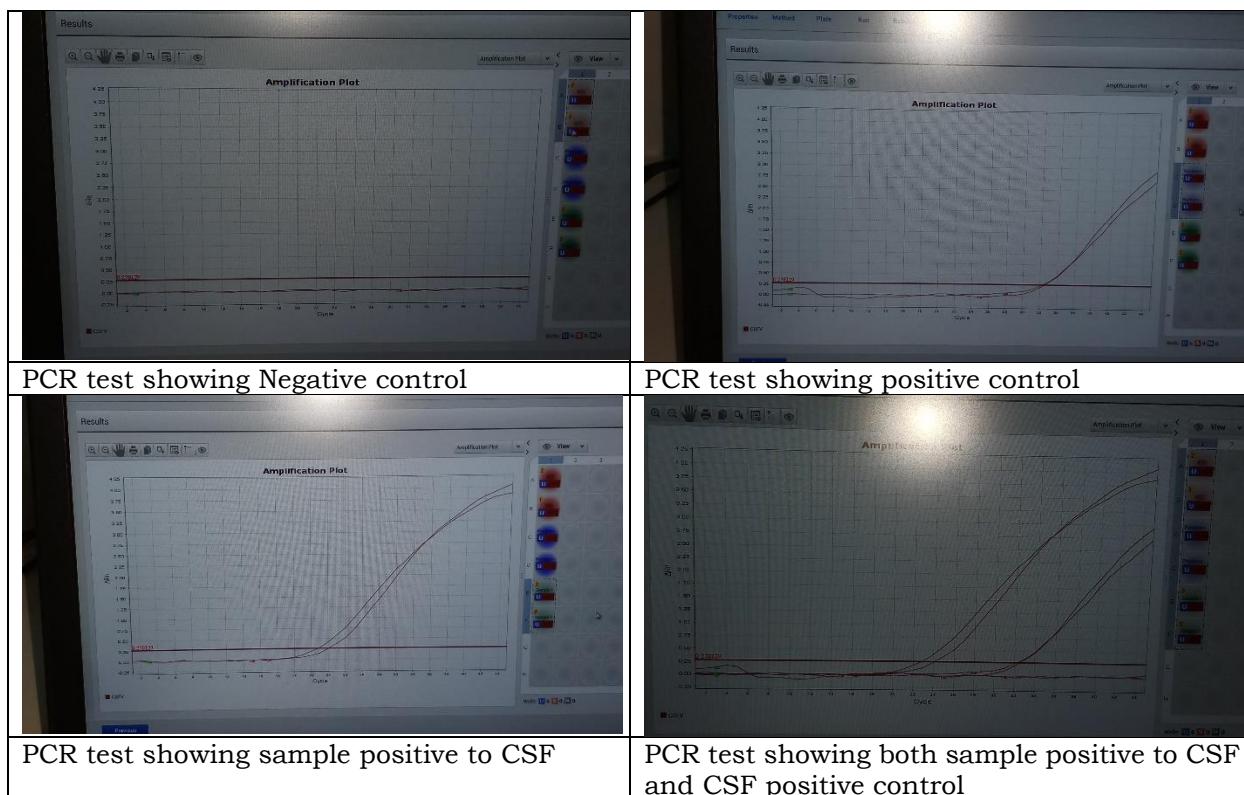




**Picture source: Drs Tenzin and NK Thapa**

#### **4. Laboratory investigation**

The samples collected during the PM examination were tested against *Classical Swine Fever* (CSF) using CSFV Antigen detection ELISA and tested positive to CSF virus. The samples also tested positive to CSF using PCR test at NCAH, Serbithang confirming CSF virus infection in pigs.



## 5. Diagnosis

As per the postmortem examinations and laboratory test using CSFV Antigen detection ELISA and PCR test, the cases were diagnosed as *Classical Swine Fever (CSF)* virus infection. The source of outbreak is unknown and is being investigated.

## 6. Vaccination history against CSF virus

The farm is following the vaccination against CSF and FMD as per the schedule. The vaccination data against CSF were collected from the farm management as follows.

Sl.no	Date	Age groups	Vaccination done (numbers)
1.	11/05/2016	Adults	94
2.	11/05/2016	Weaner	100
3.	10/01/2017	Adult	30
4.	2//02/2017 to 29/12/2017	Weaner	176
5.	03/02/3018 to 19/03/2018	Weaner	57

- The adults that were vaccinated on 10/01/2017 were due to be vaccinated on 10/01/2018, and were not vaccinated as per the management.
- Similarly the present stock of 11 numbers of growers were not vaccinated against CSF.
- All weaners were vaccinated as per the management

- There are 20 number of Suckler which are not yet time for vaccination and thus, at risk of infection. The vaccination is done is after weaning.

### **Action recommended to be taken by the farm management and the Department**

1. Immediate segregation of sick pigs from healthy ones to prevent further spread of disease
2. All the sheds need to be immediately cleaned and disinfected
3. The cleaning and disinfection of the farm has to be a regular activity by following standard operating procedure.
4. The ventilation system needs to be improved
5. The pig carcass to be submitted to NCAH for PM examination and for proper disposal
6. The bio-security measures such as foot dips, cleanliness, disinfection, restriction of visits by people in the farm should be followed as per the farm bio-security guidelines.
7. The vaccination against CSF and FMD have to be carried as per the schedule ensuring every single pig is vaccinated.
8. Recommended for treatment with antibiotic as a supportive therapy to prevent secondary bacterial infection
9. Since the pig sheds are very old, there is immediate need for maintenance and renovation with proper fencing of pig farm to enable implementation of proper bio-security measure
10. Relocation of the farm to secure place may be considered in view of its existence within Thimphu city area compromising the farm bio-security.

### **Foot and Mouth Disease (FMD) Outbreak in Cattle at Drangay Goenpa, Paro**

*Dr. Tenzin<sup>1</sup>, Dr. Yoenten Phuentshok<sup>1</sup> and Dr. Tenzin Wangchuk<sup>2</sup>*

<sup>1</sup>Disease Prevention and Control Unit, National Centre for Animal Health, Serbithang

<sup>2</sup>Dzongkhag Veterinary Hospital, Paro

#### **Background**

An outbreak of foot and mouth disease (FMD) in cattle at Drangay Goenpa village, Luni geog, Paro was notified on 7th May 2018 through flash report submitted to Regional Livestock Development Center, Tsimasham. Paro reported major outbreak of FMD in cattle between May and September 2017 affecting 8 geog, 36 villages 724 households, 764 cattle including 33 deaths and thus, this outbreak investigation was conducted to find out the source of outbreak and institute immediate containment measures. The investigation of the outbreak was conducted on 9th May 2018.

#### **History**

On 30th April, 2018, a farmer from Drangay Goenpa reported to the District Veterinary Hospital that a calf is not well, is not feeding and has started salivating excessively. The calf was treated for stomatitis since three other animals in the herd were healthy. After a week, on 7th May 2018, the same farmer reported similar signs in the three remaining cattle in her herd and upon examination, FMD was suspected through clinical signs and lesions in the oral cavity, tongue and foot. The affected animals were treated and

isolated within the house compound. On 8th May 2018, the team from Dzongkhag Livestock Sector and BAFRA, Paro visited the village and conducted a public meeting and implemented a ban on movement of animals and animal product out of the village; strict stall feeding system and restriction of free grazing of cattle in the forest.

On 9th May 2018, the outbreak was further investigated by the team from NCAH Serbithang and DVH Paro. The investigation team found out that the first clinical signs appeared on 30th April 2018 in a heifer and then spread to three other cattle in the same herd. The disease had spread to a neighbouring household and 3 cattle from the second household were reported to be showing signs of FMD with ropy salivation and slight lameness on 8th May 2018.

### **Clinical examination**

The team examined 9 animals at Drangay Goenpa belonging to 2 households.

#### **Household 1:**

No of cattle in the herd and number affected: 4 / 4 = 100% affected

- 1 female calf (heifer): showed signs suggestive of FMD on 30th April 2018 and has recovered on 7th May 2018
- 1 male calf : FMD lesions in mouth and tongue
- 1 cow: FMD lesions in mouth and tongue
- 1 cow: FMD lesions foot; is 8 months pregnant

#### **Household 2:**

- No of cattle in the herd and number affected: 3 of 4 in the herd affected
- 1 calf and 3 cows.
- 1 cow is apparently healthy at the time of examination

<b>Village</b>	<b>No HHs affected</b>	<b>Approx No animals affected</b>	<b>No. died</b>	<b>Approx No animals in the village</b>	<b>Remarks</b>
Drangzhi Goenpa, Luni, Paro	2	7	0	Hh having cattle 9 38 cattle (pigs 19) Total hh-10	Wound healing has started

### **Main findings and source of outbreak**

- All these affected cows were vaccinated in August 2017 and in February 2018. Cold chain of vaccines were adequately maintained.
- During the last vaccination, the coverage in Luni geog was 89.5% (830 of 927) as per the Paro Livestock Sector's records and report.
- The index case for this outbreak is the female calf but the source of the outbreak could not be identified. Probable source could be contamination of cattle feed/barn with FMDV meat but cannot confirm as farmers were reluctant to share the information. There are no cases of FMD reported in any of the neighbouring villages and geogs.

### **Action Taken**

- Farmers were asked to tie their animals and not to send them for grazing
- The farmers have been advised not to sell the animal products from the infected area to other areas to prevent spread of disease/infection;



- The farmers were made aware of the importance of FMD vaccination and biosecurity measures

#### **Recommendations**

- Since there is risk of further spreading the disease from the infected areas to other villages under Paro Dzongkhag, the Dzongkhag Livestock Sector, Paro and BAFRA Paro to implement disease control measures as per the National FMD Control Plan, Livestock Act of Bhutan 2001 and Livestock Rules and Regulation 2008;
- The Dzongkhag Livestock Sector (DVH, Paro and RNREC-Luni) to continuously monitor the disease situation and communicate the status to the concerned stakeholders
- The RLDC, Tsimasham to coordinate the outbreak containment measures in collaboration with DPCU, NCAH.

### **3.3.4 Coordination of World Rabies Day events**

The DPCU, NDPM & RCP of NCAH and DoPH (MoH) have coordinated the World Rabies Week starting from 28 September 2017.

### **3.3.5 Animal Health information system**

- One book on the “Status of the Notifiable Animal Diseases in Bhutan (1996-2017)” was published by DPCU, uploaded onto NCAH website and also shared the soft copies to all the stakeholders.

### **3.3.6 Coordination of One Health activities**

- Organized tick identification and molecular diagnosis training to veterinarians and laboratory technicians in collaboration with Royal Center for Disease Diagnosis, MoH
- Conducted dog bite and PEP surveillance to enhance rabies prevention and control as part of SARE exercise through visit of human hospitals in the country in collaboration with Department of Public Health, MoH
- Conducted dog bite health seeking behavior and PEP availability and accessibility study as One Health approach and GAVI exercise in collaboration with Department of Public Health, MoH
- Conducted sero-prevalence study of Rickettsial disease in animals in coordination with JDWNRH, Thimphu

- Collaborated on treatment and diagnosis of the tiger captured from Thimphu in collaboration with Department of Forest and Park Services
- Conducted post-mortem examinations of wildlife species
- Collaborated on operation of tranquilizing gun and procurement of high concentrated tranquilizing drugs for our use



### 3.3.7 Policy and Research activities

- Participated in developing framework for stepwise approach for brucellosis control program in Bhutan
- Designed and implemented risk-based brucellosis surveillance in coordination with other units in the centre
- Conducted yak dog taeniasis surveillance in Merak-Sakteng and other yak rearing areas for understanding the prevalence of Gid disease causing parasites in yak/stray dogs. Samples referred to Zurich Parasitology laboratory, Switzerland for molecular analysis
- KAP survey of dog owners in Paro and Thimphu in collaboration with NDPM & RCP
- Participatory epidemiology among PEP recipients during an outbreak of Rabies in Panbang.
- Survey of rodent borne pathogens in Bhutan
- Participated in the evaluation of International Health Regulation and development of action plan

### 3.3.8 Capacity building

- Lecture on disease outbreak investigation and response to outbreaks to animal health personnel of RLDC, Wangdue
- Conducted training of Livestock staff on scientific paper writing as resource person in collaboration with Research Extension Division, DoL
- Trained veterinarians of RLDC Wangdue and Livestock staff of Gasa on operation tranquilizing gun for control of feral dog populations at Lunana & provided technical and logistical support for the management of feral dogs in Lunana, Gasa.
- Guidance with TADinfo and VIS application and troubleshooting to field colleagues
- Tick identification training to veterinarians and laboratory technicians of RLDC, Wangdue

### 3.3.9 Other works

- Coordinated review and work planning for NCAH
- Coordinated development of Annual Performance Agreement and its implementation and participated in other policy issues

## 4. Laboratory Services Unit

The Laboratory Services Unit (LSU) is one of the four functional technical units under the National Centre for Animal Health, Serbithang. The unit has the capacity to undertake rapid diagnosis and also advanced diagnostic tests like ELISA, FAT and molecular tests for emerging and re-emerging infectious disease like FMD, highly pathogenic avian influenza, classical swine fever and rabies etc. The the lab is equipped with real time PCR machine. The unit has Biosafety level 2 plus for advanced diagnosis. The unit also functions as the National Referral Veterinary Laboratory in the country

### 4.1 Mandates of the LSU

The main mandates of the Laboratory Services Unit are:

- To provide range of veterinary diagnostic services, support clinical services, animal health programs and One-Health activities in the country;
- To serve as the national referral laboratory for diagnosis of animal diseases in the country;

The main mandates of the Laboratory Services Unit are:

1. Providing referral veterinary laboratory diagnostic services to the clients
  - Provide routine veterinary laboratory diagnostic services, support clinical services, animal health programs and One-Health activities in the country;
  - Serve as the national referral laboratory for diagnosis of animal diseases in the country
2. Major Livestock Disease Surveillance/Survey
  - To lead/coordinate and conduct laboratory based animal health research activities in the country
3. Coordination and implementation of Biosafety and Bio-security programmes
  - Implement and monitor bio-safety measures and good laboratory practices in all veterinary laboratories in the country
4. Strengthening and enhancement of laboratory diagnostic capacities
  - To serve as focal laboratory for antimicrobial resistance monitoring in animals in the country
  - To participate in regional proficiency testing for specific diagnostic methods
  - To technically backstop regional, satellite and district laboratories in the country
  - Introduction of new diagnostic tests/upgradation of diagnostic tests for the emerging and re-emerging diseases in the country
  - To liaise, collaborate and establish efficient laboratory networks with the outside agencies like Food Testing Laboratory, Bhutan Agriculture and Food Regulatory Authority; Clinical Laboratory, Jigme Dorji Wangchuck National Referral Hospital; Royal Centre for Disease Control, Department of Public Health; and Wildlife Clinic, Nature Conservation Division, Department of Forests and Park Services;
  - To liaise, collaborate and establish efficient laboratory networks with the international reference laboratories such as OIE and WHO Referral Laboratories;
5. Laboratory skill enhancement
  - To develop human resource capacity by conducting the diploma course in laboratory technology in collaboration with other relevant institutions.
  - Conduct refresher course and up-gradation courses for laboratory technicians

## 4.2 Human resources in LSU

The followings are the available human resource in the Laboratory Services Unit as on 30<sup>th</sup> June 2018 (Table 1).

**Table 1: Over all human resource capacity in LSU**

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

## 4.3 Diagnostic capacities in LSU

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

### 4.3.1 Parasitology Section

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

The Parasitology section is currently manned by the following staff.

1. Dr. Phuntsho Wangdi, AHS-I
2. Ms. Pema Tshomo Lab Assistant
3. Ms. Tshewang Dema, Assistant Laboratory Technician

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

- Identification of parasites through direct technique;
- Identification of parasites through qualitative tests (Sedimentation and Floatation methods);
- Identification of parasites through quantitative tests (Stoll method);
- Urine sedimentation test for nematodes;
- Skin scraping examination using 10% KOH digestion method;
- Blood parasite examination;
- Pepsin digestion test;
- Fecal culture (simple tube method, culture tube method, Baermann's method);
- Tick identification (stereo-zoom method);
- Recovery of nematode larvae from soil, herbage and identification;
- Prepuccial trichomoniasis test and identification of trichomonas;
- Post-mortem recovery of helminths, post mortem worm count;
- Cryptosporidium staining and identification (modified acid fast);
- Microfilaria identification from blood (modified Knott's method);
- Worm staining & preservation;
- Density estimation of flukes;
- ELISA for Fasciola;

### **4.3.2 Bacteriology Section**

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

The bacteriology section is manned by the following staffs:

1. Ms. Puspa Maya Sharma, Laboratory Officer
2. Mr. Tenzinla, Sr. Laboratory Technician

The section has the following diagnostic capacities:

- Bacterial culture and identification using sheep blood agar, MacConkey agar and other selective media and various bio-chemical tests;
- Fungal culture and identification using Sabouraud agar;
- Staining techniques - Grams, Giemsa, Methylene blue, Ziehl-Neelsen/Acid fast, Leishman, Lactophenol, Spore staining and Capsule staining;
- Species identification of important bacterial pathogens in Bhutan – *Salmonella* spp., *E. coli*, *Staphylococcus* spp., *Bacillus anthracis*, *Clostridium* species and *Streptococcus* species;
- Enumeration of bacteria - total aerobic count by pour plate technique and spread plate technique, total coli count by pour plate technique and spread plate technique, Most Probable Number (MPN) technique;
- Detection of *Mycobacterium* species by acid fast technique;
- Agglutination test (SAT, TAT, Microtitre plate agglutination test);
- Detection of mastitis in milk samples through CMT, Cell count and WST;
- Antimicrobial susceptibility test;
- Intradermal test for TB using PPD



### **4.3.3 Haematology Section**

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

The Haematology section is manned by the following staffs:

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

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

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

### **4.3.4 Bio-chemistry & Toxicology Section**

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

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

1. Dr.NK Thapa, AHS III
2. Ms. Punya Mata, Assistant Laboratory Technician
3. Ms. Ugyen Pema, Assistant Laboratory Technician

The following are the diagnostic capacities available in this section:

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

### **4.3.5 Molecular biology, Serology & Virology Section**

The section performs tests on both routine basis and also on the samples referred by the Regional/District/Satellite Laboratories in the country

This section is equipped with advanced diagnostic facilities such as real time PCR, ELISA and has the capacity to undertake rapid diagnosis of emerging diseases including the highly pathogenic avian influenza, IBD, NCD and Rabies etc.

The Molecular biology, Serology and Virology sections are manned by:

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

The diagnostic capacities available in this section are:

- Rapid antigen detection tests for AI type A, H5, NDV, IBD, FMD & Rabies;
- FAT for Rabies;
- Antibody ELISA for FMD, Brucellosis, Rabies, NCD, IBD, CSF, IBR, Leptospirosis, CBPP, CCPP, PRRS, JD and PPR;
- Antigen ELISA for CSF and PPR;
- Typing ELISA (sandwich) for FMD;
- Conventional PCR for Brucella, FMD serotyping;
- Real time PCR for AI Type A, H5N1 and ND
- Agglutination tests - HA/HI for ND and H7N9;
- Slide agglutination test for Salmonella and Mycoplasma;
- RBT for Brucella;

#### **4.3.6 Post-mortem & Pathology Section**

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

The section has the man power as follows:

1. Dr. NK Thapa, AHS-III
2. Ms. Pasang Bida, Assistant Laboratory Technician
3. Ms. Ugyen Pema, Assistant Laboratory Technician

The section is responsible for following diagnostic capacities:

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

#### **4.3.7 Bio-safety and Bio-security section**

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

The human resource in this section is as follows (both were transferred recently):

1. Ms. Puspa Maya Sharma, Laboratory Officer
2. Ms. Pema Tshomo, Lab Assistant

The section is responsible for the following:

- Planning Coordination and Implementation of Biosafety and Bio-security plans
- Technical Support on Biosafety and Biosecurity measures

- In House Training
- Reporting and Monitoring
- Samples referral to collaborating laboratories
- Routine and research laboratory test kits, reagents, consumables procurement

#### 4.4. Key achievements of LSU during FY 2017-2018

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

**Table 2: Summary of sample received and test performed**

Section	No. of sample	No. of Tests
Parasitology	1176	3286
<b>Hematology</b>	318	1225
Bio-chemistry & Toxicology	253	412
Bacteriology	723	2860
Serology/Virology	2436	4358
Post-mortem	337	261
Histo-Pathology	947	852
<b>Total</b>	<b>6190</b>	<b>13253</b>

A total of 6,190 various laboratory samples were received or collected and 13,254 various laboratory tests were performed for disease screening, surveillance and researches during the year.

#### 4.4.1 Achievements of individual sections under LSU

##### 4.4.1.1 Histopathology, Postmortem and Parasitology sections

A total of 337 animal carcasses and 947 tissue samples were received and examined in the pathology section.

In total of about 1176, samples were received and 3285 tested by parasitology section. The details of tests performed by these sections are shown in Table 3.

**Table 3: Sample collected and test performed in Pathology and Parasitology sections**

Sample		Test	
<i>Type</i>	<i>Number</i>	<i>Type</i>	<i>Number</i>
<b>Histopathology</b>			
Organs and tissue	947	H and E staining	852
<b>Postmortem</b>			

Carcass	337	Gross pathology	261
<b>Parasitology</b>			
Faeces	1141	Faecal egg count	3225
Skin scrapings	9	10% KoH	9
Intestinal contents	5	Direct smear	5
Cysts	21	Direct smear	21
Samples Referral (Parasitic egg, human, bovine, tiger cysts)			60
<b>Total</b>	<b>1176</b>		<b>3285</b>

### Significant findings

**Histopathology:** Common findings were respiratory Infections followed by septicaemic Infection, CSF, catarrhal enteritis, ND.

**Postmortem:** Swine carcasses were received in highest numbers (245) and avian (78). This section was able to diagnose many specific disease conditions from necropsy such as CSF pig breeding farm. Cyst induced neuromyopathy and renal incompetency in tanager and Acute Myocarditis was diagnosed in Tanager.

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

#### 4.4.1.2 Bacteriology Section

The details of number of samples, test types and numbers of tests performed in bacteriology are as shown in Table 4.

**Table 4: Sample and test**

Types of specimen	Number	Types of tests	Number	Remarks
Organs	82	Culture	921	
Whole blood	5	Gram stain	155	
Nasal swab	103	Motility	133	
Fecal swab	1	Biochemical test	1592	
Cloacal swab	352	Antimicrobial Sensitivity test	32	

Tracheal swab	3	LCB Stain	14	
Skin scraping	11	Leishman Stain	8	
Cultured samples	13	Acid Fast Stain	3	
Milk	1	Methylene Blue Stain	2	
FTA card	83	<b>Total</b>	<b>2860</b>	
Impression smear	6			
Oropharyngeal swab	1			
Blood smear	5			
Pus swab	1			
Egg	21			
Others	35			
<b>Total</b>	<b>723</b>			

### Significant findings

During the year, besides other routine works of the laboratory, as a part of research activities, isolation, isolation and identification of *Salmonella* from poultry birds and *Pasteurella multocida* from bovine were carried out.

Findings from AMR surveillance:

23 samples out of 289 samples tested positive to *Salmonella* sp from poultry. These isolates were archived at -80°C and performed the antimicrobial susceptible testing. The culture isolates were susceptible to: Amoxycillin, Ceftriaxone, Nalidixic acid, Co-trimoxazole and Sulphadimidine, Gentamicin, Ciprofloxacin and Chloramphenicol. Resistant to: Amoxycillin, Nalidixic acid, Co-trimoxazole & sulphadimidine, Gentamicin, Chloramphenicol,

Nine samples out of 72 nasal samples collected from bovine tested positive to *Pasteurella multocida*. Isolates were archived -80°C and performed the antimicrobial susceptible testing. The culture isolates were susceptible to: Gentamicin, Tetracycline, Streptomycin, Erythromycin, Co-trimoxazole and sulphadimidine. The culture isolates were resistant to: Streptomycin, Ampicillin and Penicillin-G

#### 4.4.1.3 Bio-Chemistry/ Toxicology and Haematology sections

The Bio-Chemistry/Toxicology section performed mycotoxin analysis in animal feeds and mineral estimation in serum sample. Minerals such as calcium, magnesium and inorganic phosphorous estimation were performed in the bio-chemistry section.

Basic haematological tests were also conducted to support the clinical diagnosis in the animals.

Details of samples and tests conducted in these sections are presented in Table 5.



**Table 5: Bio-Chemistry/Toxicology and Haematology sections**

<b>Sample</b>		<b>Test</b>	
Type	Number	Type	Number
Biochemistry and Toxicology			
Feed	204	Aflatoxin	197
		Fuminosin	43
		Ochratoxin	43
Serum	37	Calcium	35
		Magnesium	35
		Phosphorus	35
Urine	12	Urine biochemistry	24
Total	253		412
Haematology			
Blood	318	PCV	284
		HB g/dl	284
Blood smear		DLC	331
		TRCC	129
		TWCC	155
		Direct smear	21
		Knott's test	21
Total	318		1225

**Significant findings**

Mycotoxin analysis detected about 71/204 (34%) of animal feeds contained aflatoxin above permissible level. Serum chemistry indicated 35/37(94%) of the samples with low calcium, 7/37(19%) with low phosphorous, 8/37 (22%) low magnesium and 11/37(32%) with high magnesium in the submitted samples.

