

ANNUAL TECHNICAL REPORT

2064/065 (2007/2008)

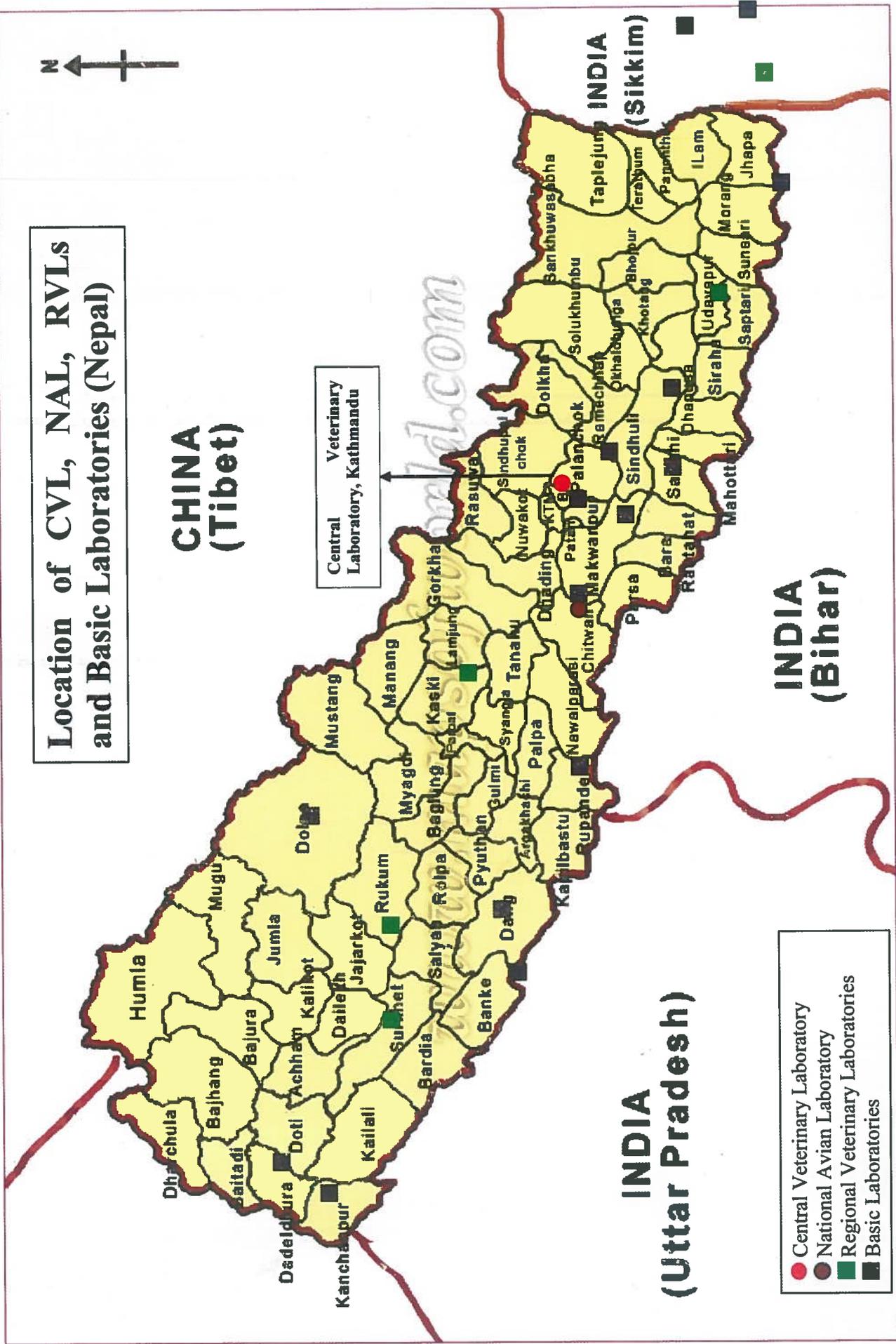


Government of Nepal
Ministry of Agriculture & Cooperatives
Department of Livestock Services
Directorate of Animal Health

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Location of CVL, NAL, RVLs and Basic Laboratories (Nepal)



CHINA (Tibet)

Central Veterinary Laboratory, Kathmandu

INDIA (Uttar Pradesh)

INDIA (Bihar)

INDIA (Sikkim)

- Central Veterinary Laboratory
- National Avian Laboratory
- Regional Veterinary Laboratories
- Basic Laboratories

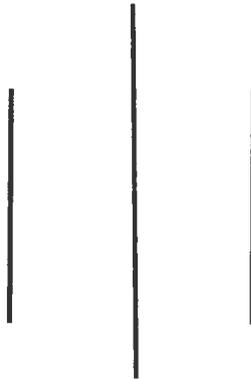
ANNUAL TECHNICAL REPORT

**F /Y 2064/065
[2007-2008]**

Editorial Board

**Dr. Poornima Manandhar
For, Chief Veterinary Officer, CVL**

**Dr. Banshi Sharma
Senior Veterinary Officer, CVL**



Published by:

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Preface

On behalf of editorial board, I am pleased to present the publication of Annual Technical Report, 2064/065 (2006/2007.) This issue subsumes complete package of activities and its progress report in tabular form from Central Veterinary Laboratory (CVL), five Regional Veterinary Laboratories (RVLs) and National Avian Laboratory (NAL).

National Veterinary Services of Nepal has done several remarkable programmes during last year such as surveillance on Avian Influenza, extension of ELISA test facility in each RVL and NAL, development of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test, setting tissue culture laboratory unit at CVL, and collaboration with international reference laboratories. Apart of all this CVL investigates on Sudden Death Syndrome in broiler chicks of Kathmandu valley. A new problem emerging in commercial broiler due to mycosis infestation which detail investigation is being suggested. Recent exoduses of neurological disorder in early age broiler in Chitwan and Kathmandu valley had yet created a new havoc in commercial broiler sector. Poultry sector become commercialized in recent year but still facing several challenges such as prevalence of bird flu in our neighbors and effort is concentrated to contain its outbreak in Nepal.

We are in the process of upgrading CVL, NAL and RVLs into Biosafety Level - II laboratory so that CVL would receive accreditation from international certification body of ISO.

I would like to express my cordial thanks to all the RVLs staffs as well as NAL staffs for providing their annual progress report and technical articles in due time. I would like to thank Dr. Banshi Sharma for working hard to shape the annual report in this form. I would like to thanks Dr. Kedar Karki, Dr. Karuna Sharma Bhattarai, Dr. Salina Manandhar, Dr. Vinaya Kumar Karna, Dr. Pragya Koirala and Dr. Krishna Raj Pandey for their support in publishing this report. My special thanks go to all the technicians of CVL for their sincere contribution and help for providing data based technical information.

Any suggestions for the improvement of its future issue will be highly appreciated.

Dr. Poornima Manandhar
For, Chief Veterinary Officer
Central Veterinary Laboratory
Tripureshwor, Kathmandu

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CENTRAL VETERINARY LABORATORY TRIPURESHWOR, KATHMANDU

1. Introduction

Central Veterinary Laboratory (CVL) focuses programme with the objective of securing healthy national flocks of animals and birds throughout the nation by mitigating the occurrence of diseases of livestock and poultry. CVL also works on epidemic investigation as well as surveillance and investigation on various diseases in its approved annual programme. The direct benefit of the performance of various laboratories has been experienced in the field of veterinary medical care based on valid laboratory test results. To achieve these multidimensional activities, CVL works with a series of laboratory test procedures through its various laboratory units; Pathology, Parasitology, Microbiology, Serology, Haematology and Biochemistry units, and Molecular Diagnosis with a considerable progress in the later. At present the molecular based diagnosis of Avian Influenza is in the course of advancement. Similarly, setting up of tissue culture laboratory unit is in progress and expected to conduct virus isolation, identification and sero-typing in future.