**4.4.1.4 Molecular Biology, Serology and Virology Section**

The section performed tests such as rapid tests: Rose Bengal Test for Brucella abortus in bovine, Influenza A antigen, H5 antigen tests in birds, rabies antigen detection test, IBD, NCD and Fluorescence Antibody Test for rabies. The other screening and confirmatory serological tests include Non-Structural Protein Enzyme Linked Immuno-Sorbent Assay (ELISA) for screening against FMD antibody, sandwich ELISA for FMD typing, antigen detection ELISA for CSF and Liquid Phase Blocking ELISA for vaccine efficacy studies. The molecular tests in this section include Multiplex conventional Polymerase Chain Reaction (PCR), real-time reverse transcriptase (RT) PCR for Influenza A, H5 & N1 and Newcastle Disease Virus, Foot and mouth disease, Classical Swine Fever (CSF), Pigeon paramyxovirus (PPMV) and foot rot disease. Details of tests performed and samples are shown in Table 6.

**Table 6: Sample and test performed in serology, virology and molecular section**

Sample Type	Number	Type of Test	Number
Serum	2216	RBT	380
		Brucella ELISA	1310
		FMD NSP(Rapid)	207
		FMD-Sero-typing (O,A,Asia-1)	16
		CSF ELISA	12
		Mycoplasma ELISA	43
		ALC ELISA	71
		<i>Salmonella pullorum</i>	136
		IBR ELISA	25
		PPR Rapid	6
		Equine Influenza Virus Rapid	9
Tissue/Epithelial/swab	180	Canine parvo virus Rapid	1
		PCR (AI,H5,N1)	107
		PCR NDV	22
		AI Rapid	53
		NDV Rapid	53
		PCR PPMV	2
		PCR FMD	2
		PCR CSF	2
		PT panel ( AI, H5, ND, CSF)	44
Brain	40	Rabies FAT	40
		Rabies Rapid	40
Sample referral (Milk, brain, Fresh droppings, epithelial tissue and serum).		NIAH, Bangkok	1692
		AAHL, Gelong, Australia	25
		HSADL, Bhopal	13
		Pirbright, UK	47
<b>Total</b>	<b>2436</b>		<b>4358</b>

**Significant findings**

A highly pathogenic avian influenza A (H5N1) virus was confirmed through PCR in the samples of poultry birds received from Zangtopelri, Samdrup Jongkhar on 11 March 2018 and Phuntshopelri, Samtse on 19 March 2018. It was also further confirmed at AAHL, Geelong Australia. NCD was confirmed through PCR in the samples from poultry birds received from Samtse and Dagana. The molecular section also confirmed Classical swine fever through real time PCR. Rabies was also confirmed by FAT at serology section from the samples received.

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

#### 4.4.1.5 Bio-safety and Bio-security section

The section facilitated and regulated various bio-safety and bio-security activities in the laboratory.

Followings are the activities carried out by the section:

- Routine Biosafety works
- Completed Technical Backstopping Missions to Participating Laboratories in Asia, conducted by Australian Animal Health Laboratory-CSIRO
- Completion of BSC testing and certification by ESCO/FAO.
  - ✓ Six Biosafety cabinets are operational and safe to use.
  - ✓ Fume hood is operational and safe to use
  - ✓ Three laminar airflow were tested. Two of them are operational and safe to use. One of the three failed.
- The section successfully conducted monthly internal auditing for the national laboratory to ensure good bio-safety and bio-security measures are in place.

#### 4.4.1.6 Clinical services provided

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

A month wise clinical services such as vaccination, treatment and spaying/neutering of domestic animals attended by LSU is shown in Table 7.

**Table 7: Clinical services provided by LSU during the period (July 2016 to June 2017)**

Months		Treatment	Deworming	Vaccination			Sterilization
				FMD	DHHPAI	Rabies	
July		04	07	10	01	08	
August		10	04			05	02
September		11	04			03	
October		03	03				
November			01				
December		03	01		01	02	
January		04			01	03	
February		02				03	
March		01	04			04	
April		08				01	
May		03	02			03	
June		05	03			04	
<b>Total</b>		<b>53</b>	<b>29</b>	<b>10</b>	<b>03</b>	<b>36</b>	<b>02</b>

#### Significant findings

Most of the calls were from veterinary hospital, Thimphu. This service is being provided to supplement the services provided by VH, Thimphu and National Animal Hospital, Chubachu, Thimphu. Most commonly attended cases were milk fever, manual removal of retained placenta and immunisation against Rabies, DHHPAI & Swine Fever.

#### 4.4.2 Introduction of new test

**In this financial year 2017-2018 the following new tests were introduced:**

**1. Rapid Tests-Pet animals**

- a. Canine Distemper
- b. Canine Leptospirosis
- c. Ehrlichia canis
- d. Heartworm
- e. Canine Anaplasma
- f. Feline Pan Leukopaenia

**2. Molecular tests**

- a. Real time RT-PCR for FMD
- b. Real time RT-PCR for Pigeon Paramyxovirus
- c. Real time RT-PCR for CSF
- d. Conventional PCR for Brucellosis

**3. Serology**

- a. Rapid test for Brucella antibody
- b. Rapid test for combined AI and ND
- c. Antibody ELISA for PPR

**4. Toxicology**

- a. Rapid test for aflatoxin

#### 4.4.3 Samples referred to international laboratories

Sl.No	Referring Laboratory	Sample type and Test	Total Number
1.	Australian Animal Health Laboratory, Geelong, Australia	Swabs - HPAI & ND	14
		Pigeon sera - PPMV1	123
2.	National Institute of High Security Animal Disease, Bhopal, India	Swabs -HPAI	14
3.	National Institute of Animal Health, Bangkok, Thailand	Serum - Brucella	188
		Tissue - Brucella	18
		Milk - Brucella	52
		Nasal Swab - Brucella	3
		Urine - Brucella	2

4.	Pirbright Animal Health Laboratory, The United Kingdom	Epithelial tissues & vesicular fluid- FMD	33
5.	Mahidol/Chulalongkorn/ National Institute of Animal Health, Kasetsart, Thailand	Swabs Serum Whole blood(Tiger)	4 1 1
6.	Institute of Parasitology, University of Zurich, Switzerland	Parasitic egg-Taenia Cysts( Bovine, Human, Tiger)- Taenia and <i>Echinococcus granulosus</i>	50 10
7.	AFIRMS, Thailand	tick samples	20
8.	NAIH, Thailand	Serum Milk	1216 191
9.	Pirbright Animal Health Laboratory, The United Kingdom	Epithelial tissues	11
	Total		1951

## 4.5. Others activities

### 4.5.1 Laboratory assessment, quality assurance audit, proficiency testing and backstopping

Technical Backstopping Missions to Participating Laboratories in Asia under project OSRO/RAP/402/USA; DATE: 8-11th January 2018  
STAFF: Andrea Certoma, Australian Animal Health laboratory-CSIRO

#### Summary

NCAH is working towards ISO17025 accreditation, they are making improvements to their infrastructure and work practices to achieve this goal. Staff should be commended for their efforts at implementing changes to improve their work practices. During this past year a new real time machine an Applied Biosystems QuantStudio5 has been purchased and staff attended a two day training session with a company technician. A key staff member Puspa Sharma is on extended maternity leave which has left a gap in technical expertise in the Virology/Bacteriology group. A new staff member has been appointed to assist with this and is due to start in the first quarter of 2018. Testing for the 2018 PT panel will be performed by Kelzing Lhamo with the support of two technicians.

#### Post Mortem Room

Changes for rabies sample collection and processing are being implemented as a result of the backstopping visit report dates 28 October 2016 as well as a visit by the



International Health regulatory body which recommended that cattle and dog heads for testing be dissected in the laboratory within a Class II cabinet and not in the field as per the current practice

Item	Improvements to PM room following observations from 2016 backstopping visit
1.	New PM table has been installed. Liquid waste drains into a bucket containing Virkon S under the table. Waste is treated for 10min as per manufacturer's recommendation. This liquid is put down the sink in the PM room
2.	Sealed drains/plumbing has been installed from the PM room to the soak pit
3.	A new desk and chair is available within the PM room for paperwork to be filled out. These records are kept in this room
4.	Floor and walls have been tiled thus sealing the floor and half way up the walls
5.	Solid waste from the post mortem room is autoclaved before disposal
6.	4oC cool room has been repaired, cracks sealed and compressor fixed. It is for the storage of carcasses if needed Reagents are no longer stored in this cool room
7.	New 4oC reagent fridge has been installed for clean kit storage
8.	New Class II cabinet has been installed. Avian submissions as well as dog heads for rabies testing are now processed within this cabinet and not on the post mortem table.
9.	Samples once collected are placed in a tube, the tubes are wiped down with 1% Virkon placed in a plastic bag. The sample bags are transported to the laboratory in a cool box with ice blocks.

## Molecular Laboratory

Two days of the visit were dedicated to trouble shooting PCR assays and training to (Mr. Dawa Tshering, Mr. P.B.Rai and Ms. Kelzang Lhamo) on the new Quantstudio 5 (QS5) machine. Initially with master mixes for Type A and H5 together with an Ag Path kit from AAHL was used to initially evaluate the machine. This run was successful as assessed by the AAHL positive control with a recorded Ct of 27.5 which is within the expected range. The machine works well when AgPath kit is used with primers and probes specifically prepared for this kit.

## Proficiency Testing

NCAH has had difficulty in consistently reporting on regional PT panels due to a lack of operational real time machines. In previous years they have relied on an ABI7500 located in the Human Health laboratory. Progress has been made with the purchase and delivery of a QuantStudio5 machine in mid-2017 to NCAH. Currently the laboratory is waiting on the delivery of specific consumables and an Agpath enzyme kit to conduct initially the avian PT panel for Type A flu followed by H5 subtype testing. It is hoped

that the laboratory will submit results for the 2017/18 PT round. Follow up communication by Andrea Certoma with the regional representative from Applied Biosystems Alison Digney and PT co-ordinator Gemma Carlile has resulted in a shipment of 0.2ml tubes and a retainer for the QS5 machine and an AgPath kit being organised for the purpose of setting up this new machine and the performance of the 2018 PT panel

### **Equipment Calibration**

Calibration of pipettes by commercial testing agencies (eg in India) is expensive and time consuming. Colourimetric dye or Gravimetric calibration of laboratory pipettes could be introduced. There is an ELISA plate reader available although this would need to be calibrated as well and a 5 figure balance is available in other institutions in the city. Consideration should be given to establishing a procedure for repairing or replacement of pipettes which fail calibration. With specific guidelines for the removal of pipettes from service. SOP's for such procedures will need to be written

### **Further staff training**

It would be of great benefit for staff if financial support can be found to send them for in-depth molecular training in the technical aspects of primer/probe rehydration, master mix preparation, the choice and use of real time enzyme kits specifically focusing on concentrations of primers and probe for different kits on the market.

### **Key Observations and Recommendations:**

Date: 8 to 11 January 2018

<b>Item</b>	<b>Observation</b>	<b>Recommendation</b>
1.	The new QS5 machine is fitted with a 0.2ml block	The correct plasticware approved by the manufacturer should be used
2.	Consumables specific for use with this machine were not available/purchased or left by the technician after the training session	The correct consumables approved by the manufacturer should be used
3.	Molecular staff were unaware of specific tubes sizes and plates required for use in the QUS5	The correct plasticware approved by the manufacturer should be used
4.	A retainer is needed when tubes are used in the QUS5	The correct plasticware approved by the manufacturer should be used
5.	0.1ml plates do not work efficiently in a 0.2ml block. 0.2ml plates must be used for this block fitted to this machine	The correct plasticware approved by the manufacturer should be used
6.	Unable to spin down strip tubes or plates to mix sample and master mix prior to loading onto the real time machine	The purchase of a benchtop centrifuge with a strip adaptor for plates should be considered to improve performance

7.	Staff do adhere to the described workflow from clean to dirty rooms, but racks appear to be moved between molecular rooms	Moving of small equipment like plate/ tube racks from dirty to clean rooms should be avoided. Plastic racks can be decontaminated in a 1% bleach bath and rinsed before returning them into the extraction room
8.	Extracted RNA is stored at -20oC until testing	RNA can degrade at -20oC and storage at -80oC is recommended for both test and positive control samples
9.	No plate or strip tube centrifuge adaptors are available in the amplification/gel room	If plate format is adopted for real time assays the master mix and sample cannot be mixed. This may affect the performance of the assays
10.	Quant Studio 5 machine testing was conducted. Using type A primers and master mixes and Ag Path enzyme kit from AAHL and new positive controls in 8 tube strip format.	During this run there was a power outage, the machine had UPS which allowed the run to continue.
11.	Reuse of 96 well real time plates	There are tendencies to reuse partially used 96 well plates which means the plates are returned to the extraction room from the gel/PCR room where the QS5 machine is located for loading of master mix and new samples. With this process there is a potential to contaminate the extraction ClassII cabinet with amplified product increasing the risk of cross contamination. For small number of samples an alternative is 0.2ml tubes specifically for the QS5 machine and a retainer plate
13.	Master mix preparation primer and probe reconstitution	Staff require further training in this area to improve their understanding of real time assays
14.	QC tracking of positive controls	There was evidence of positive control performance in laboratory books but very few runs have been done to date

#### **4.5.2 Laboratory Information Management System (LIMS)**

As recommended during the laboratory co-ordination workshop, is very essential for an efficient delivery of laboratory services both for diagnostic or research activity. The database (LIMS) developed was partial and the features like tests categories section wise is not incorporated, it was decided to incorporate the tests details sectionwise. The results was also designed to view screenshot and also printable. The results can also be viewed by the users with the password access provided. However, finalization of the program needs to be conducted to rectify issues and glitches

#### **4.5.3 Report on National Stakeholder meeting on Developing Stepwise Control of Brucellosis in Bhutan**

##### **Background**

Brucellosis is a highly contagious zoonosis caused by Brucella organisms. It is a World Organization for Animal Health (OIE) listed disease. The Food and Agriculture Organization of the United Nations (FAO) is promoting the development and implementation of stepwise/progressive approaches to the prevention and control of animal and zoonotic diseases. FAO-APHCA/DLD/CDC Brucellosis write-shop for the development of a Stepwise Approach for Brucellosis Control in Asia-Pacific Region was held on February 20-21, 2018 at the National Institute of Animal Health, Bangkok, Thailand. The brucellosis experts and participants from Bhutan, Malaysia, Myanmar and Thailand presented the relevant topics related to brucellosis prevention, control and activities in each country. The stepwise approach was reviewed and discussed to formulate the framework for developing control and prevention strategies. The participating countries are expected to apply the framework and identify a stage that corresponds to their situation. The stepwise approach is a guideline for countries to improve brucellosis control and prevention activities step-by-step from their current status until self-declaration for brucellosis freedom. As a follow up activity, the stepwise approach shall be reviewed and adapted to suit the prevailing situations in each country during brucellosis stakeholder consultation meeting in each participating country.

As scheduled the National Stakeholder Meeting on Developing Stepwise Control of Brucellosis was conducted on June 18-19, 2018 for Bhutan. The meeting was attended by international participants from France, Thailand, Australia and representative of Food and Agriculture Organization, Thailand. The national attendees were from livestock policy, experts from animal health and production, farm managers, agriculture and food regulation and public health.

##### **Objectives**

1. To identify current situation, gaps of brucellosis control and prevention;
2. To draft stepwise approach for brucellosis control and prevention;
3. To discuss and recommend way forward to the drafted stepwise approach;

**Expected outputs**

1. Current situation and gaps understood;
2. Drafted stepwise approach plan for prevention and control of Brucellosis
3. Identified roles and responsibilities in brucellosis prevention and control
4. Identified way forward to enhance brucellosis control and prevention

**Areas of expertise of participants**

1. Livestock policy (national and international)
2. Animal health (national and international)
3. Livestock production (national and international)
4. Government cattle farm management
5. Public health
6. Agriculture and Food regulation

**Funding source**

Food and Agriculture Organization in collaboration with Department of Livestock Development, Thailand financially supported in organizing the meeting. The support included:

1. Travel support for international experts
2. Per diem for workshop participants at national rate
3. Hotel accommodation for international experts
4. Working lunch for participants

**Organizer**

National Centre for Animal Health, Serbithang, Department of Livestock, Bhutan

**Resolution**

Resolutions from the National Stakeholder Meeting on Developing Stepwise Control of Brucellosis in Bhutan conducted on June 18-19, 2018 are as follows:

1. General resolutions
  - a. Recognizing the importance of control of brucellosis among ruminant herds in Bhutan and its zoonotic risk, participants agreed on the need to take up a strategic plan for prevention and control of brucellosis in line with the stepwise approach. The meeting decided to develop a stepwise prevention and control plan for brucellosis for Bhutan.
  - b. Recognizing that animal handlers, animal health workers and farm workers are at considerable risk of acquiring brucella infection from infected animals, the meeting was informed that an expert from the World Organization for Animal Health (OIE) and the Department of Livestock will revisit the Livestock Act 2001 to include legal provisions to protect and compensate people handling animals affected with brucella and other zoonotic diseases.



- c. Recognizing the importance of baseline data for initiating brucellosis surveillance, the meeting agreed that the data from recently completed risk-based brucellosis surveillance data shall be analysed and determine its usefulness in designing future research programmes on brucellosis.
  - d. Recognizing the importance of comprehensive study on brucellosis, the meeting agreed that a nationwide survey of brucellosis using milk from different epidemiological units (farmer groups and villages) using milk test be conducted. If the samples test positive, the positive animals/epidemiological units should be traced and retested using other confirmatory tests to confirm the infection. Based on the survey findings control plan should be developed and implemented nationwide.
  - e. Recognizing the risk of infection and spread of disease to other animals/farms in the country, animals on the government cattle breeding farms shall be vaccinated against brucellosis. Calf-hood vaccination at the age of 3 to 6 months should be done using Strain-19 vaccine to be administered through the conjunctival route. The adult animals should be vaccinated using RB-51 vaccine strain at 12-18 months of age (before pregnancy) and revaccinated after calving if necessary. However, pregnant animals shall not be vaccinated.
  - f. Recognizing the importance of monitoring infection status of brucellosis, pregnant animals in the infected farm (National Jersey Breeding Centre) shall be sampled one week prior to parturition and 2 weeks after calving. Routine sampling of serum and blood shall be done once every two months for the purpose of monitoring infection status.
  - g. Recognizing the risk of spread of infection, breeding of positive animals in the infected farm shall be stopped immediately.
  - h. The meeting also recommended that international agencies such as the FAO and OIE should continue to support Bhutan in its endeavor in successful implementation of the Stepwise Approach for Control of Brucellosis.
  - i. Milk test reagents and kits (Milk Ring Test-MRT and Enzyme Linked Immunosorbent Assay-ELISA) to be sourced from National Institute of Animal Health, Bangkok
2. Specific resolutions from the group work
- a. Understanding the importance of promoting inter-sectoral collaboration, the house recommended that annual conference on emerging infectious diseases including brucellosis involving relevant stakeholders of animal health, public health and wildlife health be held.
  - b. Understanding the fact that importation of live animals is the most likely risk factor to brucellosis introduction in the country, the meeting recommended that stringent mechanisms need to be put in place to ensure that infected animals are rejected at the quarantine station and allowed to import only those tested negative. These animals shall be re-tested again in the field after distribution.

- c. Understanding the importance of quality laboratory testing of brucella sample, it was recommended that the National Centre for Animal Health should coordinate and implement proficiency testing of other laboratories within the country through technical support of National Institute of Animal Health, Bangkok.
- d. Understanding the huge costs and resources required in survey and surveillance activities, the meeting recommended that samples collected for brucellosis surveillance should also be tested for other important diseases and establish baseline data.

## STEP WISE APPROACH

Strategies	Short term	Medium term	Long term
<b>Research and capacity</b>	<ul style="list-style-type: none"> <li>✓ Assessment of KAP on Brucellosis in Livestock farmers, personals, consumers and health officials</li> <li>✓ Survey on sero-prevalence and associated risk factors among livestock farms (cattle, sheep, goat, yaks and buffalo)</li> <li>✓ Conduct risk based surveillance</li> <li>✓ Risk assessment of introduction of brucellosis into brucella free area</li> <li>✓ Include brucellosis test in the existing acute febrile illness sentinel surveillance system</li> <li>✓ Training on research methodology and data analysis</li> <li>✓ Training on epidemiology of</li> </ul>	<ul style="list-style-type: none"> <li>✓ Assessment and performance evaluation of laboratory tests in collaboration with international laboratories</li> <li>✓ Conduct socio-economic impact on brucellosis</li> <li>✓ Cost effectively/benefit analysis of different control options</li> <li>✓ Brucella research/prevalence study in at-risk wild life species</li> <li>✓ Case control studies of brucellosis in human and animals (occupational hazard research)</li> </ul>	<ul style="list-style-type: none"> <li>✓ Genetic characterization of Brucella isolates at biovar level</li> </ul>

	Brucellosis (One Health)
<b>Awareness and extension</b>	<ul style="list-style-type: none"> <li>✓ Conduct advocacy and awareness at different levels</li> <li>✓ Signing of MoU between different stakeholders on brucellosis</li> <li>✓ Awareness and education at farms, schools and different institutions</li> <li>✓ Awareness on quality livestock production system including higher bio-security measures</li> </ul>
<b>Legislation and policies</b>	<ul style="list-style-type: none"> <li>❖ Develop prevention and control plan (including case definition, compensation strategy (test and cull/vaccination + test and cull/ mass vaccination), animal movement, SOPs, mandatory reporting of suspected cases to livestock offices by the owners)</li> <li>✓ Amendment of Livestock Act-2001, and Livestock Rules &amp; Regulation (with inclusion of a section on brucellosis)</li> </ul>

	<ul style="list-style-type: none"> <li>✓ Develop Action Plan in line with stepwise control pathway guidelines (objectives, milestones, road map)</li> <li>✓ Legislation for import of animals-reinforce Bhutan Health Code for import of animals</li> </ul>		
<b>Inter-sectoral collaboration</b>	<ul style="list-style-type: none"> <li>✓ IMCOH-Inter-Ministerial Committee for One Health</li> <li>✓ Task Force at Ministry level-DoL, DoPH, BAFRA, MoHCA, MoEA, DoFPS</li> <li>✓ Sharing of resources between MoH &amp; MoAF on brucellosis</li> <li>✓ Task force at district level</li> <li>✓ Routine information sharing at local, district, regional and national level</li> <li>✓ MoU drawn between relevant stakeholders</li> <li>✓ Collaboration with international agencies</li> <li>✓</li> </ul>	<ul style="list-style-type: none"> <li>✓ Joint Rapid Response Team at different level</li> <li>✓ Common trainings/workshop through One Health approach</li> <li>✓ Occupational disease surveillance for animal health worker</li> <li>✓ Inclusion of brucellosis during annual One Health conference</li> </ul>	
<b>Surveillance and laboratory diagnostics</b>	<ul style="list-style-type: none"> <li>✓ Design and implement nationwide surveillance on brucellosis</li> </ul>	<ul style="list-style-type: none"> <li>✓ Training on Sampling design and collection field staffs</li> </ul>	<ul style="list-style-type: none"> <li>✓ Accreditation and proficiency testing at national level</li> </ul>

	<ul style="list-style-type: none"> <li>✓ Conduct/reinforce surveillance at high risk areas</li> <li>✓ Assessment of laboratory diagnostic facilities at all levels of agencies</li> <li>✓ Passive surveillance for clinically suspected animals</li> <li>✓ Implementation of RBT test at District, Satellite and Regional Laboratories</li> <li>✓ Training of field staffs on SOP for sample collection, packaging and transportation</li> </ul>	<ul style="list-style-type: none"> <li>✓ Strengthen laboratory diagnostic capacities and facilities at different level</li> <li>✓ Development of SOP for laboratory diagnosis</li> <li>✓ Introduction of rapid test kits at field level</li> <li>✓ Development of external and internal quality assurance system</li> <li>✓ National level laboratory to have ELISA, CFT, PCR diagnostic facilities both in animal and human</li> </ul>	
<b>Disease prevention and control</b>	<ul style="list-style-type: none"> <li>✓ Develop strategies (pilot studies, action plan)</li> <li>✓ Develop brucella prevention and control plan (guidelines and SOPs) for both animals and humans</li> <li>✓ Diseases outbreak investigations training and field simulation exercises</li> <li>✓ Testing of animals at quarantine stations and follow up on the health status of animals</li> </ul>	<ul style="list-style-type: none"> <li>✓ Animal identification system</li> <li>✓ Inter- sectoral data sharing</li> <li>✓ Set up of milk pasteurizers in dairy cooperatives and milk testing at collection centres</li> <li>✓ Clear role of different stakeholders</li> <li>✓ Stop imports of animals and enhance livestock production within country through AI and embryo transfer technology</li> </ul>	<ul style="list-style-type: none"> <li>✓ Vaccination strategies</li> <li>✓ Capacity building of veterinarians, para-veterinarians and farmers</li> <li>✓ Farm accreditation along with the incentives for accredited farms</li> <li>✓ Resources allocation</li> </ul>



- 
- |                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                            |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>✓ Cross border movement of animals and border harmonization meetings</li> <li>✓ Public advocacy and awareness</li> <li>✓ Protocol for management of infected herds and culling procedures along with compensation schemes</li> </ul> | <ul style="list-style-type: none"> <li>✓ Establishment of Brucella free nucleus farms for input supply</li> <li>✓ Intensify the regulation on the movement of livestock and livestock products during outbreaks</li> </ul> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
- 

#### 4.5.4 Fleming Fund Project for strengthening AMR surveillance

The UK Government's Department of Health and Social Care has established the Fleming Fund to respond to the global threat of antimicrobial resistance (AMR). The aim of the Fleming Fund is to address critical gaps in surveillance of antibiotic-resistant bacteria in low- and middle-income countries (LMICs) in Asia and Sub-Saharan Africa, which are set to bear the highest burden of antibiotic-resistant infections.



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

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

While there is no formal AMR surveillance in livestock, the National Veterinary Laboratory conducts antibiotic susceptibility testing (AST) on some samples from clinically ill or dead animals. The laboratory has previously conducted a project on ESBL+ *E. coli* in pigs with the support of the Institute of Food Science and Hygiene, University of Zurich, Switzerland. Some studies have been conducted on AMR in *Salmonella* in locally produced and imported chicken meat. Bhutan has been provided with US\$15000 for a WHO Advisory Group on Integrated Surveillance of Antimicrobial (AGISAR) project to test for resistance in *Salmonella spp* in humans, livestock and food. No support other than financial is provided through this project. The regional veterinary laboratories (4 located at Chukha, Wangdiphodrang, Zhemgang and Trashigang) have the capacity to carry out culture and isolation of bacteria and conduct AST on some samples from clinically ill or dead animals. However, it was identified that there is a need strengthen the AST skills and processes in regional laboratories to produce reliable results.

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

The detailed outputs are listed as below:

#### Objective 3: Strengthen AMR and AMU surveillance in food animals

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

- Output 3.9: National Centre for Animal Health (NCAH) shares quarterly and annual reports of AMR and AMU surveillance results with the MOAF AMR and AMU Surveillance TWG, the NATC and the RLDCs.
- Output 3.10: Extended G2C database for electronically recording prescription and antibiotic use data in veterinary districts is recommended.

The grant of about £ 1-1.5m is expected to be implemented for 18 months. A second, subsequent Country Grant may be available to Bhutan, dependent upon successful implementation of the first Country Grant.

#### **4.5.5 Laboratory investigation on mortality on Tiger at WCD Taba**

A male tiger of approx. 5-8 years old was presented for necropsy on 07/04/2018. The external Examinations revealed puncture mark of own canine teeth at either side of the lips, right canine teeth was missing, a small lacerated wound in the scrotum noted. Sub-cutaneous edema was observed in the abdominal region and the neck region- as per the history, the infusions were injected sub-cutaneous. In the digestive System, liver was very dark in colour, haemorrhagic and friable, Stomach was found empty without any food material, however, no abnormalities were detected on the mucosa, extensive haemorrhages were noted in the internal mucosa of small intestine, In the respiratory System, both the lobes of the lungs were collapsed and ecchymosis were observed. In the circulatory & Lymphatic System-heart was slightly flabby and contained clotted blood; mesenteric lymph nodes were slightly enlarged and haemorrhagic, spleen appeared slightly shrivelled. In the uro genital system, both the kidneys appeared slightly enlarged and haemorrhages were noted in the cortex and medulla and urinary Bladder was fully engorged with slightly whitish coloured urine. In the nervous System, externally, no significant findings were observed however, two fluid filled cysts approximately about 1-2cm diameter were detected (one cyst slightly at the left side of the cerebellum and one at the mid part of the cerebellum).

Rapid test for Rabies, canine distemper and canine parvovirus were negative. The condition was diagnosed as Cerebellar Cyst and renal failure probably due to stress condition.

#### **4.5.6 Laboratory investigation on death of takin**

A female takin of about 4 years old was submitted for post-mortem examination on 24/02/2018 at NCAH, Serbithang. The animal was owned by Takin Preserve, Motithang. The external Examinations revealed incomplete rigor-mortis (incomplete stiffening), the carcass appeared well fed. Liver was hard and firm in consistency, gall bladder distended and filled with greenish colored bile, large intestine had inflamed internal mucosa, both the lobes of the lungs were slightly congested and froth were present in the trachea, in the heart, the pericardium was thickened and contained excessive quantities of fluid was noted. Hemorrhages were observed in both

endocardium and pericardium (Fig. A & B). Both the kidneys appeared degenerated, uterus almost involuted to normal size and shape and were found to be containing hemorrhagic locheal contents. However, no remnants of placenta were observed. In bacterial culture, no bacterial growth were observed from the heart and lungs , from Liver, Kidney & Milk- *Enterobacter aerogenes* (natural flora of gastrointestinal tract of animals) and *Staphylococcus saprophyticus* natural flora of gastrointestinal tract of animals). In HP examinations, necrosis of myocardial cells, infiltration with numerous neutrophils and also mononuclear cells noted (Fig C & D), acellular degeneration of hepatocytes were noted in liver, Hydronephrosis and degeneration of tubular cells (Fig. E) were noted in kidneys, diffuse hemorrhages in the alveoli and cellular exudation in the bronchial lumen of lungs( Fig. F)

The condition was diagnosed as acute myocarditis and since, the bacterial findings were not significant, it may not be due to infections. However, other infections cannot be ruled out,



Fig. A Heart gross lesion of hydropericardium



Fig. B Heart gross lesion-Haemorrhages in pericardium

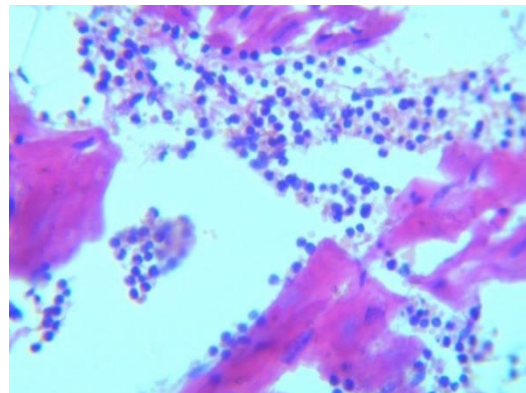
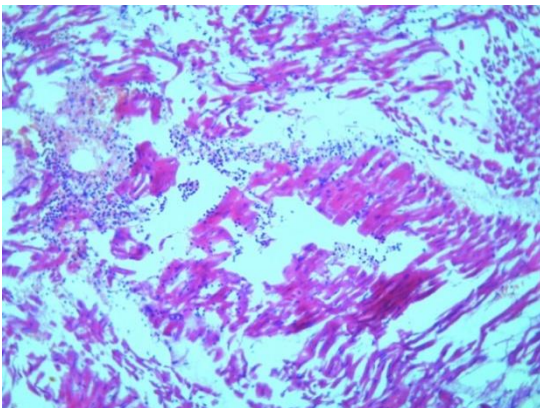




Fig. C Heart 10X: A necrosis of myocardial cells

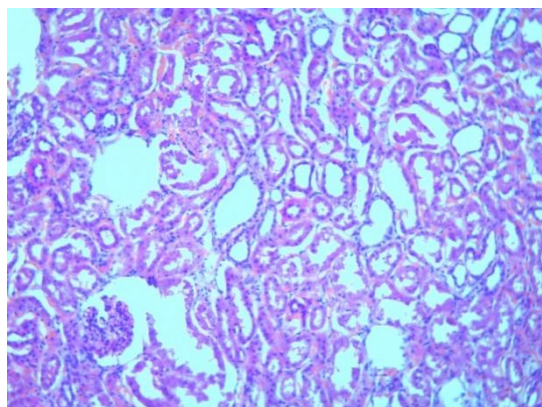


Fig. D Heart 40X: infiltration with numerous neutrophils and also mononuclear cells

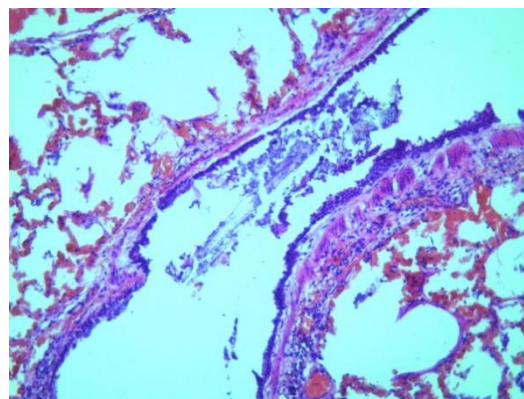


Fig. E Kidney 10X : Hydronephrosis & degeneration of tubular cells

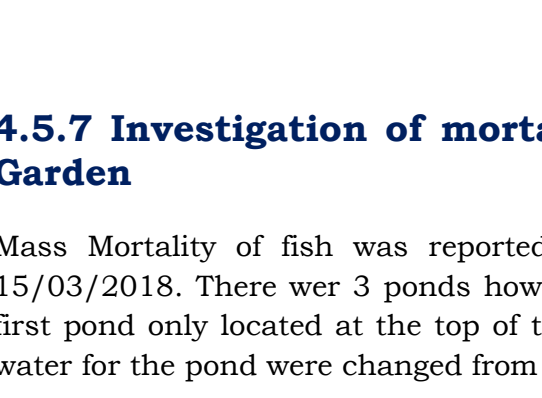
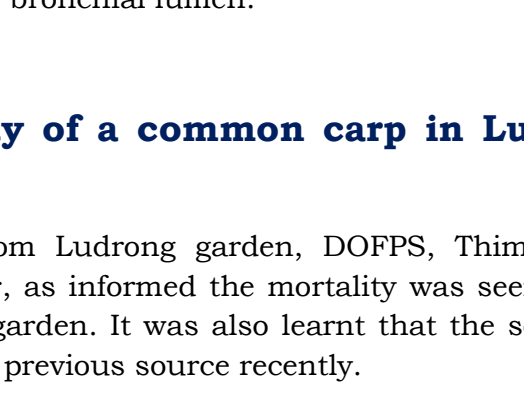






Fig. F Lungs 10X- Diffuse hemorrhages in the alveoli & cellular exudation in the bronchial lumen.



#### 4.5.7 Investigation of mortality of a common carp in Ludrong Garden

Mass Mortality of fish was reported from Ludrong garden, DOFPS, Thimphu on 15/03/2018. There were 3 ponds however, as informed the mortality was seen in the first pond only located at the top of the garden. It was also learnt that the source of water for the pond were changed from the previous source recently.

Laboratory investigation revealed that in postmortem examination, Fat depots noted, indicating well fed, all the fins of the fish had erythematous lesions in almost all the fins (Fig. C & D). Liver was dark in colour and soft in consistency and also haemorrhagic (Fig. B). Gills of all the dead fish was covered with thick mucus and appeared slightly necrosed (Fig. A). Examination of the intestinal contents and gills did not reveal any parasitic eggs or parasite like organisms. Bacterial load from water sample was  $2.0 \times 10^1$  CFU/ml (Within acceptable level), In the bacterial culture, *Micrococcus spp* was isolated (non-significant findings), from the Lungs- *Escherichia vulneris* was isolated, from fins- *Escherichia vulneris* was isolated. From fungal culture, *Microsporium nanum* was isolated from gills and fins (non-significant since, it is pathogenic to pigs and humans). Histopathological Examination revealed diffuse hemorrhages noted and diffuse infiltration of Mononuclear cells (MNC) were observed in the tubular region; Intravascular clotting of blood vessels noted with diffuse hemorrhages in the sinusoids of the liver. The disease was diagnosed as bacterial Sptecemia due to *Escherichia vulneris* infection.

			
<p>A Gills covered with thick mucus and slightly necrosed</p>	<p>Liver dark in colour</p>	<p>Haemorrhages in the tail fin</p>	<p>Haemorrhages in pectoral fins</p>

## **5. Drugs Vaccine and Equipment Unit**

### **5.1 Main mandates of DVEU**

The main mandate of the DVEU is to look after the overall management and co-ordination of Essential Veterinary Drug Program (EVDP) in the country. This mandate is implemented through various functions and activities such as:

- Timely procurement, distribution & storage of veterinary medicines, vaccines & equipment and non-drug items
- Monitoring of drugs, vaccines and equipment supply, stock position, storage at LCS & field levels
- Maintenance of veterinary equipment & cold chain equipment
- Ensure quality control and quality assurance through testing of drugs at the DRA approved laboratories
- Ensure proper management of revolving fund
- Co-ordinate/organize trainings/meetings related to EVDP
- Organize/co-ordinate NVDC meetings
- Liaise with DRA and take follow-up action in regards to drug inspection reports

### **5.2 Human Resources in DVEU**

The DVEU has 4 technical staffs to perform and carry out all the planned activities for the unit as under:

- Dr. Vijay Raika, new Head, DVEU
- Ms. Phuntsho Wangmo, Sr. Extension Supervisor
- Mr. Namgay Dorji, Sr. Livestock Production Supervisor

### **5.3. Key achievements of DVEU for FY 2017-2018**

The DVEU was involved in taking lead role and initiatives in other important activities beside the mandated functions of the unit during the FY 2017-18. The summary of the key initiatives and some of the remarkable achievements for the unit are summarized below:

#### **5.3.1 Regularization of stock of medicines, equipment and non-drug items in LCS, Phuntsholing**

- The physical, ledger and database stock verification of medicines, equipment and non-drug items was carried out in LCS, Phuntsholing. Accordingly, the ministerial approval was sought with valid justifications for differences and regularization of the stock differences.



### **5.3.2 Write-off and disposal of expired and damaged drugs in LCS, Phuntsholing**

- Write-off approval for expired medicines in LCS, Phuntsholing was sought from MoAF and damaged drugs from DoL
- The expired and damaged drugs were processed for disposal as per the pharmaceutical waste management guidelines

### **5.3.4 Training and meeting**

- Training of Trainers on G2C database for Western Region, West Central Region & East region was conducted
- Finalization of G2C Database for Animal Health(EVDP)
- National Veterinary Drug Committee Meeting was conducted at Gelephu on 11 February 2018

### **5.3.5 Procurement of medicines, vaccines and equipment during FY 2017-18**

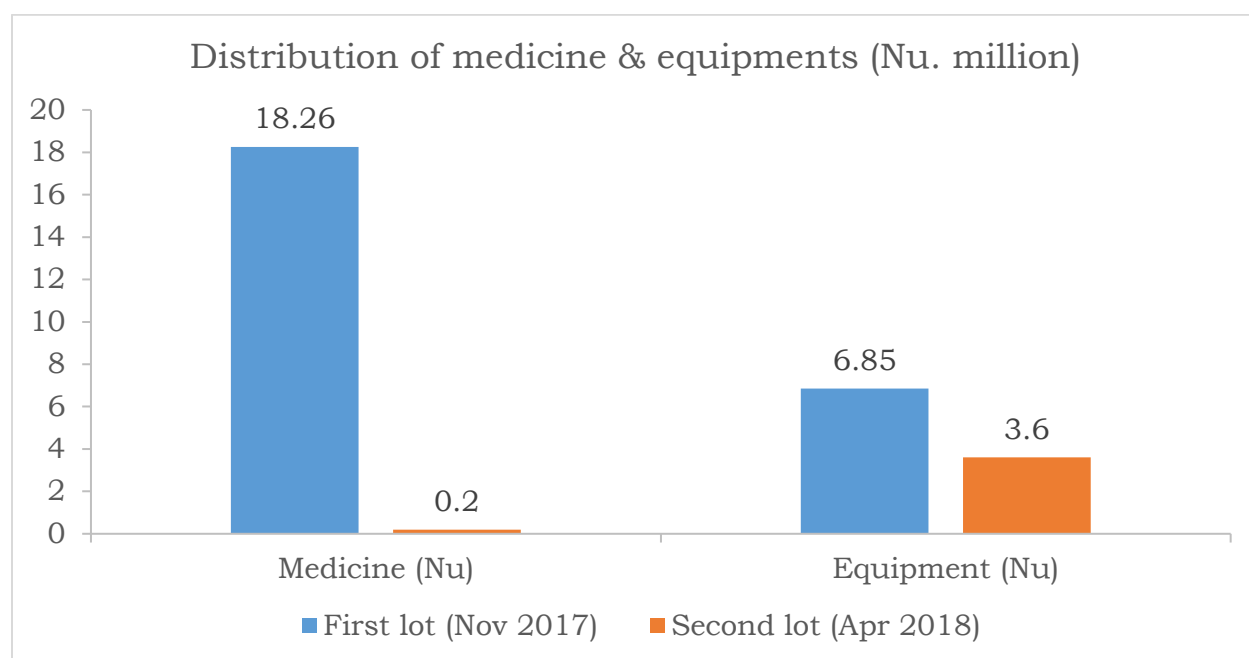
- Fast track tendering and procurement for veterinary medicines and vaccines for the FY 2016-17 was completed by June 2017 as per the revised EVDP management cycle
- Normal tendering and procurement of veterinary equipment and non-drug items was done and completed by November 2017.
- Total tendered amount for medicines and vaccines for the FY 2017-18 was worth Nu. 27 million and the supply order of medicines worth Nu 27 million were given to the substantially responsive bidders.

### **5.3.6 Verification and inspection of veterinary drugs and equipment**

- A total of 10 verification/inspection visits were conducted by the verification team during the FY 2017-18 in order to inspect the newly arrived consignments of veterinary medicines, equipment and non-drug items in LCS, Phuntsholing and NCAH, Serbithang
- Verifications and inspections were carried out for both RGOB and GoI funded medicines/equipment/non-drug items

### 5.3.7 Distribution of Veterinary Medicines, Equipment and Non-drug Items

- As per the revised modality for distribution of veterinary medicines, equipment and non-drug items, the mass distribution was done two times in a year i.e. during December 2017 and April 2018
- Distribution was done up to the DVH point for Dzongkhags and till the respective Central Farms and Agencies premises
- Ad hoc and emergency distribution of medicines and equipment was done throughout the year as and when the requirements were submitted to the DVEU by the respective units



#### Report on 1st Lot Mass Distribution of Veterinary Medicines, Equipment and Non-drug Items for FY 2017-18 (20 November to 29 December 2017) [medicine/equipments worth in Nu]

SL. No.	Name of the Agency	Medicines			Total for medicines	Equipment and non-drug items	Grand Total
		Normal Distributed Drugs	Specialist /Emergency Drugs	Nearing Expiry Drugs			
1	NSBC Bumthang	93847.9	0.00	6756.00	100603.90	36491.5	137095.40
2	NHRDC Jakar	36453.02	0.00	4504.00	40957.02	0	40957.02
3	NBSBC, Bumthang	73726.86	0.00	4504.00	78230.86	0	78230.86
4	NHBC Bumthang	77248.49	0.00	3378.00	80626.49	0	80626.49
5	RMBF Zhemgang	33210.36	0.00	4504.00	37714.36	0	37714.36
6	RLDC Zhemgang	80823.45	0.00	4504.00	85327.45	0	85327.45
7	NPoR&DC Sarpang	137782.4	0.00	0.00	137782.40	0	137782.40

8	CVH & SVL Gelephu	28020.41	0.00	11260.00	39280.41	0	39280.41
9	NPiDC Gelephu	71338.82	0.00	0.00	71338.82	0	71338.82
10	NRDCA Gelephu	52958	0.00	0.00	52958.00	0	52958.00
11	RLDC Kanglung	41145.09	0.00	0.00	41145.09	0	41145.09
12	RLDC Tshimasham	158341.9	0.00	0.00	158341.90	0	158341.90
13	RLDC Wangdue	72532.86	0.00	2252.00	74784.86	0	74784.86
14	CVH Phuntsholing	96380.82	0.00	0.00	96380.82	0	96380.82
15	CRC Wangkha	49847.96	0.00	6750.00	56597.96	450	57047.96
16	NJBC Samtse	140537.11	0.00	6756.00	147293.11	0	147293.11
17	NAH Chubachu	163077.5	0.00	1126.00	164203.50	0	164203.50
18	NPBC, Yusipang	64497.86	0.00	0.00	64497.86	2151	66648.86
19	RPPBC,Lingmethang	79724.43	0.00	0.00	79724.43	0	79724.43
20	NDR&DC, Yusipang	93576.85	0.00	180.16	93757.01	1229.4	94986.41
21	NCAH Serbithang	59921.92	0.00	0.00	59921.92	0	59921.92
22	NNBF,Tashiyangphu	51385.24	0.00	0.00	51385.24	448.5	51833.74
23	NRCR&LF, Haa	48722.87	0.00	0.00	48722.87	0	48722.87
24	RBPC Paro	60395	0.00	0.00	60395.00	0	60395.00
25	RMBF Arong	43160.88	0.00	0.00	43160.88	0	43160.88
26	SVL, Dewathang	54383.63	0.00	0.00	54383.63	0	54383.63
27	SWRRC,Jigmeling, Sarpang	34111.8	0.00	0.00	34111.80	0	34111.80
28	Native Poultry & Heifer Breeding Centre, Lhuntse	107812.53	0.00	0.00	107812.53	81297.45	189109.98
29	Zhemgang	635904.23	0.00	11260.00	647164.23	0	647164.23
30	Sarpang	817026.39	0.00	11260.00	828286.39	0	828286.39
31	Trongsa	693244.68	0.00	11260.00	704504.68	0	704504.68
32	Bumthang	329629.67	0.00	6756.00	336385.67	3700	340085.67
33	Tsirang	809805.53	15465.00	2252.00	827522.53	300	827822.53
34	Dagana	712321.26	0.00	4616.60	716937.86	0	716937.86
35	Wangdue	795546.66	158341.90	11260.00	965148.56	0	965148.56
36	Punakha	637908.84	154011.47	11260.00	803180.31	0	803180.31
37	Gasa	229813.88	0.00	9008.00	238821.88	0	238821.88
38	Thimphu	1314226.5	0.00	6756.00	1320982.50	0	1320982.50
39	Chukha	601155.19	0.00	3378.00	604533.19	0	604533.19
40	Paro	864073.7	0.00	56485.00	920558.70	52118.08	972676.78
41	Haa	303253.51	0.00	11260.00	314513.51	0	314513.51
42	Samtse	1345890.62	0.00	11260.00	1357150.62	0	1357150.62
43	Lhuntse	658355.805	0.00	29258.00	687613.81	0	687613.81
44	Mongar	1719046	0.00	96782.00	1815828.00	0	1815828.00
45	Tashigang	925981.71	0.00	58516.00	984497.71	0	984497.71
46	Tashiyangtse	597250.78	0.00	49508.00	646758.78	0	646758.78
47	Pemagatshel	579320.82	0.00	9008.00	588328.82	0	588328.82

48	Samdrupjongkhar	758023.05	0.00	31510.00	789533.05	0	789533.05
<b>Grand Total</b>		<b>17432744.79</b>	<b>327818.37</b>	<b>499127.76</b>	<b>18259690.92</b>	<b>178185.93</b>	<b>18437876.85</b>

**Report on 2nd Lot Mass Distribution of Veterinary Medicines, Equipment and Non-drug Items for FY 2017-18 (7-20 May 2018) [medicine/equipments worth in Nu]**

SL. No.	Name of the Agency	Total for medicines	Equipment and non-drug items	Grand Total
1	RLDC Wangdue	32825.54	60749.86	93575.40
2	TVH Dewathang	18048.5	45806.75	63855.25
3	RMBF Arong	19340.25	1699.75	21040.00
4	TVH Phuntsholing	82171.52	168450.04	250621.56
5	RPPBC Lingmethang	33963.58	12359.05	46322.63
6	NNBF Tashiyangphu	22761.13	8274.95	31036.08
7	NP&HBC Lhuntse	61799.06	47010.19	108809.25
8	Yak Farm, Tang	17675.4	3700.35	21375.75
9	NHBC, Bumthang	33194.69	22272.37	55467.06
10	NBSF, Bumthang	32166.18	5303.86	37470.04
11	RLDC, Zhemgang	5259.06	99550.01	104809.07
12	TVH & SL, Gelegphu	19830.65	42329.46	62160.11
13	RLDC, Tsimasham	52501.51	77280.33	129781.84
14	NRCA, Gelegphu	31750	37666	69416.00
15	NPiRDC, Gelegphu	40516.33	3984	44500.33
16	NPoRDC, Sarpang	17649.9		17649.90
17	RMBF, Zhemgang	15939.55	12726.94	28666.49
18	RLDC, Kanglung	13373.69	63093.58	76467.27
19	NJBC, Samtse	52854.84	8048.07	60902.91
20	NVH, Thimphu	96471.64	124615.29	221086.93
21	RPBC, Paro	16003	13824.9	29827.90
22	CRC, Wangkha	26790.68	21929.26	48719.94
23	NDR&DC, Yusipang	45508.064	759.68	46267.74
24	RPBC, Yusipang	28346.37	9509.65	37856.02
25	NRCR&LF, Haa	6089.1	7283.46	13372.56
26	NCAH, Serbithang	68900.95	28810.87	97711.82
27	BLDC, Samrang	29316.3	13915.76	43232.06
28	Samdrupjongkhar	322387.77	132470.33	454858.10
29	Pemagatshel	165406.29	179716.9	345123.19
30	Tashiyangtse	250586.32	127510.38	378096.70
31	Mongar	609031.84	134444.9	743476.74
32	Tashigang	424580.08	91538.62	516118.70

33	Lhuntse	180853.66	62952.74	243806.40
34	Bumthang	171328.59	100683.2	272011.79
35	Zhemgang	209087.04	110929.77	320016.81
36	Trongsa	238549.52	113943.71	352493.23
37	Tsirang	298473.96	132988.53	431462.49
38	Dagana	204178.75	99867.19	304045.94
39	Sarpang	287022.72	69811.39	356834.11
40	Lhamoidzingkha (Dungkhag)	79284.87	0	79284.87
41	Samtse	670567.96	272425.22	942993.18
42	Wangdue	286117.21	357973.92	644091.13
43	Royal Herd Punakha	46096.33		46096.33
44	Punakha	218916.19	206124.1	425040.29
45	Gasa	60628.76	73588.38	134217.14
46	Royal Herd Wangdi	63073.17		63073.17
47	Thimphu	377066.8	109486.56	486553.36
48	Paro	351596.43	138446.67	490043.10
49	Haa	123563.49	63064.65	186628.14
50	Chhukha	289343.99	98670.73	388014.72
<b>Grand Total</b>		<b>6848789.22</b>	<b>3617592.32</b>	<b>10466381.54</b>

## 5.4. Key Issues and Way Forward

- Some life-saving/essential drugs and equipments not being quoted by the suppliers
- No rational indenting for veterinary equipment and non-drug items vis-à-vis budget availability
- Over-stocking of expired drugs in LCS and RLDCs
- Weak internal mobilization of nearing expiry drugs and acute shortage drugs
- Weak monitoring and reporting system for EVDP - 2-monthly and 4-monthly monitoring and reporting system for EVDP still not being implemented by the field units
- Lack of robust database for EVDP – G2C database still at system enhancement phase

## 6. Biological Production Unit

### 6.1 Main mandates of BPU

The unit is primarily responsible for the production of viral and bacterial vaccines and other biological. The unit also imports vaccines which are not produced within the country for distribution to the field. The unit is also responsible to provide technical support and monitor the cold chain facilities in the field to ensure the effective storage of vaccine and veterinary biologicals.