Central Veterinary Laboratory is always aware in adopting modern disease diagnostic technologies. Endeavour is continuously made in improving its performance in the form of research-oriented activities rather than routine diagnostic works. We are in the process of development of Standard Operating Procedure, test protocols, quality manual, measurement traceability and biosafety system so that good laboratory practice is followed in all the diagnostic laboratories. CVL has already adopting test verification system through international reference laboratories which will help, at least, in the accreditation of CVL for international certification under ISO.

To provide diagnostic facilities throughout the country, CVL works through its five Regional Veterinary Laboratories (RVLs) located one in each of the development regions of the nation; eastern (Biratnagar), central (Janakpur), western (Pokhara), mid-western (Surkhet) and far-western (Dhangadhi) as well as through National Avian Laboratory located in Bharatpur, Chitwan. To provide the diagnostic services smoothly throughout the nation, fifteen basic laboratories established in 15 district livestock service offices (DLSOs) namely, Illam, Jhapa, Saptari, Sarlahi, Rautahat, Parsa, Makawanpur, Kabhrepalanchowk, Chitwan, Rupandehi, Dang, Banke, Jumla, Dadeldhura and Kanchanpur, and 60 primary laboratories one in each DLSOs. The basic laboratories are capable to perform microbial culture and antibiotic sensitivity test. Specimens that could not be processed in the aforementioned laboratories due to insufficient facilities are referred to central veterinary laboratory. In this way, CVL works as reference veterinary laboratory in Nepal.

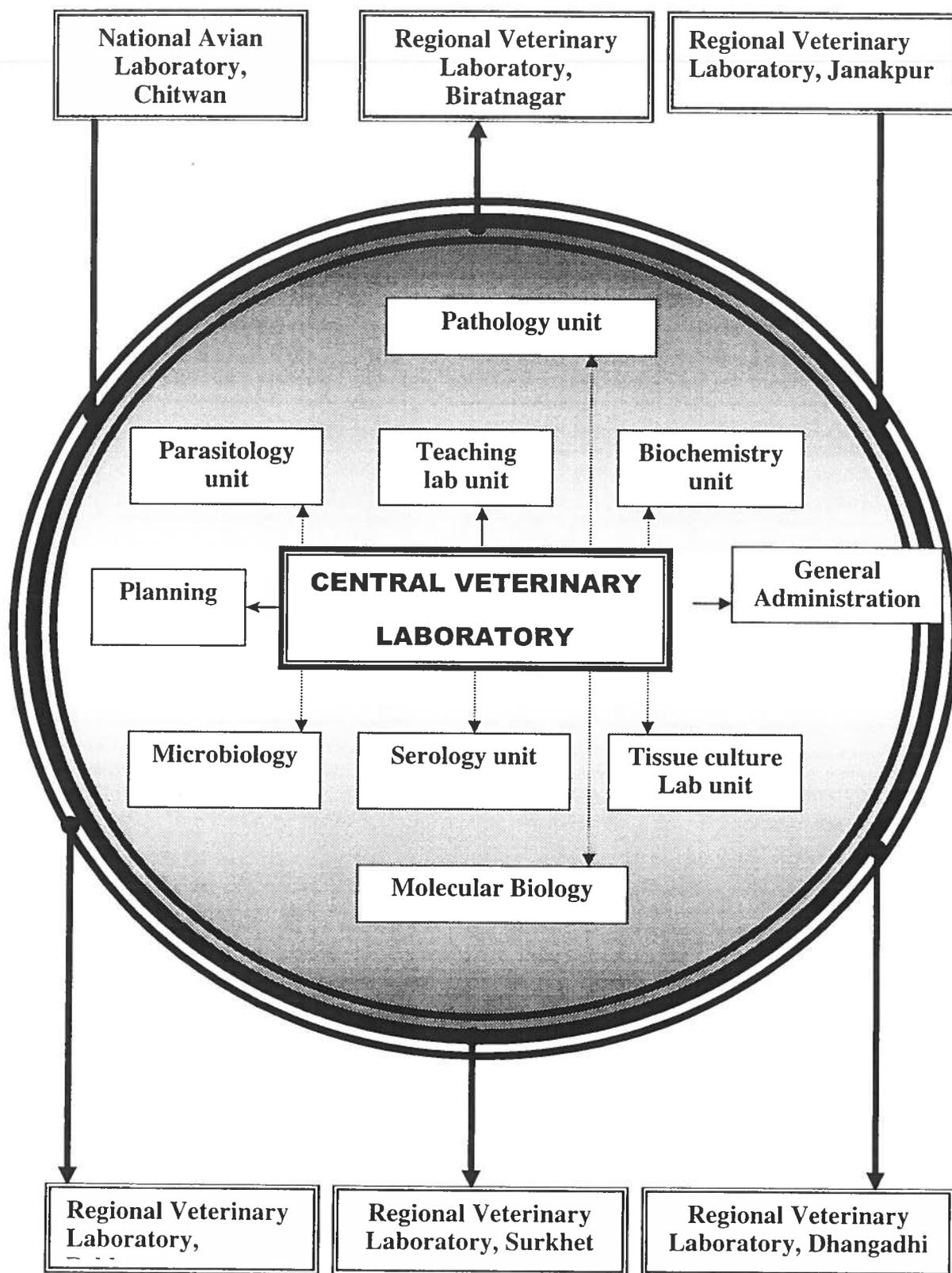
2. Objectives

The role of veterinary laboratory system has become dynamic in the advent of food safety issues, economic liberalization and trade globalization. Nepal is one of the young WTO members. As a least developed country Nepal may get many opportunities from globalization of trades. Therefore, Nepal follows the guidelines provided by Office International des Epizootics (OIE) for the provision of Sanitary and Phytosanitary (SPS) agreement under WTO that seeks scientific procedures and evidences in the course of disease diagnosis as well as production chain. The role of veterinary diagnostic laboratories are now therefore expanded and challenging in the new context. Moreover, CVL works with the following objectives in the country.

- Acts as national reference laboratory.
- Support the national disease control and surveillance programme.
- Conduct disease investigation.
- Acquire, adopt, upgrade and disseminate new as well as different diagnostic test methodologies for livestock diseases.
- Assist in formulating national epidemic control strategies.
- Capacity building of veterinarians and para-veterinarians by organizing laboratory training.
- Strengthen and coordinate regional and district laboratories.
- Disseminate information concerning animal and avian diseases to national and international organization.
- Collaboration with international reference laboratories and institutions.

To achieve the aforementioned objectives, there are a series of approved annual activities carried out by different laboratory units of the CVL, five RVLs and national avian laboratory.