### 6.2 Human resource of BPU

The unit is currently staffed with the following officials:

1. Dr. Vijay Raika, Principal Animal Health Officer (Head)
2. Harka Bahadur Tamang, Sr. L.H.S
3. Migma, Sr. Lab.Technician
4. Karma Choki, Asst. Lab.Technician
5. Mr. Sangay Nidup, Lab.Attendant

### 6.3. Key achievements of BPU during FY 2017-18

#### 6.3.1 Vaccines production and procurement

- The unit produced three batches of Classical Swine Fever vaccine totalling to 5700 doses and one batch of Anthrax vaccine.
- The unit procured 10,000 doses (500 vials x 20doses) of Classical Swine fever vaccine Tissue culture origin worth Nu.200,000.00 from Ringpu Bio-Pharmacy, Tianjin, China and 40 numbers of rabbits (weaners)- from the Directorate of Veterinary Services & Animal Husbandry, Shillong, Meghalaya for the regular production of Clasical Swine Fever vaccine
- The unit procured the following vaccines worth Nu. 5770380.00
- The unit spend Nu. 1114380.00 on poultry vaccine and Nu. 4656000.00 for the rest of other vaccines like Foot & Mouth, rabies vaccine, HSBQ and PPR.

**Table 17: Details of vaccines procured during FY 2017-18**

Vaccine type	No. of Doses procured
Gumboro (IBD) vaccine	2100000
ND B <sub>1</sub> vaccine	1000000
ND R <sub>2</sub> B vaccine	600000
Fowl Pox vaccine	750000
Mareks's vaccine	550000
FMD vaccine	240000
HS BQ combined vaccine	81000
Rabies vaccine	32000
PPR vaccine	10000

DHPPi + L vaccine	800
<b>Total</b>	<b>5363800</b>

### 6.3.2 Vaccine distribution

The Biological Production Unit carried out the routine vaccine distribution only once during the current year.

During the distribution it was ensured that:

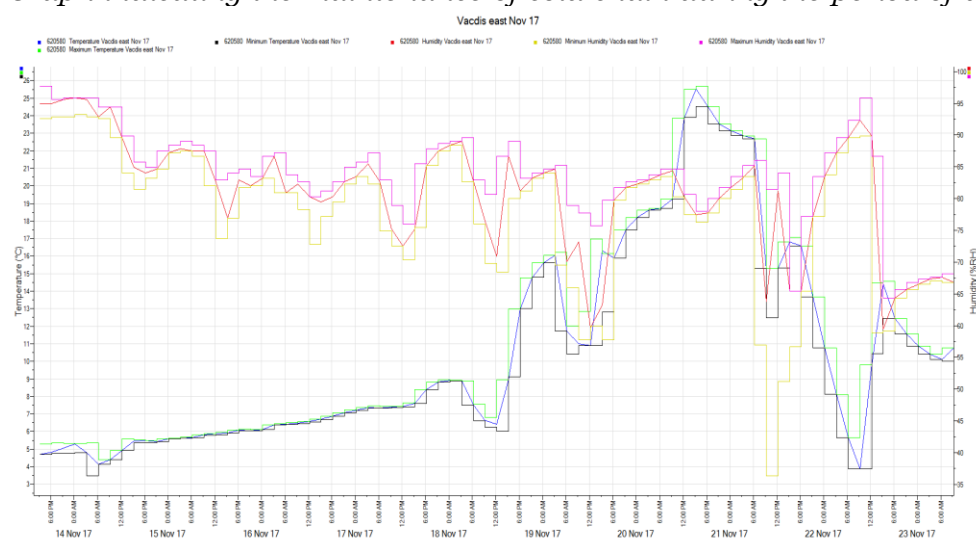
- The vaccines are delivered to the destination
- The temperature of the refrigerated van is well maintained throughout the travel using data logger
- Monitoring of the cold chain equipments ( refrigerators) in all the DVHs (as per standard format attached)are done
- Monitoring of the vaccine stock ,usages ,expiry etc (as per standard format attached)are done
- Issues if any are discussed with the In-Charge,DVH related to vaccines

The unit carried out the first quarter vaccine distribution in November 2017 starting from 14, 2017 to 23, 2017.

During the first quarter vaccine distribution, even the vaccine for the second quarter was included due to the huge demand especially for poultry vaccines.

#### Temperature of the refrigerated van

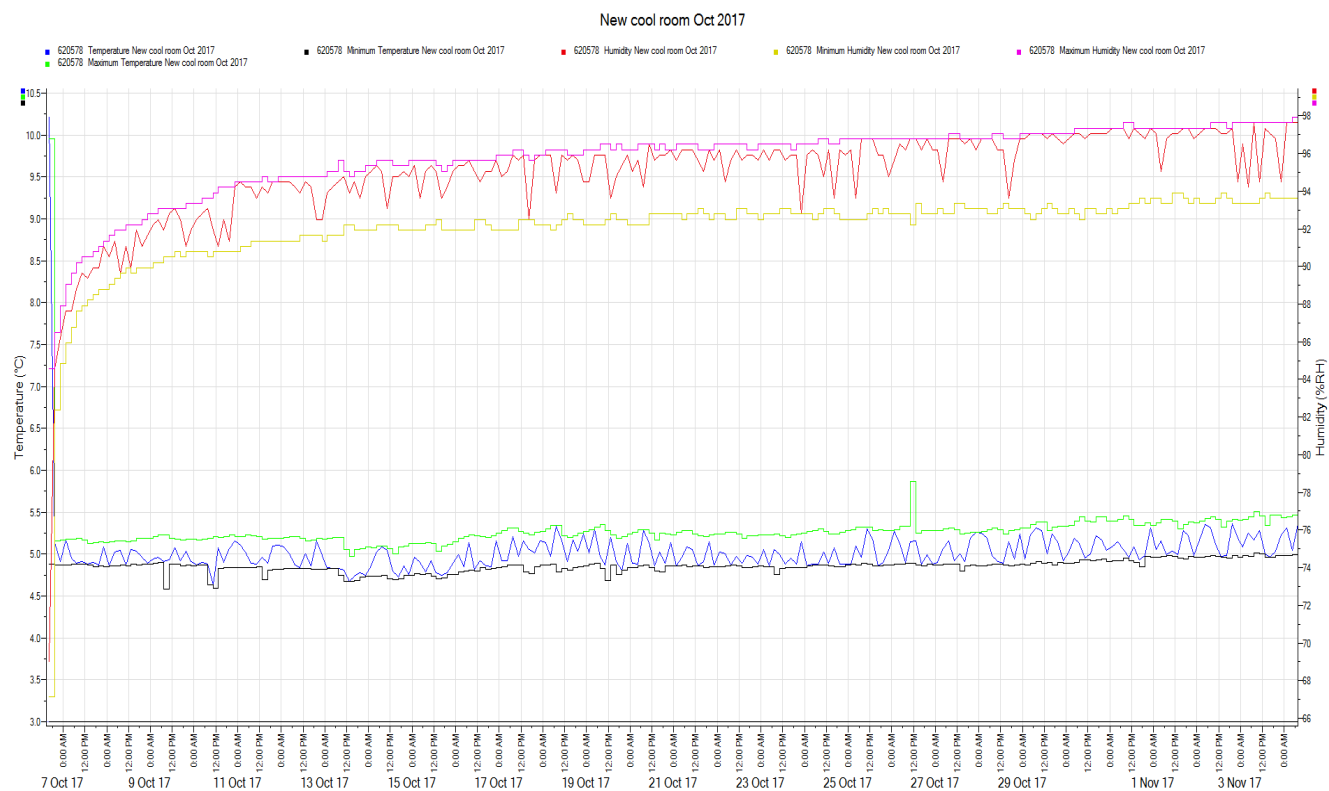
*Graph indicating the maintenance of cold chain during the period of distribution*





Apart from the routine distribution, the ad-hoc and emergency distributions were done as and when there was requirement from Dzongkhag Veterinary Hospitals, Central Units including private poultry farms.

### Temperature of the vaccine storage room using data logger



The graph above indicates the temperature recordings of the vaccine storage room for the period from October 7, 2017 to November 3, 2017 prior to distribution. It is evident that the cold chain of vaccine is maintained within the recommended temperature of +2 to 8<sup>o</sup> Celsius at all times.

All the vaccines that are produced in-house as well as procured from outside are stored in the cool room on arrival until the final distribution.

The unit have distributed various vaccines to different livestock centres in the country during FY 2017-18 (see below)

Sl. No.	Name of Dzongkhags/  Central Units	Locally Produced		Imported										
		Anthrax	SF	FMD Oil	HSBQ	IBD	Fowl Pox	ND B <sub>1</sub>	ND R <sub>2</sub> B	Mare k's	Raksh arab	DHPPI + L	PPR	Tissue Culture CSF
	<i>Dzongkhags</i>													
1	Bumthang			6000	2100	2000	2500		1000		600			
2	Chhukha			10000	510	169600	15000	118000	40000		1000			700

3	Dagana		600	8000	5400	2000		7200	3200		710			500
4	Gasa			1000	510						150	4		
5	Haa			3000	420	5800	7500	2800	4400	2000	1200	20		
6	Lhuentse			6500	3090	20000	5000	8000	7000	10000				
7	Mongar			21400	5100	40000		19000	19000		1000			
8	Paro		100	4600	1050	50000	20000	22000	20000		1300	244		
9	Pemagatshel			3000	3000	40000	2500	20000	10000	5000	700			
10	Punakha			10000	5490	29000	6000	10600	20000		1300	25		
11	Samdrup Jongkhar			10000	5100	40000	5000	12000	10000	20000	1500			
12	Samtse		300	36500	3000	310000	41000	203000	40000	20000	4000	50	800	200
13	Sarpang		600	17500	4500	792000	340000	310000	230000		2200		2000	2300
14	Thimphu			9100	900	34200	14500	22200	15500		900			
15	Trashigang			16500	5100	40000		40000	20000		2000	20		
16	Trashiyangtse			12500	3600	800	2000	600	1000	3000	700			
17	Trongsa			5500	3990	11600	1000	1000	6000		300	20		
18	Tsirang		900	11000	3000	252000	45000	125000	60000		2000	20		2000
19	Wangdue Phodrang			12000	4650	70000	25000	36000	50000	5000	2800	75		
20	Zhemgang			200	3480	21800	5500	11200	17100		1000			120
	<b>Central Units</b>													
21	NDDRC, Yusipang			150	90									
22	BSF, Bumthang			600	450									
23	NJBC, Samtse	200		400	300									
24	NNBF, Trashiyangphu			500	480						20			
25	NPoDC, Sarpang					103000	38000	20600	42000	200000				
26	RPBC, Yusipang		450	1200										1440
27	NSBC, Bumthang													
28	NPiDC, Gelephu		2200	1000										1900
29	Calf Rearing Centre, Wangkha			200	210									
30	RPPBC, Lingmethang		400	650		10000	7000	7000	3500	100000				800
31	RMBF, Arong			100	90						20			
32	RMBF, Wangdigang													

33	RPBC, Paro					14000	1000	6000	5000	53000				
34	National Animal Hospital										2700	31		
35	Private Poultry Farm					78400	5000	42600	14000	150000				
36	RDC Jakar, Samrang			350	360	76600	31000	25600	51000	5000			100	
37	RLDC Wangdue													
38	RLDC Tsimasham										850	25		
39	RLDC Zhemgang										400	70		
40	RLDC Kanglung											6		
41	NDPM & RCP (HSI)										1300			
42	Local Use / Campaign											1		
	<b>Total doses Distributed</b>	<b>200</b>	<b>5550</b>	<b>209450</b>	<b>65970</b>	<b>2212800</b>	<b>619500</b>	<b>1070400</b>	<b>689700</b>	<b>573000</b>	<b>30650</b>	<b>611</b>	<b>2900</b>	<b>9960</b>

**Note:**

1. 20 doses of tissue culture CSF vaccine used in conducting trial at RPBC, Yusipang
2. 20 doses of tissue culture CSF vaccine used in conducting trial at NPiRDC, Gelephu
3. 10 doses of Locally produced CSF vaccine used in conducting trial at RPBC, Yusipang
4. 10 doses of Locally produced CSF vaccine used in conducting trial at NpiDC, Gelephu
5. Vaccines (Raksharab & DHPPi+L) issued reflected under RLDC, Tsimasham is actually issued to CVH, Phuentsholing.
6. Vaccines (Raksharab & DHPPi+L) issued reflected under RLDC, Zhemgang is actually issued to SVL, Gelephu.
7. Vaccines (Raksharab & DHPPi+L) issued reflected under RLDC, Kanglung is actually issued to SVL, Dewathang.

### 6.3.3 Other activities

- Mobilised around 5000 doses of Foot & Mouth disease vaccine from DVHs- Wangdue, Sarpang & Samtse for mass vaccination of cattle during FMD outbreak in Paro dzongkhag in July & August 2017.
- Mobilised around 1000 doses of Anti –Rabies (Raksharab & Rabisin) vaccine from DVHs- Dagana, Sarpang & Samdrup jongkhar during Rabies outbreak in Panbang, Zhemgang for mass vaccination of dogs.
- Monitoring and evaluation of vaccine usage and cold chain inventory carried out in DVH, Jhazam and other RNR RCs within Thimphu dzongkhag during the month of September 2017.
- Conducted 2 days training for Laboratory technicians of NCAH and 4 Regional Livestock Development Centres from July 5th -6th, 2017 on “Sampling protocol for Haemorrhagic Septicaemia” with support from the University of Calgary, Canada. This training was given by Animal Health Experts who were on a visit to initiate technical collaboration for development of engineered Haemorrhagic Septicaemia vaccines in ruminants.
- The staffs from BPU were engaged on risk-based brucellosis surveillance in the country. Mr. Migma, Sr. Laboratory Technician was involved in the Questionnaire survey to understand the prevalence and risk factors of Brucella Infections in

cattle herds in dairy group farmers of Gasa & Sarpang Dzongkhags. Ms Karma Choki, Asst. Lab. Technician was involved in the Questionnaire survey to understand the prevalence and risk factors of Brucella Infections in cattle herds in dairy group farmers of Eastern Dzongkhags – Samdrup Jongkhar, Trashigang, Trashiyangtse, Lhuentse & Mongar respectively.

- The unit carried out a comparative study between the locally produced classical swine fever vaccine & imported Tissue culture classical swine fever vaccine using ten piglets each at two locations: National Pig Research Development Centre, Gelephu and Regional Pig Breeding Centre, Yusipang
- Actively involved in the Evaluation of Tender for Veterinary medicines and vaccines for the FY 2018-19 from June 18 to 27, 2018 at NCAH conference hall, Serbithang.

## **7. National Dog population Management and Rabies Control Project**

The National Dog Population Management and Rabies Control Project was launched in 2009, and has completed a total of 2 phase with the 3rd phase in its first year. During the first phase (September 2009 – June 2012), 35,689 dogs were covered under CNVR program. The term of phase two was July 2012 – June 2015, sterilizing and vaccinating approximately 25,128 dogs and cats. In phase 2 the Community Animal Birth Control Program (CABC) was initiated in order to sustain dog population management (DPM) throughout Bhutan. As per the 2015 National Survey Conducted, the national coverage as of May 2015 stands at 64.1% in urban areas and 44.7% in rural areas.

NDPM & RCP has come a long way for sustainable DPM in Bhutan. However, much needs to be accomplished in terms of streamlining CABC and ensuring on-going impact before the project can be entirely handed over to the RGOB by HSI due to which the project has been extended by a further three years (November 2015 to June 2018).

NDPM & RCP is a collaborative effort of Royal Government of Bhutan (Department of Livestock) and Humane Society International (HSI) with a 50-50 partnership, with funds flowing in both cash and kind in the last 6 years of its launch in 2009.

The NDPM & RCP, NCAH under DoL, MoAF was in its 3rd phase (July 2015-June 2018) as per the MoU signed between DoL and HSI on 9th November 2015. The partnership is based on 65% contribution from RGoB in cash and 35% contribution from HSI which are all in-kind.

The Project Management Unit (PMU) of NDPM & RCP is located in National Centre for Animal Health, Serbithang with the following staff:

- Dr Kinzang Dukpa, Project Director
- Dr Hiruka Mahat, Project Coordinator

The PMU is in constant touch with the HSI Asia office based in Ahmedabad, India with the focal person - Dr Rajesh Kumar Pandey, HSI Coordinator.

### **7.1. Key achievements of the NDPM & RCP during FY 2017-2018**

#### **7.1.1 Dog population Management**

The main mandate of the project is to carry out high volume low cost CNVR based on the concept that community takes ownership of the programme and brings in dogs to the field clinic for sterilization and rabies vaccinations. The project supports all 20 Dzongkhags during campaign through staff (Animal Welfare Officers) deployment, providing modest working lunch and drugs and equipments. From July 2016 till 30 June 2017 a total of 10,137 surgeries and vaccination were carried out both in dogs and cats in the field clinics as detailed in the graph below.

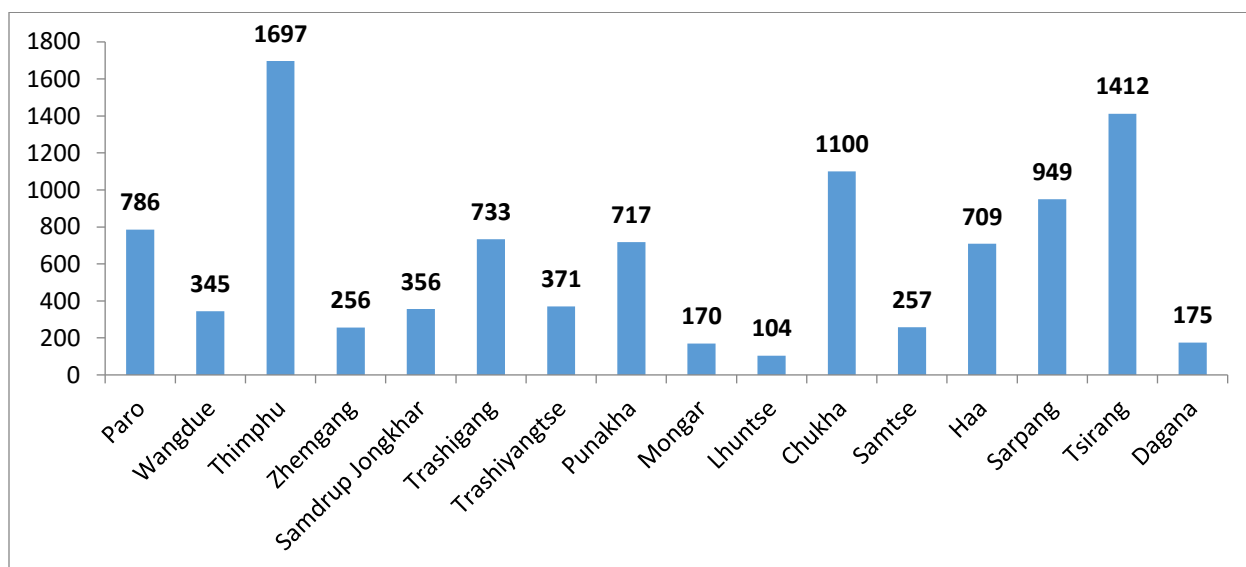


Figure 1: Dzongkhag wise sterilization and anti-rabies vaccination

Another important mandate of the project is to ensure that the dog mediated rabies is prevented and eliminated in the long run. Since the Dzongkhags along the Indo-Bhutan border are endemic to rabies a yearly Mass Dog Rabies Vaccination campaign was organized in a coordinated manner so that the vaccination coverage is high.

In the financial year 2017-18 endemic regions of Samdrup Jongkhar, Pemagatshel and Sarpang Dzongkhags were covered vaccinating 1546 dogs. Chukha and Samtse Dzongkhags were not covered as the local Dzongkhag Livestock sector had covered in the region already. The graph below depicts the numbers of dogs vaccinated against rabies.

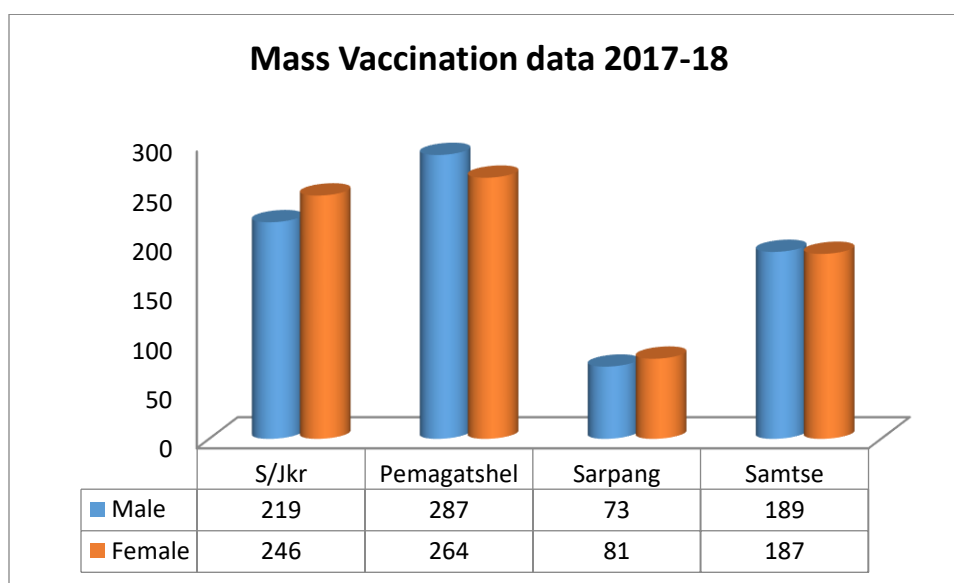


Figure 2: Dogs vaccinated in different Dzongkhag (Males and females).

**Other special achievements:**

- Bi-annual monitoring and evaluation of free roaming dogs in the country by using indicator counts in fixed tracks.
- The project supported containment of rabies outbreaks in Panbang, Zhemgang through logistic support.
- In collaboration with other stakeholders, funded the workshop on Strategic Framework for Elimination of Human Rabies Transmitted by Dogs in Bhutan
- Conducted bi-annual CABC program in Thimphu Thromdey since 2017 for greater coverage and impact in collaboration and financial support by Thimphu thromde.
- Conducted disease surveillance in stray dogs and its spill over infection in wildlife.
- Removal and translocation of problem dogs from Thimphu Dzong areas to the animal shelter in Serbithang.



## 8. Animal Disease Surveillance and Researches

### 8.1 A Review on Antimicrobial Resistance (AMR) in Livestock in Bhutan

Nirmal K Thapa<sup>1</sup>, Puspa M Sharma<sup>1</sup>, Narapati Dahal<sup>2</sup>, Surya B Chamling<sup>3</sup>, Vijay Raika<sup>1</sup>,  
Jambay Dorjee<sup>4</sup>, Ratna B Gurung<sup>1</sup> & Kinzang Dukpa<sup>1</sup>

<sup>1</sup>National Centre for Animal Health Serbithang, Thimphu, <sup>2</sup>Animal Health Division, Department of Livestock, Thimphu, <sup>3</sup>National Poultry Research & Development Centre, Sarpang, <sup>4</sup>Regional Livestock Development Centre, Zhemgang

(Published in Bhutan Journal of Animal Science 2<sup>nd</sup> edition)

#### Abstract

Antimicrobials used widely in human and animals over the decades have been known to become ineffective due to development of resistance by bacterial pathogens known as antimicrobial resistance. The resistance has been attributed to its inappropriate use in humans and livestock. Resistant pathogenic bacteria arising in humans, animals or the environment can spread from one to the other and through trade, travel and migration these resistant pathogens can spread across the globe. The tri-partite approach is made by the World Organization for Animal Health (OIE), World Health Organization (WHO), Food, and Agriculture Organization (FAO) to address such a concern at a global and regional level in combating the AMR. The OIE member states have been recommended to pursue the same issue at the national level.

The studies on imported chicken carcass during 2007 revealed prevalence of *Salmonella* as 13% with *Salmonella enteritidis* serotype (84.62%) and *Salmonella typhimurium* (15.38%). The pathogens were found to be resistant to Nalidixic acid, Amoxicillin and Cephalixin. Similarly, study conducted in the domestically produced broiler chicken carcass during 2016 also detected *Salmonella typhimurium* and *Salmonella paratyphimurium* type B with prevalence of 73.9% and 26.1% respectively with resistance to Tetracycline (95.6%), Trimethoprim (86.9%) and Amoxycillin (65.2%). The investigation of *E. coli* in three government pig-breeding farms revealed (2.4%, 2/83) ESBL producers harbouring beta lactamase genes for (CTX-M-15 and TEM-1) of which two isolates were multidrug resistant (MDR) and belonged to sequence type (ST) ST156 and ST4173, respectively. This indicates the emergence of MDR ESBL producing *E. coli* among breeding pigs in Bhutan. Likewise, common pathogens like *Escherichia coli* (33%), *Staph. Aureus* (19%), *Enterobacter* (15%), *Corynebacterium* (11%), *Streptococcus* (8%), *Pseudomonas* (5%), *Klebsiella pneumoniae* (4%) were isolated from the milk samples collected from the dairy farms from various parts of the country for the study on microbiological quality of raw milk during 2016. The pathogens were found to be resistant to antibiotics like Penicillin G (23%), Ampicillin (20%), Amoxicillin (20%), Erythromycin (17%), Tetracycline (7%), Gentamicin (7%) and Streptomycin (6%). Culture and sensitivity is conducted only at the National Veterinary Laboratory, NCAH on case-by-case basis.

Annually about 631 Kg and 441 Kg of antimicrobials were procured and distributed to the animal health facilities in the country during 2014-15 and 2015-16 respectively. Maximum of oral antimicrobials were procured and distributed by DVEU.

The regulation of antimicrobials for pharmaceutical use is done by the DRA and for the feed additives as growth promoter is done by DoL with the technical guidance of NVDC. One of the main modes of controlling AMR is promotion of its rational use. The DoL has initiated various steps like conducting of awareness workshops, preparation of antibiotic guidelines and Standard treatment guidelines. To support the AMR containment activities, NAPs have been developed for both MOH and MOAF, which has been endorsed by the cabinet very recently.

## **8.2 Identification of Echinococcus spp. in definitive and intermediate hosts in Bhutan**

*1Thapa, N. K., 1Puspa M Sharma, 2Armua-Fernandez M.T., 1Kinzang, D., 1Gurung, R. B. and 2Deplazes, P.*

1National Centre for Animal Health, Serbithang, Thimphu, Bhutan, 2Institute of Parasitology, University of Zurich, Zurich, Switzerland

*Poster presented at The 26th International Conference of the World Association for the Advancement of Veterinary Parasitology, WAAVP 2017, Kuala Lumpur, Malaysia.*

### **Abstract**

Hospital records of cystic echinococcosis in Bhutanese residents indicate the possibility of an indigenous cycle of *Echinococcus granulosus sensu lato*. Therefore, faecal samples were collected from community dogs around slaughter houses and from the capital city of Thimphu (n=138) and in the forest area around a cattle farm in central Bhutan (n=28). Samples were analyzed morphologically for the presence of taeniid eggs by the floatation and sieving method. Further analysis of samples positive for taeniid eggs by PCR/sequence revealed eleven *Echinococcus* sp. causing cystic echinococcosis infections, seven *Taenia hydatigena* and one *Hydatigera taeniaeformis* infections and additionally in nine samples DNA of *Spirometra* sp. was detected (in 9 cases double infections occurred, in 2 cases no PCR confirmation was achieved). Cysts were collected from locally slaughtered and imported beef and, by direct sequencing, seven (one fertile) and 35 cysts (four fertile), respectively, were confirmed as *E. granulosus* (G1-3). One cyst from local and one from imported cattle (both fertile) were confirmed to be *Echinococcus ortleppi* (G5). Sterile cysts were also collected from local yaks (n=10) and all revealed to be *E. granulosus* (G1-G3). The presence of *Echinococcus* spp. in dogs and ungulates in this pilot study and in ongoing investigations indicates the existence of local transmission for both *E. ortleppi* and *E. granulosus* in Bhutan.

### 8.3 A national serosurvey to determine the prevalence of paratuberculosis in cattle in Bhutan following detection of clinical cases

*Ratna B. Gurung\*, Douglas J. Begg† and Richard J. Whittington†*

\*Department of Livestock, National Centre for Animal Health, Thimphu, Bhutan and † Sydney School of Veterinary Science and School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Camden, New South Wales,

*Published in Veterinary Medicine and Science (2018)*

#### **Abstract**

Johne's disease is an economically important ruminant disease predominantly affecting cattle, sheep and goats. The economic losses are due to early culling, reduced growth rate, progressive weight loss and reduced production. It is caused by *Mycobacterium avium* subspecies paratuberculosis (MAP). Johne's disease was reported in cattle in Bhutan, based on clinical signs and histopathology; in the late 1990s samples from one mithun that was suspected to have died due to this disease was confirmed by molecular testing at the Faculty of Veterinary Science, University of Sydney, Australia. However, no detailed study on prevalence of JD has been attempted in Bhutan. Objective of this study was to conduct serosurveillance to determine the national prevalence of Johne's disease in cattle for the period 2013–2014 to provide the basis for planning a future control strategy. A national serosurvey was conducted wherein a two-stage sampling procedure was used with 95% confidence and an error level of 0.05. The sample size required for the survey was calculated using the software-Survey Toolbox for Livestock Diseases, available as Epitools at <http://www.ausvet.com.au>. A total of 1123 serum samples were collected from an administrative structure of 52 villages, 40 sub-districts and 15 districts. Serum samples were tested using commercially available antibody enzyme linked immunosorbent assay. Statistical analysis was performed using GraphPad Prism 5.0. Illustration such as maps was produced using QGIS version 2.18 'Las Palmas'. The mean national apparent prevalence of Johne's disease was found to be 2.31 (26/1123) (95% CI: 0.80–4.50) with an estimated true prevalence was found to be 8.00 (95% CI: 2.00–17.00). Trongsa district had the highest prevalence (12.96) followed by Zhemgang (4.34), Lhuntse (4.25), Sarpang (3.89), Bumthang (3.60), Trashigang (2.67) and Haa (2.63). Prevalence for all other districts was 2.00 or below. Seropositive samples were reported from all over the country with varying levels of seropositivity. In the recent past many more cattle were imported from India to boost dairy production. Nevertheless, the wide distribution of seroreactive JD cattle all over the country is a concern for future control. Therefore, in future, a detailed study on the impact of cattle import with regard to disease incursion such as Johne's disease and other diseases should be undertaken.

## 8.4 Investigation and monitoring of Brucellosis associated abortion in a dairy cattle farm in Bhutan

*Ratna Bahadur Gurung<sup>1</sup>; Kinzang Dukpa<sup>1</sup>; Bijay Raj Rai<sup>2</sup>; Tashi Dhendup<sup>3</sup>*

<sup>1</sup>National Centre for Animal Health, Serbithang, Department of Livestock, Thimphu, Bhutan

<sup>2</sup>Regional Cattle Research Farm, Wangkha, Chukha, Bhutan

<sup>3</sup>National Jersey Breeding Centre, Department of Livestock, Samtse, Bhutan

### Introduction

Bhutan is essentially an agrarian country with about 56.7% of the population engaged in agriculture and the livestock farming forms an integral part of the agriculture system with about 62% of the households rearing livestock. In late 2014, one of the cattle breeding farms - the National Jersey Breeding Centre which supplies breeding stocks reported unexpected rate of abortion (54.17%; 13/24) among the pregnant animals within a short period of time. The cause of abortion was suspected to be due to Brucellosis. The veterinary authority immediately investigated the cause of abortion.

### Materials and methods

Series of sampling and laboratory testing at five different time points were conducted over a period of two years initially to identify the cause and then monitor the status of infection. Serum, milk and abortion materials were collected. Abortion materials were collected as and when abortion occurred in the farm. Serum samples were tested for antibody using Rose Bengal Test (RBT), Enzyme Linked Immunosorbent Assay (ELISA) and Complement Fixation Test (CFT). Abortion materials and milk samples were cultured and isolates confirmed by conventional polymerase chain reaction (PCR) test. Test results were considered positive for 1+ and above agglutination in RBT; SP%  $\geq$  80 in ELISA; titre of IU  $\geq$  20 in CFT and detection of *Brucella abortus* specific amplicon in conventional PCR.

### Results

The mean results (RBT:  $32.80 \pm 2.32$ ; ELISA:  $40.11 \pm 2.42$ ; CFT:  $37.17 \pm 1.01$ ) were fairly similar (Table 1). All abortion materials tested showed the presence of *B. abortus* by culture and PCR. About 22% of the animals were found to be shedding organisms in milk in addition to heavy shedding during abortion

Table 1: Laboratory results from samples tested

Time point	Rose Bengal Test (Positive %)	ELISA (Positive %)	Culture and PCR (Positive %)	Complement Fixation Test (Positive %)
I	31.67 (38/120)	39.17 (47/120)	30.30 (10/33)	NA
II	32.23 (39/121)	49.59 (60/120)	16.67 (8/48)	NA
III	25.00 (32/128)	35.94 (46/128)	29.27 (12/41)	NA
IV	36.92 (48/130)	37.69 (49/130)	13.33 (6/45)	36.15 (47/130)
V	38.18 (42/110)	38.18 (42/110)	NA	38.18 (42/110)
Mean	$32.80 \pm 2.32$	$40.11 \pm 2.42$	$22.39 \pm 4.32$	$37.17 \pm 1.01$

## Conclusions

The investigation confirmed that the abortion was due to *Brucella abortus* infection and the infection persisted for quite a long period of time. Over a period of two years of monitoring at five different time points, the mean prevalence of 22.39% shedders is a real concern for farm as well as the occupational health safety of the farm workers. There is an urgent need to control and prevent the spread of infection. To prevent the spread of infection to human, personal protections of farm workers are enhanced and sale of unpasteurized milk banned. To prevent the spread of infection to other animals, infected animals were isolated and managed separately and distribution of breeding stock put on hold. Public health authority is in the process of screening risk group humans for possible exposure

## 8.5 Determinants of health seeking behavior of animal bite victims in rabies endemic south Bhutan: A community-based contact-tracing survey

*Kinley Penjor<sup>1\*</sup>, Tenzin Tenzin<sup>2</sup>, Rinzin Kinga Jamtsho<sup>3</sup>*

<sup>1</sup>Dewathang Hospital, Department of Medical services, Ministry of Health, Bhutan

<sup>2</sup>Disease Prevention and Control Unit, National Centre for Animal Health, Department of Livestock, Thimphu, Bhutan.

<sup>3</sup>Zoonosis Program, Department of Public Health, Ministry of Health, Thimphu, Bhutan.

## Background

Dog bites are the main source of rabies infection and death in humans, contributing up to 99% of all cases. We conducted a contact-tracing study to evaluate the health seeking and treatment compliance behaviors of people following potential exposure to rabies in rabies endemic south Bhutan.

## Methods

Using information from the rabies post exposure prophylaxis (PEP) register, animal-exposed victims who had visited five hospitals in south Bhutan between January and March 2017 were traced and further data were collected from them using a structured questionnaire. The survey was conducted between April and June 2017. Logistic regression was performed to assess factors associated with PEP-seeking and compliance behavior by the victims.

## Results

Overall, 483 people were interviewed, of which 55% were male and 45% were female. Seventy one percent (344/483) of exposure were due to animal bites of which 80% (n=365/455) were considered to be provoked incidents. Contact tracing revealed that 8% (39/483) of the victims had not reported to hospital following animal exposure.

Common reasons for not seeking PEP treatment included assumptions by the victims that risks of infection were minor if bitten by an owned or vaccinated dog and the biting animal was normal. The victims who are male (OR: 0.36; 95% CI: 0.16-0.77) and educated (OR: 0.41; 95% CI: 0.17-0.96) were less likely to seek PEP, while those that experienced unprovoked bite (OR: 5.10; 95% CI: 1.20-21.77) were more likely to seek PEP in the hospitals. Overall, 82% of the victims sought PEP from the hospitals within 24 hours after exposure. Eighty three percent completed the PEP course prescribed by the physician. The respondents living in urban areas (OR: 2.67; 95% CI: 1.34-5.30) were more likely to complete the prescribed PEP course than rural dwellers.

## Conclusions

Rabies still remains an important public health problem in southern districts in Bhutan. There exist some gaps in PEP seeking and compliance behavior of people exposed to risk of rabies infection from animals. A risk-based advocacy program is necessary to prevent dog-mediated human rabies deaths.

## 8.6 Comparison of antibody responses after vaccination with two inactivated rabies vaccines in dogs in Thimphu, Bhutan

*Tshering Yangchen<sup>1</sup>, Tenzin Tenzin<sup>2</sup>, Sangay Tenzin<sup>2</sup>, Kelzang Lhamo<sup>2</sup>, RB Gurung<sup>2</sup>, Kinzang Dukpa<sup>2</sup> and Tshering Gueltshe<sup>3</sup>*

<sup>1</sup>National Animal Hospital, Department of Livestock, Chubachu, Thimphu

<sup>2</sup>National Centre for Animal Health, Department of Livestock, Serbithang, Thimphu

<sup>3</sup>Department of Animal Science, College of Natural Resources, Royal University of Bhutan, Lobeysa

---

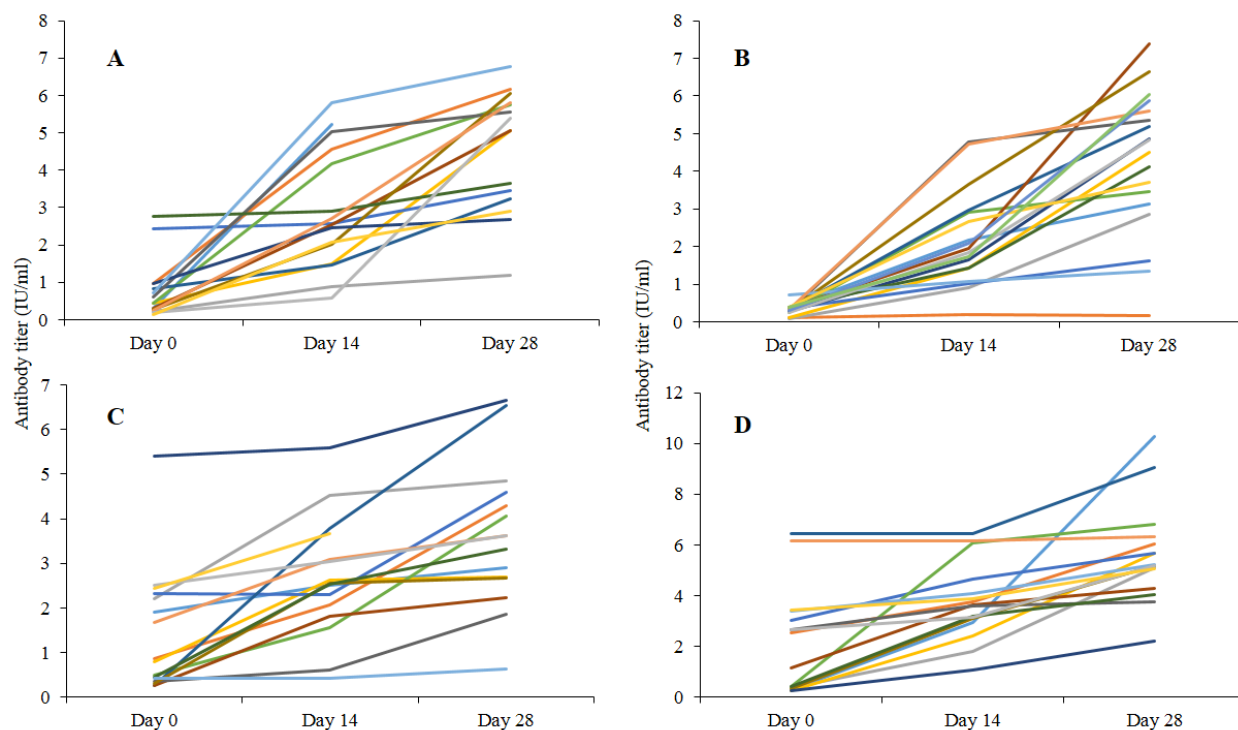
### Abstract

We compared the antibody responses after vaccination with two commercially available inactivated rabies vaccines – Rabisin (Merial, France) and Raksharab (Indian Immunologicals) in dogs in Thimphu. Fifty puppies were randomly assigned to two groups of 25 each and one group was subcutaneously vaccinated with a single dose of Rabisin and other group with Raksharab rabies vaccines as primary vaccination. Similarly, fifty adult dogs were also randomly assigned to two groups of 25 each and each group were subcutaneously vaccinated with a single dose of Rabisin and Raksharab vaccines as booster vaccination. Serum samples were collected on day 0 (prior to vaccination), 14 and 28 from all dogs. Rabies antibodies were measured over a period of 28 days using SERELISA® Rabies Ab Mono Indirect enzyme-linked immunosorbent assay (ELISA).

Of the total 100 dogs enrolled for the study, only 66 dogs (66%) were available for the subsequent sampling. Therefore, a total of 198 blood samples from 66 dogs (3 sample each on day 0, 14 and 28) were collected and analyzed in this study. Eight puppies (22.86%; 8/35; Raksharab group=7; Rabisin group=1) had demonstrated a minimum protective antibody titre ( $\geq 0.5$  IU/ml; range from 0.6 to 2.75 IU/ml) while the remaining puppies (n=27) have showed an antibody titre ranging from 0.1 to 0.45 IU/ml on day 0 (prior to primary vaccination). The antibody titre level had increased after primary vaccination in puppies ranging from 0.21 to 4.78 IU/ml and 0.16 to



7.38 IU/ml under Rabisin group on day 14 and 28, respectively and from 0.59 to 5.80 IU/ml and 1.19 to 6.76 IU/ml under Raksharab group on day 14 and 28, respectively. One puppy (female) have not responded well to the vaccination with a titre of 0.21 IU/ml at day 14 and the titre dropped to 0.16 IU/ml by day 28. In adult dogs under Rabisin booster vaccination group, 56% (9/16) of the dogs had  $\geq 0.5$  IU/ml of antibody titre (ranges: 0.25 to 6.45 IU/ml) on day 0 (before vaccination) and all dogs attained protective titre on day 14 (ranges: 1.07 to 6.46 IU/ml) and on day 28 (ranges: 2.22 to 10.26 IU/ml). Similarly, under Raksharab booster vaccination group, 56% (9/16) of the dogs had  $\geq 0.5$  IU/ml of antibody titre (ranges: 0.27 to 5.39 IU/ml) on day 0 (before vaccination). Excepting one adult male dog that failed to respond to vaccination with a titre of 0.42 IU/ml on day 14 and 0.63 IU/ml on day 28, all other dogs attained protective titre ( $\geq 0.5$  IU/ml) on day 14 (ranges: 0.62 to 5.59 IU/ml) and on day 28 (ranges: 1.86 to 6.66 IU/ml). No significant differences in the mean antibody titres were found in male and female dogs in both primary and booster vaccination group ( $p > 0.05$ ) and none of the dogs showed any adverse reactions to both the vaccines. The study findings showed that both the inactivated vaccines have elicited minimum threshold level for protection ( $\geq 0.50$  IU/ml) responses as per the recommendation of Office International des epizootic (OIE) and World Health Organization (WHO) indicating that the vaccines used in Bhutan are potent and efficient and thus acceptable for primary and booster vaccination against rabies in dogs. In conclusion, the timing of blood tests is a critical factor to determine successful serological test results after rabies vaccination.



**Figure 1:** Rabies antibody titre (IU/ml) responses before and after vaccination in experimental dogs (n = 66). (A) primary vaccination group in puppies using Raksharab vaccine; (B) primary vaccination group in puppies using Rabisin vaccine; (C) booster vaccination group in adult dogs using Raksharab vaccine; (D) booster vaccination group in adult dogs using Rabisin vaccine



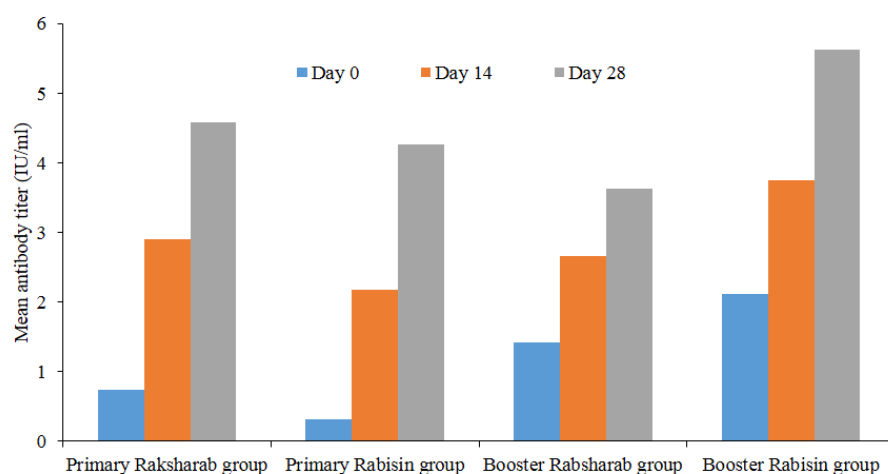
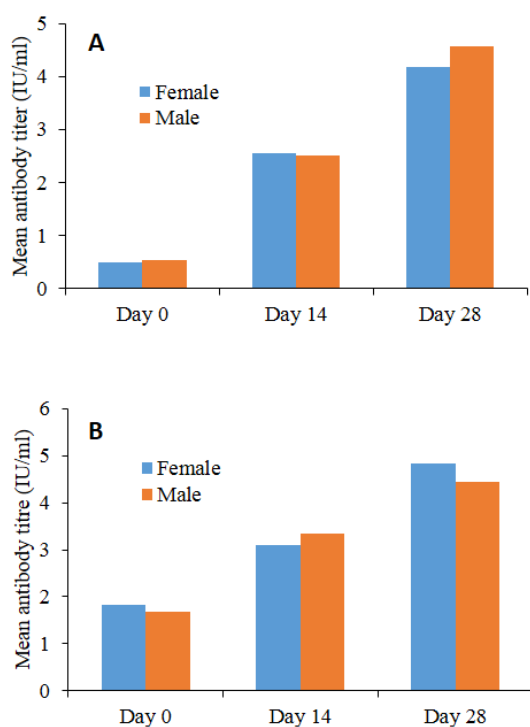


Figure 2: Comparison of mean antibody titer (IU/ml) between primary and booster vaccination group using Raksharab and Rabisin vaccine before vaccination (day 0) and after vaccination (day 14 and 28).



**Figure 3:** Mean antibody titer of male and female dogs before (day 0) and after vaccination (day 14 and 28). (A) Primary vaccination group in puppies using both Rabisin and Raksharab vaccine, and (B) booster vaccination group in adult dogs using both Rabisin and Raksharab vaccine.

## 8.7 Application of geostatistical visualisation and spatial statistics to understand the dispersion of rabies re-emergence in the highland, eastern Bhutan

**Tenzin Tenzin**<sup>1</sup>, Jamyang Namgyal<sup>2</sup>, Sangay Letho<sup>3</sup>, Pema Jamtsho<sup>2</sup>, Karma Tenzin<sup>3</sup>, Naina SinghTamang<sup>3</sup>, Kinzang Dukpa<sup>1</sup>, Karma Rinzin<sup>4</sup>

<sup>1</sup>National Centre for Animal Health, Department of Livestock, Thimphu, Bhutan;

<sup>2</sup>District Veterinary Hospital, Department of Livestock, Trashigang;

<sup>3</sup>Regional Livestock Development Centre, Khangma, Department of Livestock, Trashigang;

<sup>4</sup>Animal Health Division, Department of Livestock, Thimphu, Bhutan

*Abstract accepted for 15<sup>th</sup> Internatinal Symposium for Veterinary Epidemiology and Economics (ISVEE), Changmai, Thiland 12-16, November 2018*

### Abstract

#### Objective(s):

The main objective of this study was to generate hypotheses and understand incursion and spread of rabies (July 2016 through June 2017) in the rabies-free yak herding areas of eastern Bhutan highland (4500 masl) that shares border with India.