3. Organization Chart



Annual Work Programme & Annual Progress Of CVL (2064/065)

S.N	Activities	Unit	Target	Budget allocated	Achievement	Progress %
1.	Diagnostic Services					
1.1	Parasitology	Number	1500	140	1615	100
1.2	Microbiology	Number	3000	245	6843	100
1.3	Pathology	Number	1200	210	1204	100
1.4	Serology	Number	4500	345	4641	100
1.5	Haematology	Number	500	125	673	100
1.6	Biochemistry	Number	400	115	554	100
1.7	Molecular Diagnosis	Number	30	185	38	100
1.8	Rabies Diagnosis	Number	30	165	35	100
1.9	Tissue culture Diagnosis	Number	6	130	10	100
1.9	Dispatch of samples to other laboratories	Number	500	134	600	100
1.10	Salmonella antigen production	Times	2	195	5	100
2.	Investigation Programs					
2.1	Endemic Outbreak Investigation	Times	12	313	13	100
2.2	Serum Bank Management	Times	12	100	12	100
2.3	Investigation and surveillance of Japanese Encephalitis	Times	6	225	10	100
2.4	Teaching lab management	Times	12	154	12	100
2.7	Surveillance of Avian Influenza	Times				
3.	Teaching Lab Program					
3.1	Teaching Lab Management	Times	12	140	12	100
3.2	Training on Laboratory Technology (3 months/ JT, JTA/10 Persons)	Time	1	300	1	100
3.3	Training on Laboratory Technology (2 Week Officer level)	Time	1	0	0	0
4.	Supervision and Monitoring Program					
4.1	Follow-up & Reporting of Laboratories	Times	12	110	12	100
5.	Workshops					
5.1	Technical Workshop on disease investigation	Times	1	80	1	100
5.2	Participation in regional workshops (5 regions)	Times	5	90	5	100

5.3	Workshop on Progress. & Budget of the next F/Y	Times	1	40	1	100
6.	Publication					
6.1	Annual Technical Report including Regional & NAL	Times	1	90	1	100
7.	Contract Service					
7.1	Sweeper & Gardener/Labtechnician and Mechanics	Times	15	90	15	100
8.	Purchase					
9.1	Technical Books & Journals	Times	3	100	4	100
9.3	Computer and printer	Set	1	100	1	100
9.4	Motor cycle	Number	1	100	1	100
Total				3801		
Administrative Expense				3316		
Grand Total				68,82. 553		

5. Human resource situation, CVL (2064/065)

S. N.	Type of the Post	Class	Number	Fulfilled	Vacant
A.	Technician (Officer)				
1.	Chief Veterinary Officer	I	1	1	-
2.	Senior Veterinary Officer	II	4	4	-
3.	Veterinary Officer	III	3	3	-
B.	Technical (Non-officer)				
4.	Senior technician	I	7	7	-
5.	Stock man;	III	6	6'	-
Total Technical staff			21	21	
B.	Non-technician (Non-officer)				
1.	Junior clerk (Typist)	I	1	1	-
2.	Accountant	I	1	1	-
3.	Clerk	II	1	1	-
4.	Driver	Unclassified	1	1	-
5.	Peon	Unclassified	6	6	-
Total Administration			10	10	-
Grand Total			31	31	

Staff of Central Veterinary Laboratory (At the end of F/Y 2064/065)

S.N.	Name of Staff	Post	Class	Starting from	Remarks
1.	Dr. Rebati Man Shrestha	CVO	G.I	2062.12.6	Works as project manager in CLDP
2.	Dr. Poornima Manandhar	SVO	G.II	2057.12.22	
3.	Dr. Banshi Sharma	SVO	G. II	2064.4.6	
4.	Dr. Karuna Sharma	SVO	G.II	2061.9.11	
5.	Dr. Kedar Bahadur Karki	SVO	G.II	060.10.21	
6.	Dr. Salina Manandhar	VO	G.III	2062.9.11	
7.	Dr. Vinaya Kumar Karna	VO	G.III	059.01.22	
8.	Dr. Pragya Koirala	VO	G.III	062.3.2	
9.	Mr. Asal Bahadur Tamang	VT	NG.I	052.04.01	
10.	Mr. Ashok Pd. Shrestha	VT	NG.I	052.04.01	
11.	Mr. Prakash Devkota	VT	NG.I	060.08.01	
12.	Mr. Bal Bdr. Kunwar	VT	NG.I	053.02.24	
13.	Mr. Tek Bahadur Air	VT	NG.I	058.09.04	
14.	Mr. Gyan Bahadur Bogati	VT	NG.I	2061.12.1	
15.	Mr. Narayan Pd. Ghimire	VT	NG.I	2060.07.24	On Study Leave
S.N.	Name of Staff	Post	Class	Starting from	Remarks
16.	Mr. Laxman Sijapati	JVT	NG.II	2061.11.5	
17.	Mr. Purna Maharjan	JVT	NG.II	053.12.20	
18.	Mr. Hari Prasad Pyakurel	JVT	NG.II	054.12.02	
19.	Mr. Prahlad Basnet	JVT	NG.II	057.12.01	
20.	Mr. Hari Bhakta Karki	JVT	NG.II	059.01.01	
21.	Mr. Bhimsen Adhikari	JVT	NG.II	057.08.01	
Administration/Account					
22.	Mrs. Kamala Shrestha	Typist	NG.I	055.07.11	
23.	Mr. Nirmal Poudel	Accountant.	NG.I		
24.	Mr. Buku Prasad Acharya	Kharidar	NG.II	2064.5.28	
25.	Mr. Macha Kaji Maharjan	Driver	L. V.	055.07.01	
26.	Mrs. Chiri Maya Maharjan	Office Assistant	O.A.-5	055.10.01	
27.	Mr. Santa Raj Budathoki	Office Assistant	O.A.-5	059.11.01	

28.	Mrs. Bhima Acharya	Office Assistant	O.A.-5	055.04.01	
29.	Mr. Hari Gobinda Shrestha	Office Assistant	O.A.-2	059.11.06	
30.	Mr. Chandra Bdr. Rana	Office Assistant	O.A.-2	056.08.23	
31.	Mr. Anoj Bajracharya	Office assistant	O.A.-2	058.11.01	

7. Details of budget sanction & expenditure, CVL (2064/065)

Budget line	Budget head	Budget (Rs.)	
		Sanctioned	Expenditure
1.01	Salary	32,73,000.00	32,73,000.00
1.02	Allowance	28,000.00	13,900.00
1.03	Transfer allowance expense	15,000.00	12,489.00
1.04	Clothes	60,000.00	59,850.00
1.05	Food		
2.01	Water and electricity charge	2,40,000.00	1,73,478.16
2.02	Telecommunication charge	2,09,000.00	2,09,000.00
2.03	Office materials	5,16,094.07	5,16,094.07
2.05	Repair & maintenance	4,00,000.00	3,62,972.46
2.06	Fuel and other provision	3,85,000.00	2,15,102.42
2.07	Consultancy and other service charges	1,11,922.64	1,11,922.64
2.08	Miscellaneous	65,000.00	64,924.00
4.02	Medicine	1,20,000.00	1,19,566.00
4.03	Books and journals procurement	1,00,000.00	92,488.00
4.04	Programme cost	12,19,000.00	11,65,637.60
4.05	Travel & daily allowance (programme)	2,58,000.00	2,57,010.00
	Recurrent cost	69,46,777.35	69,46,777.35
6.02	Vehicle	96,000.00	96,000.00
6.03	Machinery equipments	99,966.00	99,966.00
	Capital expenditure	2,00,000.00	1,95,966.00
	Grand total	69,46,777.35	69,46,777.35

Source detail about Budget	Total	Released	Expenditure	Remain
Government of Nepal	76,16,000.00	73,66,339.23	73,66,339.23	0.00
Total	80,51,000.00	78,00,263.02	78,00,263.02	0.00

PATHOLOGY UNIT

1. Introduction

Pathology literally means 'discourse/study of disease' (Pathos-disease; logus-discourse) and may be defined as a study of disease. It is that branch of medicine which treats of the essential nature of disease especially of the structural and functional changes in tissues and organs of the body which cause diseases. Post Mortem unit (Necropsy examination) and histopathology unit (histological examination) are major area under the pathology laboratory unit in our Central Veterinary Laboratory. As a Reference Veterinary Laboratory, CVL receives a large number of specimens from all over the country either directly or through the respective Regional Veterinary Laboratories (RVLs) and National Avian Disease Diagnosis Laboratory. Besides District Livestock Service offices (DLSOs), Veterinary practitioners and hatcheries as well as farmers deliver specimens for the purpose of disease diagnosis.