#### Materials and methods:

We used geostatistical and spatial statistical methods to generate hypotheses and understand spread of outbreak in the villages of eastern Bhutan. Risk mapping – based on inverse distance weighing by the number of days since the report of first case on 28 July 2016 (day of onset) – was used to visualize the evolution of the outbreak. The mean center and deviational ellipse of these outbreaks was calculated weighted by day of onset and mapped (ArcGIS™ 10.2.2. Spatial Statistics, ESRI Inc.). Local clusters of outbreaks were investigated by estimating Anselin's local indicator of spatial autocorrelation statistic (ArcGIS™ 10.2.2 Spatial Statistics).

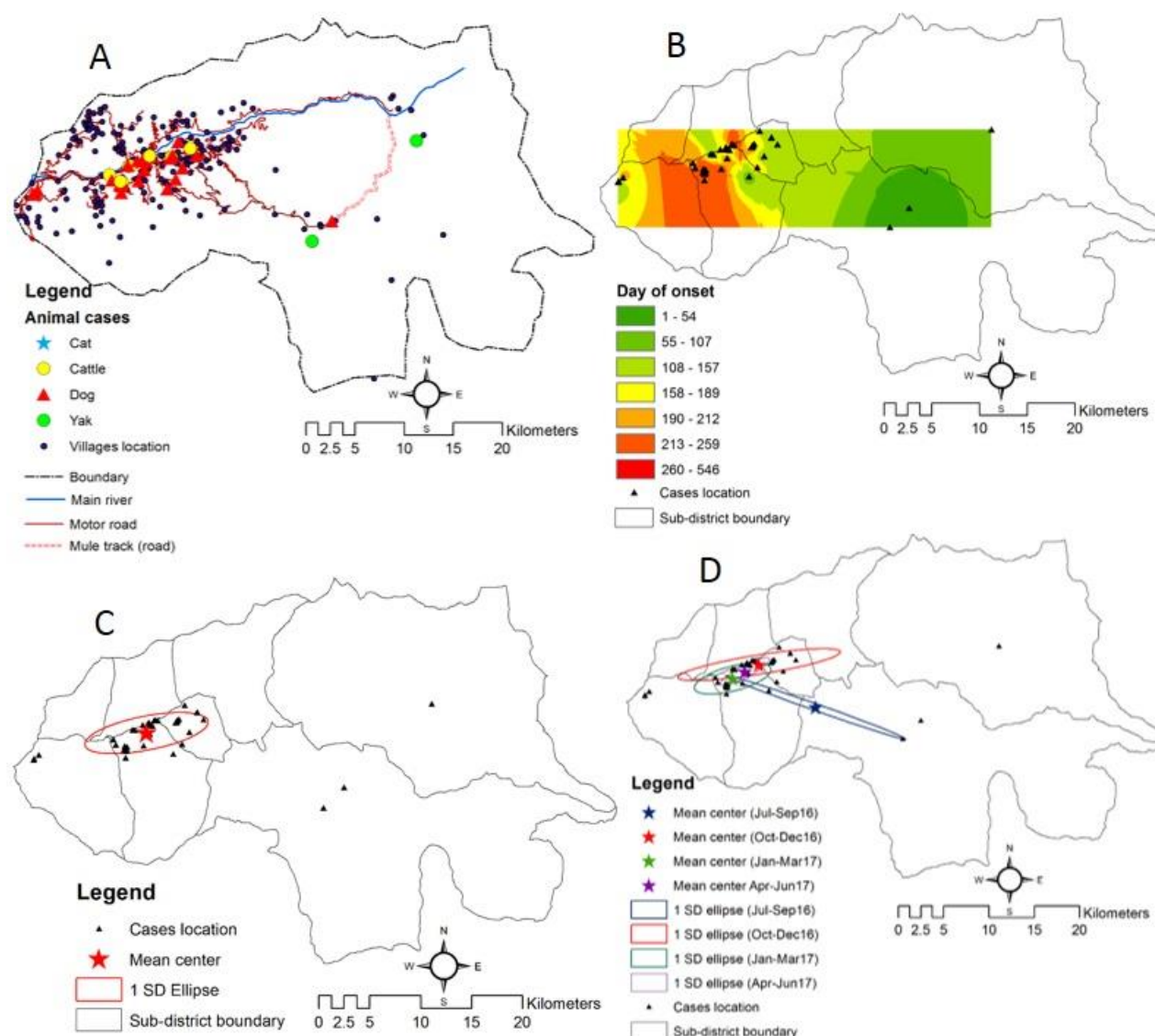
#### Results:

The outbreak resulted in the reported deaths of 38 dogs, 10 cattle, two yaks, one cat and 25 human exposure (category III bite).

#### Conclusions:

The evolution of the outbreak could be characterised into disease introduction, local spread, long-distance disease spread and sporadic outbreaks. The anthropogenic factors – movement of people and dogs – played a role in the introduction and local spread of rabies during July 2016 in the highland, and that the movement of infected dogs from the highland might have introduced the infection into lower part of valley in Sep-Oct 2016. The outbreak was clustered in Rangjung town that have high dog density and then rapidly spread to nearby villages in an east to west direction (Fig 1). The outbreak was finally controlled in June 2017 by implementing mass dog vaccination

and selective elimination of suspected and in-contact dogs through One Health approach. The lessons learned and the experience gained during containment of this outbreak will help for future preparedness planning.



*Fig 1. A) Distribution of rabies outbreaks in animals in eastern Bhutan (outbreaks location by animal species in different villages and the road networks are shown), B) evolution of spread of outbreak interpolated by day of onset (28 Jul 2017–26 Jun 2017), C) the mean center and standard deviational ellipse of the outbreak (28 Jul 2016–26 Jun 2017), D) the mean centers and standard deviational ellipses of the four outbreak phases (Jul–Sep 2016; Oct–Dec 2016; Jan–Mar 2017; Apr–Jun 2017).*

## 8.8 Evaluating rapid immunochromatographic test for diagnosis of rabies in animals in Bhutan

**Tenzin Tenzin\***, Kelzang Lhamo, Purna B Rai, Dawa Tshering, Ratna B Gurung,  
Kinzang Dukpa

National Centre for Animal Health, Department of Livestock, Thimphu, Bhutan

*Abstract accepted for 15<sup>th</sup> International Symposium for Veterinary Epidemiology and Economics (ISVEE), Changmai, Thailand 12-16, November 2018*

### **Abstract**

#### **Objective(s):**

Rapid diagnosis of rabies in animals is important for making prompt decisions for controlling rabies and for advising the exposed people for treatment. In Bhutan, the rapid rabies antigen test (*BioNote Inc, Seoul, Korea*) is being used for surveillance and diagnosis of rabies in different species of animals, and later confirmed using fluorescent antibody test (FAT). The objective of this study was to determine the test sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Rapid Ag Test compared with that of FAT for diagnosis of rabies virus in animals.

#### **Materials and Methods:**

We used 179 brain tissue samples collected during 2012-2017 from various animal species in Bhutan. The rapid test was conducted in the field (Fig 1) using fresh brain tissue samples and the remaining samples were preserved in 50% glycerol saline and immediately shipped to the laboratory for performing FAT. The rapid test was also conducted in some of the preserved samples at the time of conducting FAT in the laboratory. We determined the test sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Rapid Ag Test compared with that of FAT. The test agreement between FAT and rapid Ag test was calculated using Kappa test in Stata version 14.0 (Stata Corp, USA). Kappa measures whether a test correctly predicts an outcome.

#### **Results:**

The sensitivity, specificity, PPV and NPV of the Rapid Ag Test were found to be 92%, 100%, 100% and 84.4%, respectively. There is almost perfect agreement (test accuracy = 94.41%) between FAT and rapid Ag test (Kappa=0.874, p value <0.001).

#### **Conclusions:**

Our results shows that the rapid test is highly specific and sensitive, and the findings are comparable to other studies. A false negative result (5.6%, 10/179) by Rapid Ag test may be due to the relatively low detection limit of the kit and also could have been influenced by the experiences of the technicians who performed the test. Nevertheless, our finding demonstrated that Rapid Ag test

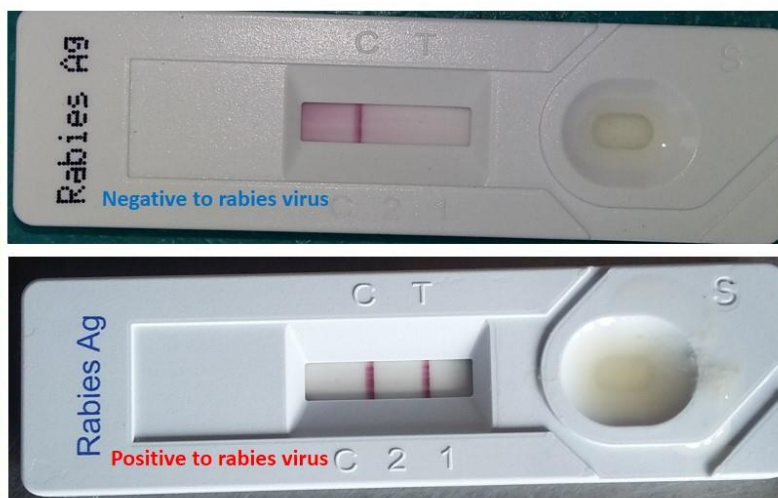
could be used to help make rapid decisions for detecting and controlling rabies in the field since Rapid Ag test is inexpensive, rapid and easy to perform in the field.

### Acknowledgments

We would like to thank all field veterinarians and laboratory technicians for collecting samples from animals suspected of having rabies and also conducting test in the field.



Collecting brain tissue sample to conduct rapid Rabies Ag test in the field



## 8.9 Self-assessment of Bhutans' Rabies Prevention and Control programme using SARE tool

**Tenzin Tenzin**<sup>1\*</sup>, Karma Rinzin<sup>2</sup>, Kinley Penjor<sup>3</sup>, Kinzang Dukpa<sup>1</sup>, Rinzin Kinga Jamtsho<sup>4</sup>, Yoenten Phuentshok<sup>1</sup>, Hiruka Mahat<sup>1</sup>, Kinley Choden<sup>5</sup>, Kuenzang Gyeltshen<sup>6</sup>, Jit Bdr Darnal<sup>7</sup>

<sup>1</sup> National Centre for Animal Health, Department of Livestock, Thimphu, Bhutan;

<sup>2</sup> Animal Health Division, Department of Livestock, Thimphu, Bhutan;

<sup>3</sup> Military Hospital, Ministry of Health, Deothang, Bhutan;

<sup>4</sup> Department of Public Health, Ministry of Health, Thimphu, Bhutan;

<sup>5</sup> Wildlife Conservation Division, Department of Forests and Park Services, Thimphu, Bhutan;

<sup>6</sup> Bhutan Agriculture and Food Regulatory Authority, Phuentsholing, Bhutan;

<sup>7</sup> Royal Centre for Disease Control, Department of Public Health, Ministry of Health, Thimphu, Bhutan

*Abstract accepted for 15<sup>th</sup> International Symposium for Veterinary Epidemiology and Economics (ISVEE), Changmai, Thailand 12-16, November 2018*

## **Abstract**

### **Objective(s):**

Bhutan has implemented One Health approach to eliminate dog-mediated rabies before the global target of 2030. The objective of this study was to evaluate the national rabies control program and determine future needs for rabies control in Bhutan using the Stepwise Approach towards Rabies Elimination (SARE) tool.

### **Materials and methods:**

The SARE tool, developed by FAO and the Global Alliance for Rabies Control provides a self-assessment guide for the evaluation of the current rabies prevention and control activities and to define what is required to move forward for rabies control programmes in respective countries. The SARE tool provides a measurable steps to progress from Stage 0 to Stage 5 towards becoming canine-rabies free country (Fig. 1), and is divided into seven categories and 120 stage-specific activities, viz: legislation (13 specific activities), data collection and analysis (22), laboratory diagnosis (13), information, education and communication (21), prevention and control (26), dog population related matters (13), and cross cutting issues (12). Each stage-specific activities under each category needs to be addressed and then determined whether a country progresses to the next Stage. With the goal of strengthening a One Health-based strategy for rabies control in Bhutan, technical experts from Ministry of Agriculture and Forests and Ministry of Health held a collaborative consultative meeting on World Rabies day – 28 September 2017 – and evaluated our national rabies control program using the Stepwise Approach towards Rabies Elimination (SARE) tool.

### **Results:**

Our assessment indicated that 107 of the 120 Stage-specific activities were accomplished and have progressed to Stage 3.5. Nevertheless, several critical gaps have been identified during the assessment. Based on this assessment, we drafted a National Strategic Action Plan to achieve zero human rabies deaths by 2023 (before the global target of 2030) through One Health approach.

### **Conclusions:**

Our assessment demonstrated the status of national rabies control program in the country and some of the critical issues that needs to be addressed for achieving dog-mediated human rabies elimination in line with the global target.



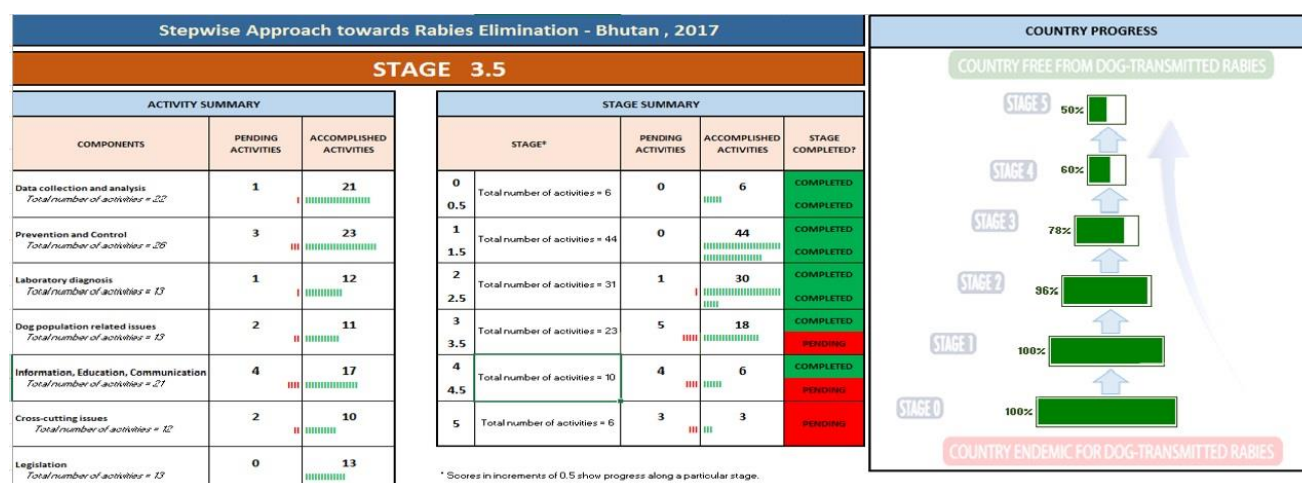
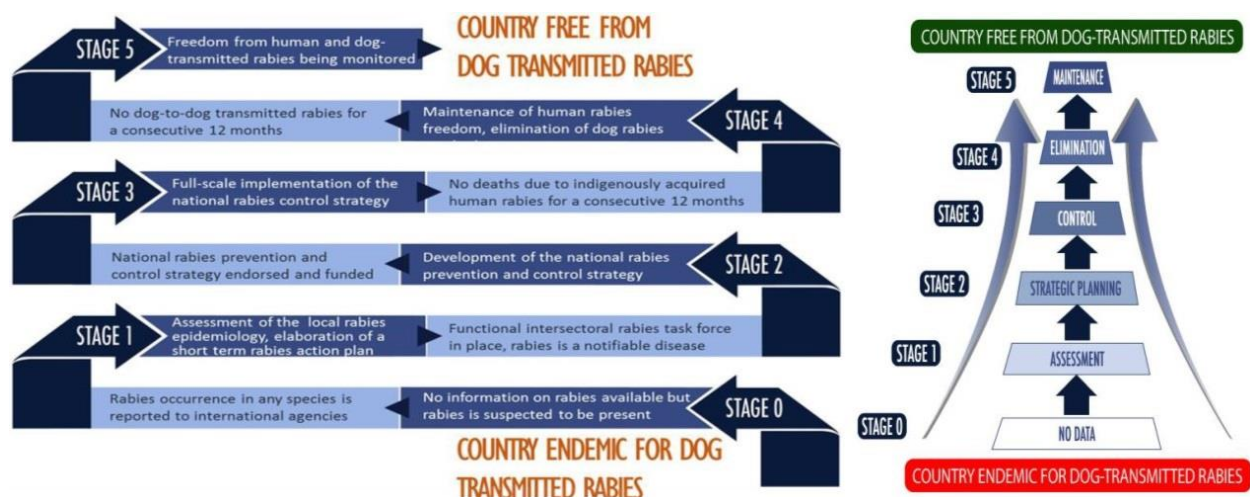


Fig.1: SARE keys to progress through each stage towards freedom from dog-mediated rabies (A and B) and summary of the SARE result in Bhutan (C and D).



## 8.10 A Qualitative Risk Assessment for Re-introduction of Rabies into Rabies-free Areas of Bhutan

Sangay Rinchen<sup>1</sup>, Tenzin Tenzin<sup>2</sup>, Kinzang Dukpa<sup>2</sup>, David Hall<sup>3</sup>, Susan Cork<sup>3</sup>

<sup>1</sup>Regional Livestock Development Centre, Department of Livestock, Tsimasham, Chukha

<sup>2</sup> National Centre for Animal Health, Department of Livestock, Serbithang, Thimphu

<sup>3</sup> Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada

*Abstract accepted for 15<sup>th</sup> International Symposium for Veterinary Epidemiology and Economics (ISVEE), Changmai, Thailand 12-16, November 2018*

### Abstract

**Objective(s):** Bhutan has eliminated rabies from its northern and central regions. However, rabies remains endemic in the South and incursions are reported from other parts of Bhutan that share a porous border with the neighboring states of India. Anthropogenic factors such as increasing human settlements along highways, increased animal transportation, and the complex and changing human-pet relationship have increased the likelihood of rabies reintroduction from endemic areas to the rabies-free areas of the country. The objective of this qualitative risk assessment was to estimate the risk of rabies re-introduction and to identify the most effective risk mitigation options.

**Materials and methods:** The assessment was conducted for three risk pathways under scenarios in which the current risk mitigation measures were in place and in which no risk mitigation measures were in place. They were pet dog pathway, stray dog pathway, and cattle pathway. The likelihood of an event occurring was assigned using available literature. Where gaps in knowledge existed, expert opinion was used.

**Results:** Under the scenario in which no risk mitigation measures were in place, the risk of rabies reintroduction was estimated to be medium for the stray dog pathway with a low level of uncertainty, low for pet dog pathway with a low level of uncertainty, and very low for the cattle pathway with a medium level of uncertainty. When current risk-mitigation measures were included, the risk of rabies reintroduction was estimated to be very low for the stray dog pathway with a medium level of uncertainty, low for pet dog pathway with a low level of uncertainty, and extremely low for the cattle pathway with a medium level of uncertainty.

**Conclusions:** The risk of rabies re-introduction through all the pathways was greater than negligible. These findings highlight the importance of maintaining and enhancing current risk mitigation measures to prevent re-introduction of rabies into rabies low-risk areas.

### **8.11 Community-based survey of ecological and epidemiological risks associated with free roaming and feral dog populations in buffer zone of a protected area in Bhutan**

*Tsheirng Dorji<sup>1,2</sup>, Michel de Garine Wichatitsky<sup>2,3</sup>, Waraphon Phimprapai<sup>3</sup>, Tenzin Tenzin<sup>4</sup>, Karma Rinzin<sup>5</sup>*

<sup>1</sup> Dzongkhag Livestock Sector, Gasa, Bhutan, <sup>2</sup>CIRAD, France, <sup>3</sup>Kasetsart University, Thailand, <sup>4</sup> National Centre for Animal Health, Bhutan, <sup>5</sup>Department of Livestock, Bhutan

*Abstract of a part of Master Thesis for Tshering Dorji (M1)*

#### **Abstract**

Large number of free roaming and stray dogs pose health hazard to human, livestock and wildlife. Understanding community knowledge, attitudes and practices are important for management of free roaming dogs and its impact at human-animal-wildlife interface. Therefore, a cross sectional study was conducted during March-April 2018 in buffer zone of Jigme Khesar Strict Nature Reserve, Haa Dzongkhag, Western Bhutan to evaluate the community knowledge on ecology and epidemiology of free roaming dogs. A total of 139 household respondents from two geogs (Katsho and Uesu) were selected and interviewed of which 57.5 % (n=80) were female and 42.5% (n=59) were male. Majority of the respondents 71% were farmers with mean age of 45.83 years old. Of 34 different lists of diseases in dogs, rabies was ranked high by the respondents (68%). There is positive knowledge, perception and practices on dog population management through animal birth control program. The majority of the respondents believed that free roaming dogs is a problem in the community and felt that it was important to control the dog population. The respondents mentioned that there are around 7.2 feral dogs in each village. The threat (attack and bite) due to free-roaming and feral dogs to wildlife was found to be higher than livestock. The knowledge on transmission of diseases from dogs to human and livestock amongst the respondents were poor indicating that there exists a knowledge gap about impacts of dogs in the community and could be improved by creating an awareness education.

### **8.12 A Case-Control Study of an outbreak of Newcastle Disease in poultry at the farm level in Pemagatshel district, eastern Bhutan**

*Lungten Lungten<sup>1\*</sup>, Tenzin Tenzin<sup>2</sup>, Karoon Chanachai<sup>3</sup>, Tshewang Rabjay<sup>1</sup>*

<sup>1</sup>Thromdue Veterinary Hospital & Satellite Laboratory, Department of Livestock, Dewathang, Samdrup Jongkhar, Bhutan

<sup>2</sup>National Centre for Animal Health, Department of Livestock, Thimphu, Bhutan

<sup>3</sup>Bureau of Disease Control and Veterinary Services, Department of Livestock Development, Bangkok, Thailand

*Abstract accepted for 15<sup>th</sup> International Symposium for Veterinary Epidemiology and Economics (ISVEE), Changmai, Thailand 12-16, November 2018*

## **Abstract**

**Objectives:** Newcastle disease (ND) is a highly contagious viral disease of domestic and wild birds, and have huge economic impacts to the poultry farmers. The objective of this study was to understand risk factors for ND outbreak in backyard poultry farms.

## **Methods**

Using a case control study design, we identified farm level risk factors for ND outbreak in two villages under Pemagatshel district, eastern Bhutan. Thirty households that experienced an ND outbreaks in 2014 and 2016 were identified as case. For each case two controls were selected from the same village. The data were retrospectively collected using questionnaire in February and March 2017. A univariable and multivariable logistic regression model were built to identify risk factors of ND outbreaks.

## **Results**

Ninety household (30 case and 60 control) were selected and interviewed. Twelve of the 48 variables were found to be associated with ND outbreak on univariable analysis while the final model (multivariable analysis) identified three variables to be a risk factors for ND outbreaks. The odd of ND occurrences in a farm that were easily accessible by wild birds was 13.08 times (95% CI: (2.96-57.78) more than those farm that are not accessible to wild bird. Significant higher odds (adjusted odd ratio: 10.66; 95%CI: 2.3-49.5) of outbreak were reported in the farms that have larger flock size (>10) than the smaller farm (<10). The farm which were located near to the road (<500m) were 3.85 times (95% CI: 0.95-15.63) more likely to get the disease comparing to those that are located far from road (>500m). Routine cleaning of litter materials from the shed [(OR: 0.16 (95%CI: 0.04-0.66)] and farmers knowledge on vaccinations [(OR: 0.16 (95% CI: 0.03-0.79)] had protective effects to ND outbreaks.

## **Conclusions**

Improving the farm biosecurity and cleanliness would reduce the incidences of ND outbreak. Awareness and regular vaccination against ND should be conducted to prevent disease outbreaks.