Necropsy examination is the first step of disease diagnosis in case of dead animals. The history, clinical findings, epidemiological surveillance informations are also helpful to necropsy examination for diagnosis of diseases. So all the records as history, clinical findings, hematological and biochemical parameters, line of treatment and its response should be sent together with the dead animals to the pathology unit. Veterinary Pathologist during Post Mortem examination may send the sample for other unit as histology, microbiology, molecular biology, parasitology, tissue culture for further investigation of diseases. So the necropsy examination plays very important role in the procurement of different specimens suitable for various diagnosis techniques. In this way Post Mortem Unit provides sizable number of samples to CVL. So the pathological test procedure in a biomedical diagnostic laboratory acts as the opening door where from process of disease diagnosis begins.

In histopathology unit, the tissues received from biopsy and necropsies are processed by wax technique method which will takes a week to provide the result. Nowadays, this technique is regarded as an absolute test procedure. Its value as a diagnostic procedure is still high in the diagnosis of diseases of neoplastic origin, chronic diseases, some of the viral diseases and disease of prion origin. The wax techniques processing methods includes the following steps: Fixation, Dehydration, Clearing, Impregnation, Blocking, Section cutting, Staining and mounting.

The total numbers of cases received for necropsy examination during fiscal year 2064/65 were 651. Out of them 610(93.7%) cases were of chickens which includes commercial broilers, layers and parents. Among 651 cases the remaining 41(6.3%) cases of animals and birds other than chickens. Out of 610 cases of chickens 496(81.3%) were broilers, 94(15.4%) layers and 20(3.3%) parents. Rest of the 41 cases comprised of 19 cases of pigs followed by 5 cases of dog, 1 case of pigeon, goat, sheep and Alpaca each. The monthly distribution of cases for necropsy of Chickens at CVL has been given in table 1. Similarly, the monthly distribution of cases for necropsy of animals and birds other than chickens is given in table 2.

The necropsy examination shows that in duck mycosis is the major disease followed by Colibacillosis, Pasteurellosis, Coccidiosis, Gout, Enteritis and HPPGE. Similarly in pig Swine fever is the most common disease followed by Hemorrhagic septicemia. In dog CVGE and Parvo-viral infection are common followed by cirrhosis. The pigeon was suffered by Colibacillosis, goat by poisoning and sheep by pneumonia. In Alpaca chronic fascioliasis was the problem.

Table 2: Monthly distribution of cases of necropsy examination of animals and birds other than Poultry at CVL

Animals/birds	Diseases/Conditions	4	5	6	7	8	9	10	11	12	1	2	3	Total
Dog	CVGE												2	5
	Parvo viral infection											2		
	Cirrhosis						1							
Swine	Haemorrhagic Septicaemia					2	1							13
	Swine Fever	1		1				2		1	1	1		
	Swine Fever+Mycosis									1				
	PUO								1		1			
Duck	Colibacillosis		1									1	1	19
	Pasteurellosis			2								1		
	Mycosis	1					1		1	1		1	1	
	Coccidiosis							2			1			
	HPPGE			1										
	Enteritis								1					
	Gout					2								
Pigeon	Colibacillosis							1					1	
Goat	Poisoning				1								1	
Sheep	Pneumonia										1		1	
Alpaca	Chronic Fascioliasis						1						1	
Total														41

Biochemistry Unit

The Biochemistry unit of Central Veterinary Laboratory (CVL), Tripureshwor mainly deals with urine and serum samples analysis. These samples are either directly collected from the farmers or send to laboratory from farmers, DLSO, RVL or collected from field during the field outbreak of diseases. These samples are processed in laboratory following the operating protocol and Standard operating procedure (SOP) of the unit

Biochemistry the following test are the performed

- 1) Calcium, Magnesium, Zinc and Phosphorous estimation
- 2) SGOT, SGPT
- 3) Glucose
- 4) Protein and albumin estimation

Urine Analysis

- 1) Dipstick method (Multistick/Unistick)
- 2) Microscopic examination

Urine samples are analysed using Dipstick method and microscopic examination. Urine sample is examined for specific gravity, phosphorous, sugar, ketone bodies, albumin, total protein, bilirubin, triple phosphate, calcium oxalate, RBC and pus cells. A total number of urine samples were 250 (Dogs and cattle) analysed during this fiscal year 2064/2065.

The urine samples were mostly send when there is haematuria. In case of dog if diabetes is suspected and in diagnosis of urinary calculi, kidney function impairment, jundice, ascites and haemoglobinuria this test is requested.

For microscopic examination 23 samples were test where RBC is found in 7, pus cells 4, crystals, 5, epithelial cell 3 and bacteria in 2 test samples.

Five hundred and fifty four samples were tested fore phosphorous, calcium, magnesium, protein and glucose estimation of 554 samples are presented in Table No 3.

Table 3 Total blood estimation and urine analysis of different species

Sl no	Sample tested	No of samples	Dog	Cattle	Buff	Goat
1	Urine analysis	250	110	120	20	
2	Calcium estimation	125	15	80	20	10
3	Phosphorous estimation	125	-	80	20	25
4	Glucose estimation	25	20	5	-	
5	Zinc	-	-	-	-	
6	Magnesium	135	10	80	20	25
7	Protein	144	30	80	20	14
8	Others (urine)	32	7	25	-	-
9	Total	586	75	325	80	74

Hematology Unit

The actual meaning of Haem is blood and study of blood in Greek language is called haematology. Therefore, the responsibility of this unit is to analyse the parameter of whole blood samples collected in EDTA and also blood smear received in the unit or send from District Livestock Service Office (DLSO), Regional Veterinary Laboratory, (RVL) Central Veterinary Hospital (CVH) and collected during disease out break.

Haematology

- 1) ESR
- 2) PCV
- 3) Hb
- 4) Total RBC Count
- 5) Total leukocyte Count
- 6) Total platelets Count
- 7) Differential Count

Six hundred and seventy three samples were tested during fiscal year 2064/2065 eighty six numbers of samples were subjected to identify blood protozoan parasite by using Giemsa stain. Out of tested samples only 19 were positive for babesiosis, anaplasmosis and theilaria.

Table 4: Parameter of tested Samples of various species

SI No	Species	Total samples	Hb	PCV	ESR	TLC	DLC	Blood protozoa +ve case	Remarks
1	Cattle	120	25	30	15	65	49	19	
2	Buffalo	23	15	23	5	27	25		
3	Dog	300	60	55	10	75	62		
4	Horse	150	45	48	6	50	50		
5	Poultry	80	22	25	-	27	27		
6	Total	673	167	181	36	244	188		

MICROBIOLOGY UNIT

1. Introduction

This unit is responsible for bacterial, fungal, and viral diseases diagnosis and investigation of epidemics. In addition, it involves in research and on development activities such as Antigen production and development of test procedure; penside test for Peste des Petitis Ruminant (PPR) diagnosis. The produced Salmonella Antigen is supplied to the different five Regional Veterinary Laboratories (RVLs), National Avian Laboratory (NAL) and private practitioners on demand. Similarly, this unit is also involved in the thesis guidance of graduate and post-graduate students received various academic institutions. Recently, the role and responsibilities of this unit has been expanded in the surveillance, isolation and diagnosis of avian influenza, strain identification of Newcastle disease and diagnosis of swine fever.