## **8.13 Serological evidence of Rickettsia, Orientia and Coxiella in domestic animals from Bhutan: preliminary findings**

Tshokey Tshokey<sup>1,2,3</sup>, John Stenos<sup>2</sup>, Tenzin Tenzin<sup>4</sup>, Kinzang Dukpa<sup>4</sup>, Ratna Bahadur Gurung<sup>4</sup>, Stephen R Graves<sup>1,2</sup>

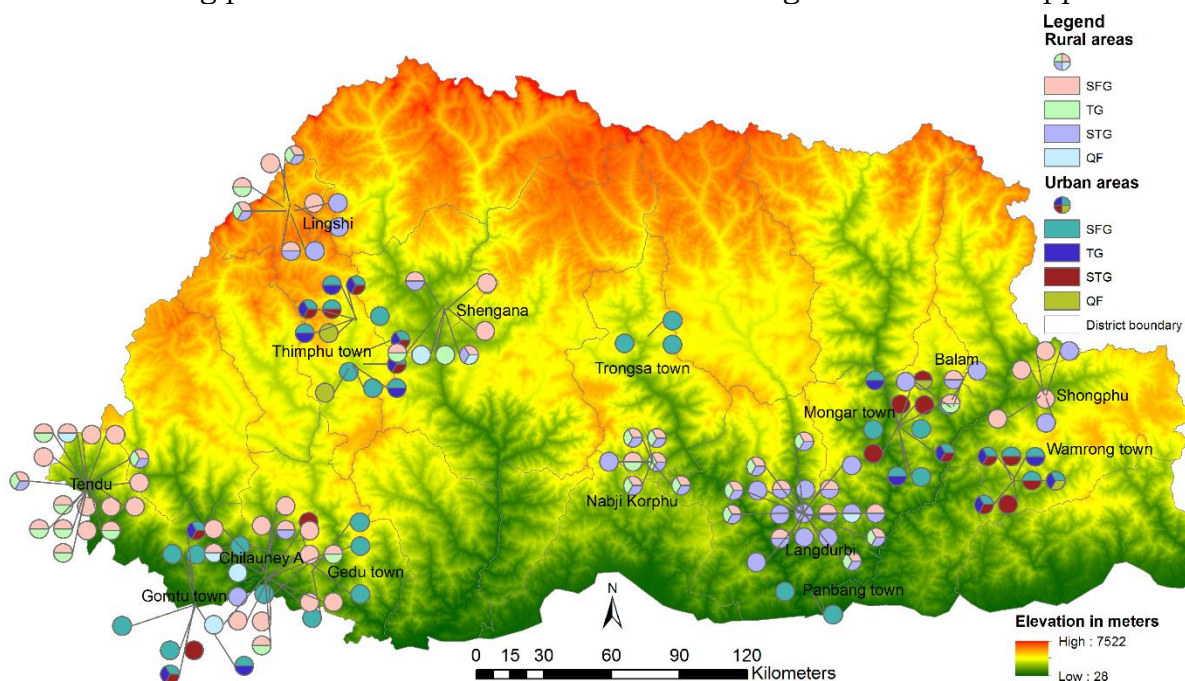
<sup>1</sup>Faculty of Health and Medicine, University of Newcastle, Newcastle, NSW, Australia

<sup>2</sup>Australian Rickettsial Reference Laboratory, University Hospital Geelong, VIC, Australia, <sup>3</sup>Department of Laboratory Medicine, Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Thimphu, Bhutan, <sup>4</sup>National Centre for Animal Health (NCAH), Department of Livestock, Thimphu, Bhutan

*Published in Vetctor Borne and Zoonotic Diseases*

## Abstract

There is no information on rickettsial diseases in domestic animals in Bhutan. We provide the first preliminary serological report of domestic animals against *Rickettsia*, *Orientia* and *Coxiella*. Animal sera were collected from Bhutan and tested in the Australian Rickettsial Reference Laboratory for IgG antibodies against Spotted fever group (SFG) and Typhus group (TG) *Rickettsia*, scrub typhus group (STG) and Q fever (QF). Of the 294 animals tested, 136 (46%) showed serological evidence of past exposure to one or more rickettsial agents: 106 (36%), 62 (21%), 45 (15%) and 11 (4%) being positive against SFG rickettsia, *Orientia*, TG rickettsia and *Coxiella* respectively. Dogs appeared to exhibit the highest seropositivity against SFG (55%) and TG rickettsia (45%), horses against STG (91%) while goats were mostly positive for Q fever (9%). Dogs also appeared to have high risk of being exposed to SFG rickettsia (OR 5.71, 95% CI 3.02, 10.80,  $p < 0.001$ ), TG rickettsia (OR 48.74, 95% CI 11.29, 210.32,  $p < 0.001$ ) and STG (OR 6.80, 95% CI 3.32, 13.95,  $p < 0.001$ ) but not against QF (OR 1.95, 95% CI 0.42, 8.95,  $p = 0.390$ ). Differences in seropositivity rates between animal species may have been significant for SFG, TG and STG but not for QF. The differences in the seropositivity rates of the four infections between districts appeared to be significant for TG and STG but not for SFG and QF. The seropositivity rates of domestic animals to the four rickettsial infections were consistent with similar studies in the human population in the same areas and probably demonstrate a high prevalence of rickettsial diseases in Bhutan. These preliminary findings constitute the baseline data for Bhutan. The findings of this study call for an increased human-livestock sector collaboration in rickettsial diseases research aimed at developing diagnostic and therapeutic guidelines and formulating preventive and control measures through a One Health approach.



### 8.14 A large-scale study of a poultry trading network in Bangladesh: implications for control and surveillance of avian influenza Viruses

*N. Moyen<sup>1</sup>, G. Ahmed<sup>3</sup>, S. Gupta<sup>2,3</sup>, T. Tenzin<sup>4</sup>, R. Khan<sup>3</sup>, T. Khan<sup>3</sup>, N. Debnath<sup>3</sup>, M. Yamage<sup>3</sup>, D.U. Pfeiffer<sup>1,5</sup> and G. Fournie<sup>1</sup>*

<sup>1</sup>Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, Hertfordshire AL9 7TA, UK. <sup>2</sup>School of Veterinary Science, The University of Queensland, Gatton 4343, Qld, Australia. <sup>3</sup>Emergency Centre for Transboundary Animal Diseases, Food and Agriculture Organisation of the United Nations, Dhaka, Bangladesh. <sup>4</sup>National Centre for Animal Health, Thimphu, Bhutan. <sup>5</sup>College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong.

*Published in BMC Veterinary Research (2018) 14:12.*

#### **Abstract**

**Background:** Since its first report in 2007, avian influenza (AI) has been endemic in Bangladesh. While live poultry marketing is widespread throughout the country and known to influence AI dissemination and persistence, trading patterns have not been described. The aim of this study is to assess poultry trading practices and features of the poultry trading networks which could promote AI spread, and their potential implications for disease control and surveillance. Data on poultry trading practices was collected from 849 poultry traders during a cross-sectional survey in 138 live bird markets (LBMs) across 17 different districts of Bangladesh. The quantity and origins of traded poultry were assessed for each poultry type in surveyed LBMs. The network of contacts between farms and LBMs resulting from commercial movements of live poultry was constructed to assess its connectivity and to identify the key premises influencing it.

**Results:** Poultry trading practices varied according to the size of the LBMs and to the type of poultry traded. Industrial broiler chickens, the most commonly traded poultry, were generally sold in LBMs close to their production areas, whereas ducks and backyard chickens were moved over longer distances, and their transport involved several intermediates. The poultry trading network composed of 445 nodes (73.2% were LBMs) was highly connected and disassortative. However, the removal of only 5.6% of the nodes (25 LBMs with the highest betweenness scores), reduced the network's connectedness, and the maximum size of output and input domains by more than 50%.

**Conclusions:** Poultry types need to be discriminated in order to understand the way in which poultry trading networks are shaped, and the level of risk of disease spread that these networks may promote. Knowledge of the network structure could be used to target control and surveillance interventions to a small number of LBMs.



### 8.15 Spatio-temporal epidemiology of Japanese encephalitis in Nepal, 2007-2015

*Dhan Kumar Pant*<sup>1,2</sup>, *Tenzin Tenzin*<sup>3</sup>, *Rakesh Chand*<sup>2</sup>, *Barun Kumar Sharma*<sup>4</sup>, *Padam Raj Bist*<sup>2</sup>

1 Institute of Medicine, Tribhuvan University, Kathmandu, Nepal, 2 National Zoonoses and Food Hygiene Research Centre, Kathmandu, Nepal, 3 National Centre for Animal Health, Thimphu, Bhutan, 4 Ministry of Livestock Development, Government of Nepal, Kathmandu, Nepal

*Published in PLoS ONE 12(7): e0180591.*

#### **Abstract**

Japanese encephalitis (JE) is a major public health problem in Nepal. For the effective management and surveillance of JE, a clear understanding of its epidemiology is essential.

Therefore, we conducted descriptive and spatial analyses to understand the spatio-temporal distribution of JE in human in Nepal. From 2007 to 2015, 1,823 JE cases were reported with a cumulative mean incidence of 0.735/100,000 population and a case fatality rate of 6.6%. The death rate in the up-to-24 years of age group was 74%. The JE cases were most commonly reported in the age group of 1±14 years. There is a strong seasonal pattern of JE occurrence in Nepal which peaked in August and declined by October each year, which corresponds to the monsoon season. The JE cases were reported in 63 of 75 districts (84%), expanding in the mountain and hill regions. There was a strong clustering of JE incidence in the south-western and south-eastern Terai region, which is endemic for JE. Therefore, the JE surveillance system should be improved to better understand the drivers of disease expansion in Nepal for instituting a control program.

### 8.16 Study on *Taenia multiceps* infection in yak dogs in Merak-Sakteng, Trashigang

Coenurosis or Gid is a disease of the central nervous system in yaks and other ruminants, caused by *Coenurus cerebralis*, the larval stage of a canine tapeworm *Taenia multiceps*. In Bhutan, yak dogs are considered as the principle definitive host responsible for transmission of Gid disease in yaks. Yak herdsman use dogs to control and protect yaks from wild carnivores and in the process yaks get infected by grazing on pastures contaminated by yak dogs faeces infested with eggs of *T.multiceps*. Gid disease is reported in yak rearing Dzongkhags of Haa, Paro, Thimphu, Gasa, and Bumthang but was brought under control in Wangdue. However, the disease has not been reported from other yak rearing areas in eastern Dzongkhags of Trashigang, Tashiyangtse and Lhuentse. In order to find out presence/absence of gid causing parasites (*T.multiceps*) in yak dog and stray dogs in Merak-Sakteng and Bumdeling yak rearing areas, yak dog/stray fecal sample collection and laboratory analysis is being initiated. A molecular analysis will be conducted to find out the presence/absence of *T.multiceps* in the taenia positive samples. In addition, a questionnaire survey has been

conducted among the yak herders to corroborate the laboratory findings. The findings from this study will guide policy decision on gid control program in the country.

### **8.17 First Report of detection and phylogenetic analysis of important zoonotic pathogens from rodents in Gedu, Bhutan**

Yoenten PHUENTSHOK,<sup>1,3\*</sup> Kezang DORJI,<sup>1</sup> Tandin ZANGPO,<sup>1</sup> Silas A. DAVIDSON,<sup>2</sup> Ratree TAKHAMPUNYA,<sup>2</sup> Tenzinla TENZINLA,<sup>3</sup> Chencho DORJEE,<sup>4</sup> Roger MORRIS,<sup>5</sup> Peter JOLLY,<sup>6</sup> Sithar DORJEE,<sup>4</sup> and Joanna McKENZIE.<sup>6</sup>

<sup>1</sup>One Health Epidemiology Fellowship Program, Massey University, Palmerston North, New Zealand. <sup>2</sup>Entomology Department, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. <sup>3</sup>National Centre for Animal Health, Department of Livestock, Ministry of Agriculture and Forests, Serbithang, Bhutan. <sup>4</sup>Khesar Gyalpo University of Medical Sciences of Bhutan, Thimphu, Bhutan. <sup>5</sup>MorVet Ltd, Consultancy services in health risk management and food safety policy, Masterton, New Zealand. <sup>6</sup>International Development Group, Institute of Veterinary Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

#### **Abstract**

Rodents are well known reservoirs and vectors of many emerging and re-emerging infectious diseases, but little is known about their role in zoonotic disease transmission in Bhutan. A cross-sectional study of zoonotic disease pathogens in rodents was performed in Chukha district, Bhutan where a high incidence of scrub typhus and acute undifferentiated febrile illness cases had been reported in people during the preceding four to six months. Twelve rodents were trapped using live wire mesh traps. Following euthanasia, liver and kidney tissues were removed and tested by PCR for *Orientia tsutsugamushi* and other bacterial and rickettsial pathogens causing bartonellosis, borreliosis, human monocytic ehrlichiosis, human granulocytic anaplasmosis, leptospirosis, and rickettsiosis. A phylogenetic analysis was performed on all rodent species captured and pathogens detected. Four out of the twelve rodents (33.3%) tested positive by PCR for zoonotic pathogens. *Anaplasma phagocytophilum*, *Bartonella grahamii*, and *B. queenslandensis* were identified for the first time in Bhutan. *Leptospira interrogans* was also detected for the first time from rodents in Bhutan. Although the scrub typhus pathogen was not detected, the relatively high rates of infection indicate that rodents may be commonly infected with zoonotic pathogens in Bhutan and play an important role in disease transmission.

### **8.18 Bovine Papillomatosis (wart) treatment in Paro and Trongsa Dzongkhag**

<sup>1</sup>V. R Monger, <sup>1</sup>Migma, <sup>2</sup>B.B. Gurung and <sup>3</sup>L.B. Rai

<sup>1</sup>National Centre for Animal Health, Serbithang, <sup>2</sup>Dzongkhag Veterinary Hospital, Paro,

<sup>3</sup>Dzongkhag Veterinary Hospital, Trongsa

#### **Abstract**

Animal papillomavirus (Papoviridae) such as bovine papillomavirus (BPV) have long been known to induce tumours in their host species. Infectious papillomatosis (warts)



are contagious in the animals in which they naturally occur. These lesions may be regarded as either hyperplasia or benign neoplasms since they do not metastasize and kill the host. Bovine papilloma virus (BPV) is distributed worldwide in cattle herds and is recognized as an etiological agent associated with several forms of benign tumors, e.g., cutaneous fibropapillomas, genital fibromas, and urinary bladder tumors, as well as benign fibroplasias in other tissues such as teats and esophagus. Bovine cutaneous papillomas are observed mainly on the head and neck, but in some animals localization in other parts of body has also been reported such as teats. The teat wart leads to severe milking problems in individual cows. The disease is common in cattle of all ages but is most common in young cattle under 2 years. In Bhutan, quite a number of cattle are being affected by papillomatosis in various districts with different clinical outcomes, although there is no official record or study. However, the presence and incidence of BPV infections have not been well documented. This study was carried out with the aim to see the efficacy of the autogenous vaccine and simultaneously compare the alternative treatment regimens against papillomatosis.

The study was conducted in Paro and Trongsa where the wart cases were reported. The animals in the study comprised of dairy animals with rice grain like fibropapilloma on the udder and teats with ulceration and some with chronic papilloma on the shoulder and neck region.

Animals with warts in the udder were subjected to auto-haemotherapy, using its own blood by drawing 10-20 ml venous blood from the jugular vein and injecting back subcutaneously on both sides of the lateral neck region by taking sterile precautions. The treatment was repeated several times (2-4 times) at weekly interval depending upon the recovery time. The animals with chronic papilloma on the shoulder and neck region were subjected to autogenous vaccine therapy. The autogenous vaccine was prepared by collecting 5 grams of fresh active growth (tissue) from affected animal and homogenizing in glycerine-saline/normal saline solution and giving at the dose rate of 10-15 ml per animal subcutaneously several times at weekly intervals. Some animals were treated with topical application of 40% formalin.

In most of the animals treated with either of the above treatment therapy, there was complete recovery after therapy of three to four times on weekly interval. The findings of this study revealed that along with autogenous vaccine, auto-haemotherapy can also be effectively employed to treat papillomatosis of udder and teat in cattle. In many studies, the treatments with autogenous vaccine were effective after 2-3 times treatment on weekly interval.



Figure 1. Wart on the neck and teat is cattle

## On-going surveillance and researches

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

### 1. Risk-based surveillance of bovine brucellosis in cattle in Bhutan

(sampling and testing completed; referred samples outside for re-testing and results awaiting)

### 2. Study on *Taenia* infection in dogs in Bhutan

Bhutan has a large population of free-roaming dogs which is a concern for the general public, including tourists. A diverse range of zoonotic infections, including parasitic, bacterial, viral and fungal diseases, can be transmitted from dogs to humans. Like in other countries, eggs of *Taenia* and *Toxocara* spp. have been found in dogs during routine faecal microscopy in laboratories in Bhutan. Besides, the dogs have been found to be acting as definitive hosts for *Taenia multiceps* in yaks causing gid disease and also cystic echinococcosis in humans. Human cases of cystic echinococcosis have been recorded in the surgical records of JDWNRH. Therefore, the study aimed to study the *Taenia* infection and their molecular characterization in dogs in Bhutan. The environmental sampling collected from all the Dzongkhags and analysed for taeniid eggs at NCAH. 50 positives samples were identified microscopically in the second batch and sent to Institute of Parasitology, University of Zurich for molecular analysis.

### 3. One Health approach to determine Antibiotic Susceptibility profile of *Salmonella* in human, animals and food products in Bhutan

*Salmonella* has been recognized as an important zoonotic pathogen of economic significance in animals and humans. Salmonellosis is most common and widely distributed food-borne disease and increasing antimicrobial resistance in non-typhoid *Salmonella* species has been a serious concern for public health worldwide. Typhoid is listed as notifiable disease in Bhutan and there were 49 confirmed cases of Typhoid in human in 2015. A study on prevalence of *Salmonella* in imported chicken carcasses in Bhutan showed 13% prevalence. *Salmonella enteritidis* dominated with a prevalence of 80.7% and 40 of the 42 isolates harboured two or more resistance determinants. A recent study concluded the prevalence of *Salmonella* at 20.3% and 27.1% in imported and locally produced beef and pork respectively. These isolates were not tested for antimicrobial resistance. Thus, the antibiotic susceptibility profile of these organisms is unknown.

The overall objective of the study is to develop ABST profile for *Salmonella* isolates in Bhutan while enhancing national capacities for laboratory surveillance and antimicrobial resistance monitoring through One Health Approach. This will also enable Bhutan to provide representative and internationally comparable information that can be used for rational management of antimicrobial use in human and animals.

So far a total of 289 samples are collected from different poultry farms from Chukha, Tsirang, Sarpang and Thimphu out of which 23 samples are positive to *Salmonella* sp. Antimicrobial susceptible tests were performed on these 23 positive samples and

archived at -80°C. The culture isolates were susceptible to: Amoxycillin, Ceftriaxone, Nalidixic acid, Co-trimoxazole and Sulphadimidine, Gentamicin, Ciprofloxacin and Chloramphenicol. Resistant to: Amoxycillin, Nalidixic acid, Co-trimoxazole & Sulphadimidine, Gentamicin, Chloramphenicol. Sample collection from various sampling sites and laboratory tests are still on going.

## **9. Human Resource & Capacity Development**

The following training/workshop were organized by NCAH during the FY 2017-18 to enhance the capacity of the livestock and medical professions.

The NCAH organized various incountry training, workshop and seminar as follows.

### **9.1 Workshop for revision of SOP and development of new Standard Operating Procedure (SOPs) for veterinary laboratory diagnosis towards maintaining GLP**

First edition of laboratory standard operating procedures (SOP) were prepared during 2013. Since it was prepared about four years ago, the need was felt to revise the SOP with inclusion, addition and modification of the techniques based on the emergence and re-emergence of the animal diseases in the country and also the advancement in the laboratory technologies (proven through researches). It was also recommended by the OIE PVS laboratory mission and also the external expertise. The need was also highlighted during in-country Laboratory Co-ordination workshop held during 2016. Hence, the workshop was conducted at Gelephu on 8th to 11th February 2018. This was followed by another workshop for final editing of the SOPs at Paro from 11-13 June 2018.

### **9.2 Workshop for Development of National Rabies Action Plan**

The expert team have drafted a national rabies action plan using Stepwise Approach towards Rabies Elimination (SARE) tool to guide elimination of dog mediated human rabies in Bhutan by 2023. The workshop was conducted at Paro from 28-30 September 2017.



### **9.3 International Health Regulation (IHR) Workshop**

The IHR focal point from NCAH participated in the workshop organized by MoH and WHO to evaluate the IHR activities in the country. The International Health Regulations (2005) or “IHR (2005)” are an international law which helps countries work together to save lives and livelihoods caused by the international spread of diseases and other

health risks. They entered into force on 15 June 2007 and are binding on 194 countries across the globe, including Bhutan.

The IHR (2005) aim to prevent, protect against, control and respond to the international spread of disease while avoiding unnecessary interference with international traffic and trade. The IHR (2005) are also designed to reduce the risk of disease spread at international airports, ports and ground crossings.

The IHR (2005) establish a set of rules to support the global outbreak alert and response system and to require countries to improve international surveillance and reporting mechanisms for public health events and to strengthen their national surveillance and response capacities.



## 9.4 National Stakeholder Meeting on Developing Stepwise Control of Brucellosis in Bhutan

A National Stakeholder Meeting on Developing Stepwise Control of Brucellosis was conducted on June 18-19, 2018 at Paro with fund and technical support from the Food and Agriculture Organization of the United Nations (FAO). The meeting was attended by international participants from France, Thailand, Australia and representative of Food and Agriculture Organization, Thailand. The national attendees were from livestock policy, experts from animal health and production, farm managers, agriculture and food regulation and public health. The main objectives of the meeting were to:

1. To identify current situation, gaps of brucellosis control and prevention;
2. To draft stepwise approach for brucellosis control and prevention;
3. To discuss and recommend way forward to the drafted stepwise approach;



## 9.5 Developed G2C datababse for management of veterinary drugs and condcucted trainings for focal livestock officials from the Dzongkhags and livestock frams/agencies



## 10. Visitors to NCAH, Serbithang during FY 2017-18

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

SI No.	Date	Visitor's Name	Purpose for visit
1.	10.10.2017	Dr. Kihins Karane Dr.Yukokunegni Dr. Kaku Dr. Morikawa Dr. Akiko	Consultative meeting on project on infectious zoonosis, National Institute of Infectious Disease, Japan
2.	14.12.2017	Hents Omel, FAO Peter Rzeszotarski Fredesik Copper, CDC John Wood, UK	As a part of external evaluation of animal health components to conduct Joint External Evaluation (JEE), organized by MoH and WHO
3.	20.12.2017	Namgay TsheringNorbu TsheringSamdrup NorbuGyeltshen	RNR Professional Development Program

		TsheringDendup NamgaySherpa	
4.	16.01.2018	Jonna Mckenzie VikasAggarval	Scoping visit for fleming fund for AMR
5.	20.03.2018	SitharDorji JonnaMckenzie, RinzinPem	Positioning activity visit for fleming fund group
6.	30.04.2018	Namgay RinchenWangmo Leki Kinley Tenzin Cheku SangayDorji Kabita	RNR Professional Development Program
7.	24.04.2018	Mr. Kunzang Dorji Mr. Tashi Dhendup	From DRA for GMP Inspection at BPU
8.	24.04.2018	Madame Françoise Moreau-Lalanne	Agricultural Counselor for India and South Asia at Embassy of France visited the National Centre for Animal Health to understand animal health services delivery and system in Bhutan as part of a delegation led by the French Ambassador to India.
9.	04.06.2018	Ms Tshering Yangzom Mr. Masaaki Osawa	From Thimphu Thromde to inspect the Incinerator at NCAH
10.	18.06.2018	Dr. Rinzin Pem Dr. Chendu Dorji Dr. Tshewang Gembo Mr. Kaka Wangchuk Dr. Jambay Dorji	The Drug and Vaccine Evaluation team to evaluate for FY 2018-19

## Annexure

### Annexure 1. List of Staff at NCAH as of June 30 2017

Sl. No.	Name List of Staff	Position Title	EID No.	P/L	Remarks
<b>Veterinary Doctors</b>					
1	Dr. Kinzang Dukpa	Program Director	9603005	P1	
2	Dr. Phuntsho Wangdi	Animal Health Specialist-I	8202026	ES 1	
3	Dr. N.K. Thapa	Animal Health Specialist-III	9302007	ES 3	
4	Dr. R B Gurung	Animal Health Specialist-III	9603028	ES 3	