This unit receives wide variety of samples from fields, veterinary hospitals, farms, DLSO, Animal Quarantine Check-post and post mortem unit of CVL itself. Besides, it also receives samples/ primary isolates from Regional Laboratories as well as National Avian Laboratory for test verification and result verification.

Microbiological unit comprises four main sub-units, Bacteriology and Mycology, Virology, Rabies Diagnosis and Washing and Sterilization unit through which the various activities are performed.

2. Programs and Progress of Various Sub-units

2.1 Bacteriology and Mycology Unit

This unit is responsible for isolation and identification of bacteria and fungi from various samples. It also performs the drug sensitivity test to isolate organism that facilitate the proper line of treatment. The major samples include milk, various tissues, blood and urine followed by swabs, pus and egg from different species of animal. Similarly, water samples are also received from different hatcheries for the appreciation of microbes present therein. This unit also play, a special role in mastitis control by identifying organism and drug in time.

Progress

During the fiscal year 2064/065, a total of 2,230 samples were received from different species of animals. Among these, only 1,840 samples were found positive from which different organism were isolated through different types of culture methods. Out of these positive samples, different species of fungus were isolated from 344 samples.

A total post mortem sample of different species received from PM section of CVL itself was 1,196. Out of these, 1,135 samples were received from poultry and rest from swine (11), bovine (08), ovine (10) and canine (32). Various organisms like *E. coli*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *pasteurella*, etc. were isolated from 1,098 positive samples as shown in **Table 5**. According to this table, number of isolates has been isolated from one sample.

Table 5: Result of Bacteriological Analysis of Post Mortem Samples

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Poultry	1135	1055	80	<i>E.coli</i> <i>Salmonella</i> <i>Staphylococcus</i> <i>Streptococcus</i> <i>Klebseilla</i> Others (<i>Pseudomonas</i> , <i>Pasteurella</i> , <i>Haemophilus</i> , <i>Bacillus</i> , <i>proteus</i>)	565 194 225 69 12 20
3.	Swine	11	9	2	<i>E.coli</i> <i>Staphylococcus</i>	04 05
4.	Bovine	08	06	2	<i>E.coli</i> <i>Pasteurella</i>	01 05
7.	Ovine	10	08	02	<i>E.coli</i> <i>Staphylococcus</i> <i>Pasteurella</i> <i>Cryptococcus</i>	03 02 01 02
8.	Canine	32	20	12	<i>E.coli</i> <i>Staphylococcus</i> <i>Streptococcus</i> <i>Pseudomonas</i>	02 14 03 01
	Total	1196	1098	98		

A total of 329 milk samples received from field. Out of these, 248 samples were found positive for California Mastitis Test (CMT). The major organisms isolated from milk were *Staphylococcus*, *E.coli*, *Streptococcus* and *Klebsiella etc.* Cryptococcus was also isolated from few milk samples, the result of which has been shown in Table 6.

Table 6: Result of Bacteriological Analysis of Milk Samples

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	328	247	81	<i>E.coli</i> <i>Staphylococcus</i> <i>Streptococcus</i> <i>Klebsiella</i> <i>Bacillus</i> <i>Cryptococcus</i>	74 124 33 18 02 01
2.	Horse	1	1	-	<i>Staphylococcus</i>	01
	Total	329	248	81		

Similarly, total of 22 vaginal swabs samples of bovine were received from field. The organism isolated from these 18 samples was *Staphylococcus*, *Steptococcus*, *E.coli*, and *Bacillus* the detail of the result has been shown in Table 7.

Table 7: Result of Bacterial Culture of Vaginal Swabs Samples

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	22	18	04	<i>Staphylococcus</i> <i>Streptococcus</i> <i>E.coli</i> <i>Bacillus</i>	10 04 02 05
	Total	22	18	04		

Total blood samples received from field were 6. None of them were found positive. The detail of the result has been given in **Table 8**.

Table 8: Result of Bacterial Culture of Blood Samples

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
2.	Canine	06	-	06	-	-
	Total	06	-	06		

A total urine sample of bovine received from field condition was 12. Out of these, only 7 samples were found positive. *Staphylococcus sps* and *Streptococcus sps* were the main organism isolated from these positive samples. The results bacterial culture has been shown in **Table 9**.

Table 9: Result of Urine Culture

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	12	07	05	<i>Staphylococcus</i> <i>Streptococcus</i>	04 03
	Total	12	07	05	-	

The Laboratory also received water samples from different hatcheries. During the fiscal year 2064/065, a total of 75 water samples were received. Out of these, 53 samples were found positive for *E.coli*, *Staphylococcus*, *Streptococcus* and other organism. The results are given in **Table 10**.

Table 10: Result of Bacterial Culture of Water

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
2.	Water	75	53	22	<i>Staphylococcus</i> <i>E.coli</i> <i>Streptococcus</i> <i>Klebseilla</i> <i>Bacillus</i> <i>Haemophilus</i>	20 26 05 02 06 02
	Total	75	53	22	-	

During the fiscal year 2064/065, the laboratory also received different types of samples like poultry shed swabs (61), vaccines (10) and embryo (1). Almost all the samples were found positive. The results are given in **Table 11**.

Table 11: Result of Bacterial Culture of Miscellaneous

S.No	Objectives	Total	Positive	Negative	Isolate Organism	No.
1.	Poultry Shed Swab	61	61	-	<i>Staphylococcus</i> <i>E.coli</i> <i>Klebseilla</i> <i>Bacillus</i> <i>Proteus</i>	27 10 02 18 04
2.	Vaccine	10	10	-	<i>Acinetobacter</i> <i>E.coli</i> <i>Streptococcus</i> <i>Bacillus</i>	01 03 04 04
3.	Embryo	1	1	-	<i>Bacillus</i>	01
	Total	72	72	-	-	

The total samples received from field for fungal culture were 518. Out of these, 311 samples were received from avian and rest from canine, bovine and poultry feed and fodders. The results of isolated fungus are shown in given **Table 12**.

Table 12: Result of Fungal Culture of different species of animals

S.NO.	Species	Total	Positive	Negative	Isolate Fungus	No.
1.	Avian	467	311	156	<i>Penicillium</i> <i>Candida</i> <i>Aspergillus</i>	180 31 100
2.	Canine	29	12	17	<i>Penicillium</i> <i>Candida</i>	09 03
3.	Bovine	01	-	01	-	-
4.	Poultry feed/fodder leaf	21	21	-	<i>Penicillium</i> <i>Candida</i>	20 01
	Total	518	344	174		

2.2 Virology Unit

This unit is responsible for diagnosis of viral diseases applying pathgenecity test method and strain identification of different viruses. It receives the samples mainly from post-mortem section of CVL itself and a few from field.

PPR diagnosis is done by Penside test, which can be done in field condition. Ocular and nasal swabs of goat are required for this test

This unit does have great contribution in the diagnosis of avian influenza. Rapid test was used for the purpose followed by virus isolation in the embryonated chicken eggs.

Progress

During the fiscal year 2064/65, a total of 2,489 samples from chicks, ducks, wild birds and goat were tested for different viral diseases. Out of this only 137 samples were found positive.

A total of 37 samples suspected for New Castle Disease were received from Kathmandu, Morang, Kaski, Surkhet, Jhapa and Bharatpur districts. Only 2 samples were found positive which is shown in **Table 13**.