5	Dr. Vijay Raika Monger	Animal Health Specialist-III	9411039	ES 3	
6	Dr. Tenzin	Principal Livestock Health Officer	2001032	P1	
7	Dr. Hiruka Mahat	Dy. Chief Veterinary Officer	200501113	P2	
8	Dr. Yoenten Phuentshok	Sr. Veterinary Officer	201201031	P3	
<b>Technical Staff</b>					
9	Puspa Maya Sharma	Laboratory Officer	20140103185	P4	
10	Dechen Wangmo	Laboratory Officer	20150105019	P4	
11	Purna Bdr. Rai	Sr. Laboratory Technician II	8806138	SS3	
12	Harka Bdr. Tamang	Sr. Livestock Health Supervisor II	8307007	SS3	
13	Namgay Dorji	Sr. Livestock Health Supervisor II	200208011	SS3	
14	Migma	Sr. Laboratory Technician II	9801103	SS3	
15	Phuntsho Wangmo	Sr. Extension Supervisor III	200308065	SS3	
16	Tenzinla	Sr. Laboratory Technician III	9901013	SS3	
17	Dawa Tshering	Sr. Laboratory Technician III	9901014	SS3	
18	Kinzang Namgay	Livestock Health Supervisor	8604131	S1	
19	Ugyen Pema	Asstt. Laboratory Technician I	2109009	S2	
20	Kelzang Lhamo	Asstt. Laboratory Technician I	200310013	S2	
21	Tshewang Dema	Asstt. Laboratory Technician I	200407360	S2	
22	Karma Choki	Asstt. Laboratory Technician I	2108008	S2	
23	Pasang Bida	Asstt. Laboratory Technician I	2109008	S2	
<b>Administrative Section</b>					
24	Pari Chhetri	Accounts Assistant II	9709051	SS3	
25	Tshewang Dakpa	Accounts Assistant III	8712024	SS4	
26	Karma Dekar	Sr. Administrative Assistant IV	9507009	S1	
27	Rinzin Dorji	Store Keeper	9910107	S4	
28	Pemo	Sr. Telephone Operator II	9904051	O1	
29	Phuntsho Choden	Administrative Asst II	200712003	S4	
<b>Drivers</b>					
30	Penjor	Driver	9906003	O1	
31	Tashi Gayleg	Driver	2006039	O1	
32	Pema Wangdi	Driver	2106032	O1	
33	Sangay Tshering	Driver	9902017	O1	
34	Tshewang Rinzin	Driver II	201108012	O3	
<b>Attendants</b>					
35	Sonam	Night Guard	ESP		
36	Karna Kumar Tamang	Sweeper	ESP		



37	Sangay Nidup	Helper (Lab Utility)	ESP		
38	Man Bir Lama	Helper (Lab Utility)	ESP		
39	Chimi Wangmo	Helper (Lab Utility)	ESP		

### **Annexure 2: Staff transferred to and from NCAH, Serbithang**

1. Kinzang Namgay, Livestock Health Supervisor transferred from DoL to NCAH
2. Sonam Dolma, LPO transferred to NPRDC, Sarpang
3. Punya Mata Sanyasi, Laboratory Technician transferred to NVH, Thimphu
4. Pema Tshomo, Laboratory Assistant transferred to NVH, Thimphu
5. Dechen Wangmo, Laboratory Officer transferred from BLDC to NCAH
6. Kuenzang Dorji, Sr. Store Keeper transferred to Directorate Services, MoAF, Thimphu
7. Sangay Tenzin, Sr. Laboratory Officer voluntary resigned
8. Sonam Dorji, Sr. Livestock Health Supervisor, superannuated
9. Tashi Penjor, Sr. Technician, Superannuated
10. Rinchen Tshering, Driver, Superannuated
11. Karma Phuntsho, ESP-Superannuated
12. Man Bir Lama, ESP –Newly recruited
13. Chimi Wangmo, ESP-Newly Recruited

### **Annexure 3: Promotion acquired during the FY 2017-2018**

14. Dr. R B Gurung, Specialist III
15. Dr. Vijay Raika Monger, Specialist III
16. Dr. Yoenten Phuentshok, Sr. Veterinary Officer, P3
17. Tenzinla, Sr. Laboratory Technician II, SS3
18. Dawa Tshering, Sr. Laboratory Technician II, SS3
19. Phuntsho Wangmo, Sr. Extension Supervisor II, SS3
20. Kuenzang Dorji, Sr. Store Keeper, S3

### **Annexure 4: Details of Infrastructure**

Sl. No	Class of Building	No. of Unit
1	Office building (Administrative Block)	1
2	Laboratory	2
3	Biological Production building	1
4	Old Laboratory building (Store)	1
5	Generator House	1
6	Refrigerator Workshop	1
7	Small animal house	1
8	Sheep shed	1
9	Garage	1
10	Animal potency test	1
11	Guard house	1
12	Res. Quarter, Class II	3
13	Res. Quarter, Class III	1
14	Res. quarter, Class IV	4
15	Drivers quarter	2

16	Res. quarter, old hostel	5
----	--------------------------	---

### Annexure 5: Details of vehicle

Sl. No.	RSTA Reg. No	Type	Make	Model	Engine No	Chassis No	Funded by	Present condition
1	BG-1-A1601	Scorpio	Mahindra Co	2008	21622	84152	EU	Running
2	BG-1-A1602	Bolero pick up	Mahindra Co	2008	19010	40083	EU	Running
3	BG-A-A1603	Scorpio	Mahindra Co	2008	21655	92060056	EU	Off road
4	BG-1-A0217	Bike	Bajaj co	2008	22511	15826	EU	Running
5	BG-1-A0612	Bus	Eicher	2009	208022	183632	GOI	Running
6	BG-1-A1887	Refrigerator Van	Toyota Co	2010	1112063 2308	JTELB T1 J807	GOI	Running
7	BG-1-A2290	Hilux	Toyota	2013	2KD-U32- 9542	MROFR- 22G2007 5997	HSI	Running
8	BG-1-A2291	Hilux	Toyota	2013			HSI	Running
9	BG-1-A1812	Mini Force Van					HSI	Off road
10	BG-1-A1952	Bolero DC	Mahindra Co	2011	BKB4c1 1125	MATSS4B 2C71246	HSI	Running
11	BG-1-A3076	Bolero /DC Turbo	Mahindra Co	2018	BKJ4A9 5373	MAISS4B KCJ2A55 676	HSI	Running

### Annexure 6: Vehicle Expenditure for maintenance and spare parts

Sl. No	Vehicle No.	Funding	Cost (Nu.)
1	BG-1-A1601	RGoB	Nu. 11,190.00
2	BG-1-A1602	RGoB	Nu. 21,260.00
3	BG-1-A1603	RGoB	Nu. 54,305.00
4	BG-1-A1887	RGoB	Nu. 7230.00
5	BG-1-A0612	RGoB	Nu. 50,845.00
6	BG-1-A2291	HSI Project	Nu. 67,433.00
7	BG-1-A2290	HSI Project	Nu. 1,42,473.00
8	BG-1-A1952	HSI Project	Nu. 2,06,911.00
9	BG-1-A3076	HSI Project	Nu. 4252.00
<b>Total Amount:</b>			<b>Nu. 5,65,899.00</b>

### Annexure 7: Construction & Renovation works carried out

1. Installation of CCTV- Nu. 2,07,780/-
2. Installation of Street Lighting- Nu. 99,612/-
3. Construction of parking

## Annexure 8. Budget expenditure statement as of 30 June 2017 (FY 2017-18)

ACT	SACT	FIC	OBC	TITLE	Approved budget	Expenditure	Balance	% utilization
001				<b>DIRECTION SERVICES (NCAH)</b>				
	01			<b>OPERATION &amp; MANAGEMENT SERVICES</b>				
		0001		RGOB Financing				
			01.01	Pay and Allowances	13.012	12.981	0.031	99.76
			02.01	Other Personnel Emoluments	0.420	0.420		100.00
			11.01	Travel - Incountry	1.771	1.771	0.000	100.00
			12.01	Utilities -Telephones, Telex, Fax, E-mail, Internet	0.300	0.300	0.000	99.98
			12.02	Utilities -Telegram, Wireless Transmission, Postage	0.002	0.002	0.000	109.80
			12.03	Utilities - Electricity, Water, Sewerage	0.333	0.330	0.003	99.09
			12.05	Utilities - Fuelwood	0.024	0.023	0.001	95.87
			14.01	S & M - Office Supplies, Printing, Publications	0.137	0.137	0.000	99.98
			14.06	S & M - Uniforms, Extension Kits, Linens	0.051	0.051	0.000	99.02
			15.01	Maintenance of Property - Buildings	0.055	0.055	0.000	100.00
			15.02	Maintenance of Property - Vehicles	1.036	1.032	0.004	99.59
			15.07	Maintenance of Property - Computers	0.050	0.048	0.002	95.64
			15.09	Maintenance of Property - Water supply, Sewerage, Playfield				
			17.01	Op. Exp. - Advertising	0.070	0.070	0.000	99.79
			17.02	Op. Exp. - Taxes, Duties, Royalties, Fees, Handling Charges, Bank Charges	0.105	0.102	0.003	97.31
			17.08	Op. Exp. - Incountry Meetings and Celebrations	0.131	0.131		100.00
			24.03	Contributions - Provident Fund	1.161	1.121	0.040	96.54
			25.01	Retirement Benefits	2.256	2.225	0.031	98.61
			52.04	Plant & Equipt. - Telecommunications				
			54.03	Computers & Peripherals	0.040	0.036	0.004	91.00
				TOTAL OF FIC 0001	<b>20.954</b>	<b>20.834</b>	<b>0.120</b>	99.43
				TOTAL OF Sact 01	<b>20.954</b>	<b>20.834</b>	<b>0.120</b>	99.43
	02			<b>OIE GENERAL SESSION &amp; CONFERENCE</b>				
		0001		RGOB Financing				
			11.02	Travel - Outside Bhutan	0.248	0.248		100.00
				TOTAL OF Sact 02	<b>0.248</b>	<b>0.248</b>		100.00
	03			<b>INSTALLATION OF STREET LIGHTS</b>				
		0001		RGOB Financing				

			52.03	Plant & Equip. - Power Trans. & Dist.	0.100	0.100	0.000	99.61
				TOTAL OF SAct 03	<b>0.100</b>	<b>0.100</b>	<b>0.000</b>	99.61
				TOTAL OF Act 001	<b>21.302</b>	<b>21.182</b>	<b>0.120</b>	99.44
002				DRUGS, VACCINES AND EQUIPMENT UNIT (DVEU)				
	01			PROCUREMENT, DISTRIBUTION AND MANAGEMENT OF VETERINARY MEDICINES, VACCINES & EQUIPMENT				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.400	0.400		100.00
			14.02	S & M - Medicines & Laboratory Consumables	27.000	27.000	0.000	100.00
			17.01	Op. Exp. - Advertising	0.050	0.034	0.016	67.55
			17.03	Op. Exp. - Transportation	0.110	0.110	0.000	100.00
			52.07	Plant & Equip. - Hospital/Lab. Equipment	4.000	4.000	0.000	100.00
				TOTAL OF SAct 01	<b>31.560</b>	<b>31.544</b>	<b>0.016</b>	99.95
	02			STRENGTHENING & ENHANCEMENT OF DRUGS, VACCINES AND EQUIPMENT DELIVERY SERVICES				
		0001		RGOB Financing				#DIV/0!
			11.01	Travel - Incountry	0.200	0.200		100.00
				TOTAL OF SAct 02	<b>0.200</b>	<b>0.200</b>		100.00
	04			CONDUCT FIELD STUDY/RESEARCH RELATED TO VACCINE				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.050	0.049	0.001	98.86
			17.09	Op. Exp. - Survey/Census	0.050	0.048	0.002	96.87
				TOTAL OF SAct 04	<b>0.100</b>	<b>0.098</b>	<b>0.002</b>	97.86
				TOTAL OF Act 002	<b>31.860</b>	<b>31.842</b>	<b>0.018</b>	99.94
003				LABORATORY SERVICE UNIT				
	01			TEST KITS VALIDATION AND TEST STANDARDIZATION FOR SEROLOGY AND MOLECULAR				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.100	0.100		100.00
			14.02	S & M - Medicines & Laboratory Consumables	0.050	0.050		100.00
				TOTAL OF SAct 01	<b>0.150</b>	<b>0.150</b>		100.00
	02			EMERGENCY FIELD VISIT, SAMPLE COLLECTION				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.100	0.100		100.00
				TOTAL OF SAct 02	<b>0.100</b>	<b>0.100</b>		100.00

	03			PROVIDING REFERRAL LABORATORY DIAGNOSTIC SERVICES TO CLIENTS				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.405	0.405		100.00
			14.01	S & M - Office Supplies, Printing, Publications	0.030	0.021	0.009	71.61
			14.02	S & M - Medicines & Laboratory Consumables	0.400	0.400	0.000	100.00
			52.07	Plant & Equipt. - Hospital/Lab. Equipment	0.300	0.280	0.020	93.39
				TOTAL OF Sact 03	<b>1.135</b>	<b>1.107</b>	<b>0.028</b>	97.50
	04			STRENGTHENING AND ENHANCEMENT OF LABORATORY DIAGNOSTIC CAPACITIES				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.200	0.200		100.00
			14.01	S & M - Office Supplies, Printing, Publications				
			14.02	S & M - Medicines & Laboratory Consumables	0.200	0.200	0.000	99.78
				TOTAL OF Sact 04	<b>0.400</b>	<b>0.400</b>	<b>0.000</b>	99.89
	05			MAJOR LIVESTOCK DISEASE SURVEILLANCE/SURVEY				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.240	0.240		100.00
			14.01	S & M - Office Supplies, Printing, Publications				
			14.02	S & M - Medicines & Laboratory Consumables	0.200	0.200	0.000	100.00
				TOTAL OF Sact 05	<b>0.440</b>	<b>0.440</b>	<b>0.000</b>	100.00
	06			LABORATORY CO-ORDINATION & SKILL ENHANCEMENT				
		0001		RGOB Financing				
			14.07	S & M - Text Books, Library Books, Stationeries & Sports Item	0.020	0.020	0.000	97.70
			17.08	Op. Exp. - Incountry Meetings and Celebrations	0.320	0.320	0.000	99.99
				TOTAL OF Sact 06	<b>0.340</b>	<b>0.340</b>	<b>0.000</b>	99.86
	07			COORDINATION AND IMPLEMENTATION OF BIOSAFETY AND BIO-SECURITY PROGRAMMES				
		0001		RGOB Financing				
			14.01	S & M - Office Supplies, Printing, Publications				
			14.02	S & M - Medicines & Laboratory Consumables	0.094	0.093	0.001	99.25
			15.05	Maintenance of Property - Equipment	0.156	0.155	0.001	99.57
				TOTAL OF Sact 07	<b>0.250</b>	<b>0.249</b>	<b>0.001</b>	99.45
	08			MONITORING AND REPORTING				
		0001		RGOB Financing				
			12.02	Utilities -Telegram, Wireless Transmission, Postage				

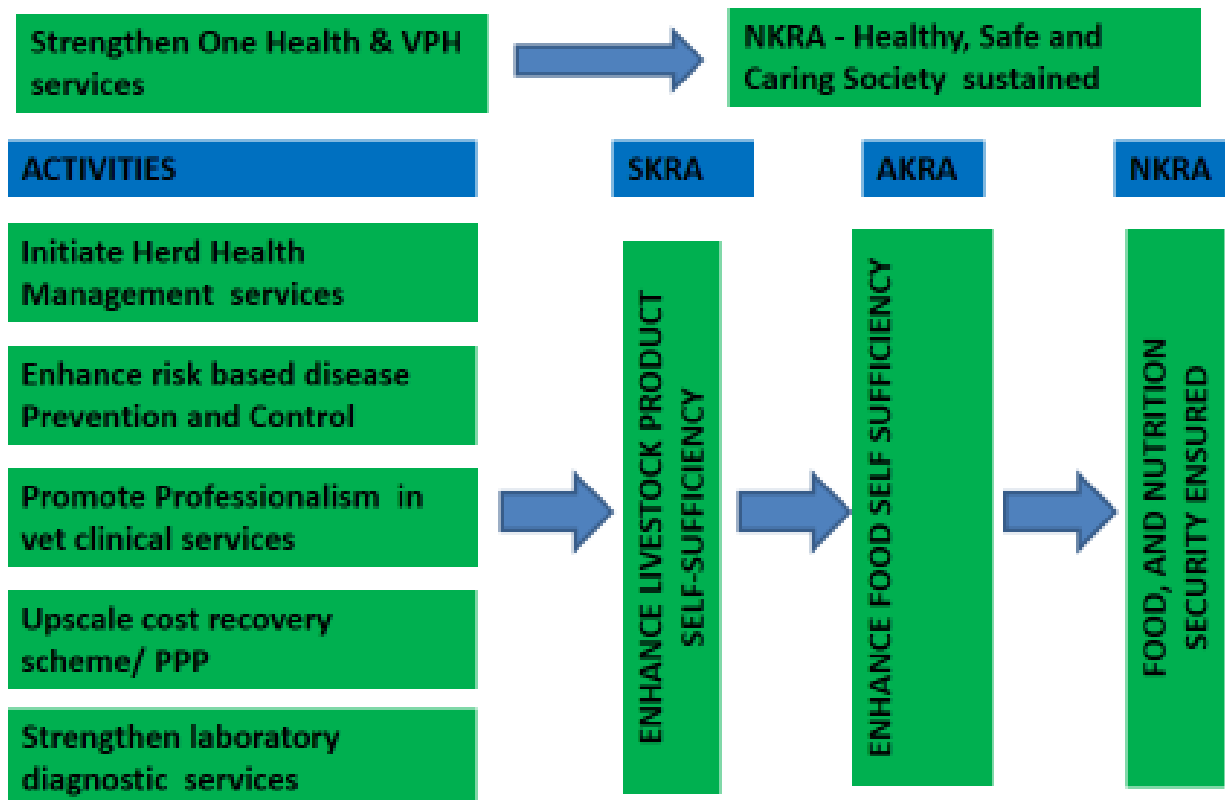
			17.02	Op. Exp. - Taxes, Duties, Royalties, Fees, Handling Charges, Bank Charges	0.060	0.011	0.049	18.12
				TOTAL OF SAct 08	<b>0.060</b>	<b>0.011</b>	<b>0.049</b>	18.12
	09			ONE HEALTH APPROACH TO DETERMINE ANTIBIOTICS SUSCEPTIBILITY PROFILE OF SALMONELLA IN HUMAN, ANIMAL AND FOOD PRODUCTS IN BHUTAN				
		2979		Promoting Health Through The Life Course				
			11.01	Travel - Incountry	0.202	0.202	0.000	99.98
			17.08	Op. Exp. - Incountry Meetings and Celebrations	0.278	0.278		100.00
				TOTAL OF FIC 2979	<b>0.480</b>	<b>0.480</b>	<b>0.000</b>	99.99
				TOTAL OF SAct 09	<b>0.480</b>	<b>0.480</b>	<b>0.000</b>	99.99
				TOTAL OF Act 003	<b>3.355</b>	<b>3.275</b>	<b>0.080</b>	97.62
004				DISEASE PREVENTION AND CONTROL UNIT				
	01			NATIONAL FOOT AND MOUTH DISEASE PREVENTION & CONTROL				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.140	0.140		100.00
			17.09	Op. Exp. - Survey/Census	0.090	0.090		100.00
				TOTAL OF SAct 01	<b>0.230</b>	<b>0.230</b>		100.00
	02			NATIONAL AVIAN INFLUENZA (BIRD FLU) PREVENTION & CONTROL				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.120	0.120	0.000	100.00
			14.01	S & M - Office Supplies, Printing, Publications	0.020	0.020		100.00
			17.01	Op. Exp. - Advertising				
				TOTAL OF SAct 02	<b>0.140</b>	<b>0.140</b>	<b>0.000</b>	100.00
	03			NATIONAL GID DISEASE PREVENTION & CONTROL				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.100	0.100		100.00
			14.02	S & M - Medicines & Laboratory Consumables				
			17.09	Op. Exp. - Survey/Census	0.150	0.150	0.000	100.00
				TOTAL OF SAct 03	<b>0.250</b>	<b>0.250</b>	<b>0.000</b>	100.00
	04			NATIONAL RABIES PREVENTION & CONTROL				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.100	0.100	0.000	99.68
			17.01	Op. Exp. - Advertising				
			17.09	Op. Exp. - Survey/Census	0.040	0.038	0.002	95.27
				TOTAL OF SAct 04	<b>0.140</b>	<b>0.138</b>	<b>0.002</b>	98.42

	05			ANIMAL HEALTH RESEARCH ON ZOONOTIC DISEASES				
		0001		RGOB Financing				
			17.08	Op. Exp. - Incountry Meetings and Celebrations	0.050	0.050		100.00
			17.09	Op. Exp. - Survey/Census	0.050	0.050		100.00
				TOTAL OF SAct 05	<b>0.100</b>	<b>0.100</b>		100.00
	06			ANIMAL HEALTH INFORMATION SYSTEM				
		0001		RGOB Financing				
			14.01	S & M - Office Supplies, Printing, Publications	0.050	0.049	0.001	98.00
				TOTAL OF SAct 06	<b>0.050</b>	<b>0.049</b>	<b>0.001</b>	98.00
				TOTAL OF Act 004	<b>0.910</b>	<b>0.907</b>	<b>0.003</b>	99.65
005				BIOLOGICAL PRODUCTION UNIT				
	01			PRODUCTION OF ANIMAL VACCINES				
		0001		RGOB Financing				
			14.02	S & M - Medicines & Laboratory Consumables	0.150	0.150		100.00
			14.05	S & M - Animal Feeds	0.150	0.148	0.002	98.84
				TOTAL OF SAct 01	<b>0.300</b>	<b>0.298</b>	<b>0.002</b>	99.42
	02			PROCUREMENT OF ANIMAL VACCINES				
		0002		RGOB Contribution				
			14.02	S & M - Medicines & Laboratory Consumables	3.300	3.300		100.00
				TOTAL OF SAct 02	<b>3.300</b>	<b>3.300</b>		100.00
	05			PROCUREMENT OF LABORATORY ANIMALS FOR FOR VACCINE PRODUCTION				
		0001		RGOB Financing				
			52.06	Plant & Equipt. - Livestock	0.150	0.150		100.00
				TOTAL OF SAct 05	<b>0.150</b>	<b>0.150</b>		100.00
	07			CONDUCT FIELD STUDY/RESEARCH RELATED TO VACCINE				
		0001		RGOB Financing				
			11.01	Travel - Incountry	0.050	0.050		100.00
			14.01	S & M - Office Supplies, Printing, Publications	0.010	0.006	0.004	62.00
			17.09	Op. Exp. - Survey/Census	0.040		0.040	0.00
				TOTAL OF SAct 07	<b>0.100</b>	<b>0.056</b>	<b>0.044</b>	56.20
				TOTAL OF Act 005	<b>3.850</b>	<b>3.804</b>	<b>0.046</b>	98.82
006				NATIONAL DOG POPULATION MANAGEMENT & RABIES COUNTR0L PROGRAM				
	01			OPERATIONAL AND MANAGEMENT SERVICE				
		0002		RGOB Contribution				
			12.01	Utilities -Telephones, Telex, Fax, E-mail, Internet	0.050	0.050	0.000	99.78
			14.01	S & M - Office Supplies, Printing, Publications	0.100	0.096	0.004	96.31



			15.02	Maintenance of Property - Vehicles	0.625	0.625	0.000	99.99
				TOTAL OF SAct 01	<b>0.775</b>	<b>0.771</b>	<b>0.004</b>	99.50
	02			MASS RABIES VACCINATION CAMPAIGN				
		0002		RGOB Contribution				
			11.01	Travel - Incountry	0.100	0.100		100.00
			14.02	S & M - Medicines & Laboratory Consumables	0.150	0.148	0.002	98.87
			17.01	Op. Exp. - Advertising	0.050	0.050	0.000	99.97
			17.08	Op. Exp. - Incountry Meetings and Celebrations	0.050	0.050		100.00
				TOTAL OF SAct 02	<b>0.350</b>	<b>0.348</b>	<b>0.002</b>	99.51
	03			OBSERVATION OF WORLD RABIES DAY AND AWARENESS CAMPAIGN				
		0002		RGOB Contribution				
			11.01	Travel - Incountry	0.031	0.031		100.00
			14.01	S & M - Office Supplies, Printing, Publications	0.005	0.005	0.000	91.54
			17.08	Op. Exp. - Incountry Meetings and Celebrations	0.189	0.189	0.000	99.96
				TOTAL OF SAct 03	<b>0.225</b>	<b>0.225</b>	<b>0.000</b>	99.78
	04			RESERCH COMPONENT : SPILL OVER INFECTION RESERCH AND PUBLICATION				
		0002		RGOB Contribution				
			11.01	Travel - Incountry	0.050	0.050		100.00
			14.01	S & M - Office Supplies, Printing, Publications	0.040	0.040		100.00
				TOTAL OF SAct 04	<b>0.090</b>	<b>0.090</b>		100.00
	05			IMPLEMENTATION OF CABC				#DIV/0!
		0002		RGOB Contribution				#DIV/0!
			11.01	Travel - Incountry	0.400	0.400	0.000	99.96
			14.01	S & M - Office Supplies, Printing, Publications	0.030	0.030		100.00
			14.02	S & M - Medicines & Laboratory Consumables	0.200	0.200		100.00
			17.03	Op. Exp. - Transportation	0.100	0.099	0.001	98.58
				TOTAL OF SAct 05	<b>0.730</b>	<b>0.728</b>	<b>0.002</b>	99.79
	06			KAP SURVEY AND M&E FOR CNVR				
		0002		RGOB Contribution				
			17.09	Op. Exp. - Survey/Census	0.350	0.350	0.000	99.94
				TOTAL OF SAct 06	<b>0.350</b>	<b>0.350</b>	<b>0.000</b>	99.94
				TOTAL OF Act 006	<b>2.520</b>	<b>2.512</b>	<b>0.008</b>	99.69
				TOTAL OF SPrg 027	<b>63.797</b>	<b>63.522</b>	<b>0.275</b>	99.57

## Canopy view of Animal Health Strategies in the 12<sup>th</sup> FYP



*Happy Reading and Tashi Delek*