Table 13: Test Results of New Castle Disease by Egg Inoculation Method

S.No.	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Avian	17	1
2.	Morang	„	03	0
3.	Kaski	„	02	0
4.	Surkhet	„	06	0
5.	Jhapa	„	02	0
6.	Bharatpur	„	07	01
	Total		37	02

A total of 24 serum samples received from different districts like Bharatpur, Jhapa and Kathmandu were tested for Newcastle disease applying HI method. Out of these, 9 samples were found positive which is given in **Table 14**.

Table 14: Test Results of New Castle Disease by HI method

S.No.	District	Species	No. of sample tested	No. of sample positive
1.	Bharatpur	Duck	10	0
2.	Jhapa	„	09	9
3.	Kathmandu	„	05	0
	Total		24	9

Similarly, a total of 9 samples suspected for IBD were received from Kathmandu, Sunsari, Surkhet, Morang, Bharatpur and Sarlahi districts. Out of them only 2 samples were found positive as shown in **Table 15**.

Table 15: Test Results of IBD

S.No.	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Avian	01	0
2.	Sunsari	„	01	0
3.	Surkhet	„	03	0
4.	Morang	„	01	0
5.	Bharatpur	„	01	0
6.	Sarlahi	„	02	2
	Total		09	2

Similarly, a total of 4 samples suspected for swine fever were received from Kathmandu. All were found positive which is given in **Table 16**.

Table 16:

S.No.	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Avian	04	04
	Total		04	04

This unit also received 97 samples from various district of the country like Kathmandu, Lalitpur and Dang for the diagnosis of PPR. These samples were tested through Penside test. A total of 93 samples were found positive. The test result has been shown in **Table 17**.

Table 17: Examination of PPR disease by Penside test method

S.No.	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Goat	12	9
2.	Lalitpur	,,	04	4
3.	Dang	,,	81	80
	Total		97	93

A total of 2,233 samples (tracheal/cloacal swabs) from duck and poultry were received from different district of Nepal which was tested for avian influenza through rapid test method. None of them were found positive which as shown in **Table 18**.

Table 18: Test Results of Avian Influenza by Rapid Test method

S.No	District	Species	Type of Samples	No. of sample tested	No. of sample positive	Remarks
1	Rupendhehi	Duck/Poultry	Tracheal/Cloacal Swabs	309	All samples were Negative	
2	Bharatpur	,,	,,	529		
3	Kaski	,,	,,	152		
4	Morang	Duck/Poultry	,,	133		
5	Kavre	,,	,,	1		
6	Dang	,,	,,	74		
7	Banke	Duck/Poultry	,,	205		
8	Jhapa	,,	,,	41		
9	Surkhet	Poultry	,,	21		
10	Kapilbastu	Poultry/Duck	,,	220		
11	Nawalparasi	,,	,,	86		
12	Bhaktapur	,,	,,	6		
13	Saptari	,,	,,	10		
14	Siraha	,,	,,	31		
15	Ilam	,,	,,	15		
16	Sunsari	Poultry/Duck	,,	235		
17	Lalitpur	,,	,,	50		
18	Kathmandu	,,	,,	31		
19	Kailali	Duck/Poultry	,,	84		
	Total			2233		

But, a total of 54 serum samples were received for HI test. Out of them only 10 samples were found for HI positive which is given in **Table 19**.

Table 19: Test Results of AI by HI method

S.No.	District	Species	No. of sample tested	No. of sample positive
1.	Bharatpur	Duck	32	04
2.	Nuwakot	Poultry	05	01
3.	Bardia	Duck/Wild bird	05	05
4.	Sunsari	Duck	12	00
	Total		54	10

A total of 31 samples were tested through egg inoculation method. Out of them 17 samples were positive for H9 which is shown in table 20.

Table 20: Test Results of Avian Influenza by Egg Inoculation method

S.No	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Duck	04	02
2.	Bardia	Duck/Poultry	15	00
3.	Sunsari	Duck	12	02
4.	Jhapa	Duck/Poultry	14	01
5.	Bharatpur	Poultry/Duck	25	03
6.	Nawalparasi	„	06	02
7.	Nuwakot	„	10	06
8.	Kathmandu	„	04	00
9.	Kapilbastu	„	01	00
10.	Siraha	„	10	00
11.	Morang	Duck	02	02
	Total		31	17

RABIES DIAGNOSIS UNIT

Rabies important from public health aspect and is a part of virology. Virology section of CVL, this unit has been separated as different unit. For the diagnosis of Rabies this unit is using different tests methods are Rapid test, Negri body test, Fluorescence antibody test and Biological test.

During F/Y 2064/065 a total of 32 brain samples were submitted from different field to CVL. Out of those samples 26 were Dogs, 4 were goats, 1 cattle and 1 buffalo. The results are given in following table

Table No. 21

S. No.	Districts	Total Sample of different Animals				Test Results		Remarks
		Dogs	Goats	Cattle	Buffalo	Postive	Negative	
1	Kathmandu	20	3	0	0	14	9	12 dogs & 2 goats positive
2	Bhaktapur	0	0	1	0	1	0	
3	Lalitpur	2	0	0	0	1	1	
4	Pokhara	1	0	0	0	1	0	
5	Dhading	1	0	0	0	1	0	
6	Chitwan	0	0	0	1	1	0	
7	Surkhet	1	1	0	0	1	1	1 Dog positive
8	Kalikot	1	0	0	0	1	0	
	Total	26	4	1	1	21	11	

WASHING AND STERILIZATION UNIT

This unit is the backbone of Central Veterinary Laboratory and so for Microbiology Unit. It provides the clean and sterilized glassware for media preparation and reagent preparation.

SEROLOGY

Introduction

At CVL, serology unit is responsible to conduct various serological tests to detect antigen and antibody for the purpose of diagnosis, screening, monitoring and surveillance of animal and poultry mainly associated with viral and bacterial diseases. This unit possesses capacity and facility of Competitive enzyme-linked immunosorbent assay (ELISA), Indirect ELISA, Agar gel immunodiffusion (AGID) test, Plate agglutination test (PAT) and a solid phase immuno assay (Immuno comb). Most of the samples are submitted to this unit by Regional Veterinary laboratories, Districts Livestock Services Offices, Quarantine check posts, farmers and staff of CVL during disease outbreak investigations well as routine diagnosis. Samples thus received are tested in serology laboratory unit by above test method. This unit is supporting to National Peste des petits ruminants (PPR) Control Programme by testing sera collected from sheep and goat to detect antibodies against infection and to monitor antibodies in vaccinated goats.

2. Programmes and progress

The result of different test for specific disease has been presented from table 22-29 with the overall progress in table 29. A total number of 5,645 sera were tested during 2064/065. Of the total samples, 3,265 sera were tested for seromonitoring to detect antibody titer in response to PPR live vaccine. Vaccine works on 81.28% of goats (Table 26). A total 990 serum samples were tested for the cases of disease outbreak of PPR infection. Out of 990, 323 samples were positive for PPR (Table 27). Screening for Brucellosis was conducted by plate agglutination test (PAT) in fifteen districts by testing 126 samples collected from cattle, buffaloes, pigs, horse and dogs. One sample was found to be positive (Table 22). Three hundred and sixty eight and two twenty samples were tested by PAT method to detect salmonella and mycoplasma infection respectively. Out of those samples 170 for salmonella and 19 for mycoplasma were found positive (Table 23). Out of 351 samples tested by immunocomb method in vaccinated flock for antibodies against Infectious Bursal Disease (IBD), Newcastle Disease (ND) and Infectious Bronchitis (IB) were detected 40, 98 and 96 samples respectively (Table 24). Similarly, antibodies against *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) of 48 serum samples were found negative for both immunocomb test method (Table 25). A total 277 serum samples were tested for Rinder Pest (RP) seromonitoring by C-ELISA method. No samples showed antibody titer against RP (Table 28).

In this fiscal year (064/65), 3805 serum samples were stored at CVL (Table 29). Storage of large quantity of serum for longer time is problem for serum bank. Number of sera is increasing every year but storage capacity is limited. Therefore this is right time to think to remove and dispose the old sera to provide enough space for newly collected samples.

Table No. 22 Plate Agglutination test for Brucellosis (F.Y. 2064-065)

S.No.	District	Animal	Brucella (PAT)		
		Species	Sample Tested	Positive	Negative
1	Bhaktapur	Goat	6	0	6
2	Rupandehi	Cattle	1	0	1
3	Rupandehi	Buffalo	4	0	4
4	Kathmandu	Cattle	3	0	3
5	Kathmandu	Cow	15	0	15
7	Parsa	Buffalo	12	0	12
8	Kanchanpur	Goat	38	0	38
9	Lalitpur	Cow	1	0	1
10	Makawanpur	Pig	6	0	6
11	Ilam	Cattle	3	1	2
12	Kavre	Cattle	9	0	9
13	Banke	Cattle	5	0	5
14	Banke	Buffalo	5	0	5
15	Sankhuwasabha	Horse	18	0	18
Total			126	1	125

Table No. 23: Plate Agglutination test for Salmonella pullorum & Mycoplasmosis in poultry (F.Y. 2064-065)

S.No.	District	Salmonella (PAT)			Mycoplasma (PAT)		
		Sample Tested	Positive	Negative	Sample Tested	Positive	Negative
1	Kathmandu	44	6	38	42	0	42
2	Chitwan	281	170	111	135	17	118
3	Sunsari	33	0	33	33	2	31
4	Bhaktapur	5	2	3	5	0	5
5	Lalitpur	5	0	5	5	0	5
Total		368	178	190	220	19	201

Table 24: Sero- monitoring of Poultry Disease (IBD, ND, IB) by Immuno-comb test (F.Y. 2064-065)

S. N.	District	IBD			ND			IB		
		Sample Tested	Posi tive	Neg ative	Sample Tested	Posi tive	Neg ative	Sample Tested	Posi tive	Neg ative
1	Mohottari	6	2	4	6	3	3	6	6	0
2	Dhanusha	6	1	5	6	2	4	6	2	4
3	Kathmandu	12	3	9	12	11	1	12	5	7
4	Chitwan	93	63	30	93	74	19	93	83	10
Total		117	69	48	117	90	27	117	96	21

Table 25: Sero- monitoring of Poultry Disease by Immuno-comb test (F.Y. 2064-065)

S.No.	District	MG			MS		
		sample Tested	Positive	Negative	Sample Tested	Positive	Negative
1	Mohottari	6	0	6	6	0	6
2	Dhanusha	6	0	6	6	0	6
3	Kathmandu	12	0	12	12	0	12
Total		24	0	24	24	0	24

Table No. 26 PPR Sero-monitoring record in vaccinated goats by C-ELISA (F.Y.064/065)

S. No.	District	Tested samples	Test Results		Positive %
			Positive	Negative	
1	Parsa	70	53	17	76
2	Siraha	40	33	7	83
3	Jhapa	80	66	14	83
4	Sunsari	165	134	31	81
5	Saptari	72	57	15	79
6	Udaypur	140	114	26	82
7	Rupandehi	40	34	6	85
8	Morang	110	99	11	90
9	Bhojpur	70	58	12	83
10	Rauthat	56	48	8	86
11	Lalitpur	90	74	16	82
12	Kathmandu	72	57	15	79
13	Ramechhap	96	86	10	90
14	Doti	90	78	12	87
15	Dadeldhura	40	34	6	85
16	Sindhupalchok	104	85	19	82
17	Kavre	108	93	15	86
18	Shyangja	136	112	24	82
19	Kapilbastu	40	32	8	88
20	Gorakha	120	99	21	83
21	Terathum	60	47	13	79
22	Surkhet	52	43	9	83
23	Khotang	60	44	16	73
24	Taplejung	60	50	10	83
25	Mohottari	60	46	14	77
26	Bara	44	32	12	73
28	Kailali	30	28	2	93
29	Rukum	80	66	14	83
30	Kanchanpur	90	72	18	80
31	Dang	75	58	17	77
32	Banke	51	31	20	69
33	Makwanpur	110	88	22	88
34	Bhaktapur	80	68	12	85
35	Sankhuwasabha	48	36	12	75
36	Dhankutta	46	37	9	88

37	Jajarkot	80	64	16	88
38	Nawalparasi	160	122	38	76
39	Nuwakot	110	91	19	83
40	Dailekh	40	31	9	78
41	Salyan	40	32	8	88
42	Dhading	20	15	5	75
43	Dhanusha	80	68	12	85
44	Bajura	50	39	11	78
Total		3265	2654	611	81.28 % (Pos.%)

**Table No. 27: C-ELISA test for diagnosis of PPR in Sheep and Goats
(Cases of Disease outbreak) F.Y. 064-065**

S. No.	District	Sample Tested	Test Results	
			Positive	Negative
1	Parsa	16	9	7
2	Siraha	97	64	33
3	Jhapa	22	7	15
4	Sunsari	30	4	26
5	Saptari	65	23	42
6	Udaypur	10	1	9
7	Rupandehi	51	15	36
8	Morang	59	10	49
9	Bhojpur	10	2	8
10	Rauthat	15	10	5
11	Lalitpur	1	0	1
12	Kathmandu	18	1	17
13	Ramechhap	13	6	7
14	Doti	5	5	0
15	Dadeldhura	16	5	11
16	Sindhupalchok	88	12	76
17	Kavre	40	17	23
18	Shyangja	7	3	4
19	Kapilbastu	13	9	4
20	Gorakha	10	5	5
21	Terathum	33	9	24
22	Surkhet	2	2	0
23	Khotang	10	2	8
24	Taplejung	10	0	10
25	Mohottari	22	9	13
26	Bara	31	11	20
28	Kailali	23	12	11
29	Rukum	14	7	7
30	Kanchanpur	12	3	9
31	Dang	30	1	29
32	Banke	28	6	22
33	Makwanpur	10	1	9

34	Bhaktapur	21	9	12
35	Sankhuwasabha	11	0	11
36	Dhankutta	15	4	11
37	Jajarkot	20	4	16
38	Nawalparasi	5	3	2
39	Nuwakot	10	3	7
40	Dailekh	20	2	18
41	Salyan	20	8	12
42	Dhading	14	5	9
43	Dhanusha	1	0	1
44	Bajura	15	5	10
45	Saralahi	27	9	18
Total		990	323	667

Table No. 28 Rinder Pest (RP) Sero-monitoring in ruminant animals by C-ELISA (F.Y.064/065)

S.No.	District	Species	SampleType	SampleTested	Test Results	
					Positive	Negative
1	Nawalparasi	C,B,G	Serum	50	0	50
2	Banke	C,B	Serum	42	0	42
3	Bara	B	Serum	50	0	50
4	Morang	C,B	Serum	41	0	41
5	Lalitpur	C,B	Serum	6	0	6
6	Kavre	C	Serum	8	0	8
7	A.Q.C.P.Banke	G	Serum	40	0	40
8	Myagdi	C,B	Serum	40	0	40
TOTAL				277	0	277

Note: C = Cattle, B = Buffalo, G = Goat

Table No. 29 Serum Storage Record (F./Y. 2064-065)

S.No.	District	Animal Species	No.of Serum Storage
1	Rauthat	Goat	81
2	Arghakhanchi	„	80
3	Kavre	„	150
4	Ramechap	„	71
5	Rasuwa	„	60
6	Nuwakot	„	70
7	Dhading	„	160
8	Kathmandu	„	70
9	Lalitpur	„	70
10	Bhaktapur	„	70
11	Chitwan	„	215
12	Sindhupalchowk	„	140

13	Mohattari	„	81
14	Dhanusha	„	81
15	Saralahi	„	81
16	Sindhuli	„	143
17	Makmanpur	„	194
18	Kaski	„	60
19	Parbat	„	60
20	Tanahun	„	60
21	Shyanja	„	60
22	Myagdi	„	60
23	Baglung	„	60
24	Morang	„	212
25	Jhapa	„	212
26	Dhankutta	„	135
27	Saptari	„	343
28	Sunsari	„	218
29	Ilam	„	219
30	Udayapur	„	289
Total			3805

MOLECULAR BIOLOGY UNIT

1. Introduction

Molecular techniques are very sensitive, fast and reliable tools for diseases disease diagnosis and research works. Therefore, molecular biology unit of central veterinary laboratory has started to diagnose the bacterial and viral diseases by extracting DNA and RNA with the use of polymerase chain reaction (PCR) test and reverse transcriptase polymerase chain reaction (RT-PCR) techniques. The unit is responsible for diagnosis of avian influenza by RT-PCR.

2. Activities and progress

In the F/Y 2064/065, total 35 of avian influenza suspected samples were tested and found all negative for the same.

RT-PCR was performed for both cDNA synthesis and PCR amplification in a single tube using gene-specific primers (Recommended by WHO H₅ Reference Laboratory Network).

The PCR product so prepared was subjected to electrophoresis using 2% agarose gel and observed under ultraviolet light in which the samples showed no positive band of HPAI H₅N₁ virus at 219 base pair and therefore, all the samples were negative.

PARASITOLOGY UNIT

The Parasitology unit is involved in routine examination as well as investigation of different digestive tract parasites and non-digestive tract endo-parasites of animals and birds causing adverse effects on livestock and poultry health as well in production. Faecal samples, skin scrapings, blood samples from different animals and birds are examined by adopting standard test protocols. They are done mainly for identification of eggs/ova of different nematodes, cestodes, trematodes and other common parasites found in gastrointestinal system of livestock.

Qualitative test is done by the technique of double floatation for detection and identification of the eggs of gastrointestinal parasites whereas the quantitative test is performed by the modified Mc Master's counting methods for the determination of number of eggs per grams in the feces which helps in the evaluation of the extent of parasitic burden in a particular animal species. Furthermore, this unit also carries out larvae culture for the identification of nematodes. Similarly, skin scrapings for the presence of mites, blood samples for the presence of blood parasites are routinely carried out. All these laboratory works are being conducted in collaboration with RVLs and animal health research division of Nepal agriculture research. In addition, this unit is also involved in the surveillance of parasitic infestations in various wild and zoo animals regularly since past few years.

Samples from districts, private practitioners are also being examined too assess the magnitude of parasites and parasitism. Since last few years this unit is actively involved in collaborative research work and study programme of graduate and post-graduate study of Trivuwani University and Purbanchal University in field of Parasitology. With this collaboration, the Unit has been able to profiling the specieswise prevalence of parasite. So far the unit has prepared the profile of different parasite spp present in goat, buffaloes, Monkey, Captive Elephant. As this serves as baseline information on the background of this unit intended to conduct the EPG, Larva culture. Test result of parasitological examination has been presented in following table. Over the last fiscal year 3,250 samples of cattle, buffaloes, goats, dogs, poultry and monkeys were tested out of which 1,885 were found positive for parasites. The various types of parasitological examination conducted during the F/Y 2064/065 have been presented in tabular form as shown below.

Table: Results of various types of parasitological examination (2064/065)

(**Note:** The numbers in the head of the table is indicative of Nepalese fiscal years. The digit 4 represents Shrawan and so forth.)

S. N.	Type of Parasites	Species	4	5	6	7	8	9	10	11	12	1	2	3	Total	
1	Fasciola spp	Cow	22	10	23	25	32	45	56	53	67	30	15	23	401	
		Bull	1	-	-	-	-	-	-	-	-	-	-	-	-	1
		Goat	7	6	25	20	45	76	43	10	54	12	10	12	320	
		Sheep	-	-	-	1	-	-	-	-	-	-	-	-	-	1
		Buffalo	40	34	32	64	30	25	40	57	40	50	86	65	563	
		Dog	-	-	-	-	-	-	-	-	-	-	-	-	-	1
2	Paramphistomum spp	Cow	17	7	2	1	-	9	2	12	-	-	-	-	50	
		Goat	1	-	-	1	-	-	-	-	-	-	-	-	2	
		Buffalo	2	-	-	-	-	-	-	-	-	-	-	-	2	
		Sheep	-	-	-	1	-	-	-	-	-	-	-	-	1	
		Monkey	-	-	-	-	-	-	-	1	-	-	-	-	1	
3	Strongyles	Cow	11	11	-	1	-	-	-	12	-	-	-	-	35	
		Goat	19	22	43	23	31	12	16	1	10	18	20	24	239	
		Buffalo	2		-	-	-	-	-	9	-	-	-	-	11	
		Pig	-	10	-	-	-	-	-	-	-	-	-	-	10	
		Horse	-	-	-	-	-	7	-	-	-	-	-	-	7	
		Monkey	-	-	-	-	-	-	-	29	-	47	90	40	206	
4	Skin scrapping	Dog	8	-	-	-	-	-	3	-	-	-	-	11		
5	Blood protozoa	cow	-	2	-	-	-	-	-	-	-	-	-	-		
		Dog	-	-	-	-	-	-	-	1	-	-	-	-	1	
6	coccidia	Poultry	-	-	-	-	-	-	-	-	-	-	-	-		
7	Ascasis	Pig	-	7	-	-	-	-	-	-	-	-	-	-	7	
		Dog	-	-	1	-	-	-	-	-	-	-	1	-	2	
Positive			130	109	126	156	138	174	157	181	171	157	221	165	1885	
Negative			54	60	90	123	120	124	120	201	90	145	109	129	1365	
Total sample			184	169	216	279	258	298	277	382	261	302	330	294	3250	

1. IDENTIFICATION OF HELMINTH EGGS OF GOATS IN KATHMANDU Valley 2064/2065:

During examination of winter samples 46% samples were found positive among 100 samples and 90.3% samples of summer were found positive among 124 samples.

The total number of genera observed during examination was 21 in number: 3 genera of trematode, 2 genera of Cestode and 16 genera of Nematodes in general. Seasonally, 2 genera each in case of Trematode and Cestode during winter and summer and 11 and 15 genera of Nematodes were found during winter and summer respectively. Identification of eggs of helminthes was done on the basis of their morphology and the characters.

Observed genera of different Classes:

Srl. No.	Class	Genera of Helminth
1.	Trematode	<i>Dicrocoelium sp.</i>
2.		<i>Fasciola sp.</i>
3.		<i>Schistosoma sp.</i>
4.	Cestode	<i>Moniezia sp.</i>
5.		<i>Taenia sp.</i>
6.	Nematode	<i>Ancylostoma sp.</i>
7.		<i>Ascaris sp.</i>
8.		<i>Bunostomum sp.</i>
9.		<i>Capillaria sp.</i>
10.		<i>Chabertia sp.</i>
11.		<i>Diactophyma sp.</i>
12.		<i>Dictyocaulus sp.</i>
13.		<i>Gnathostoma sp.</i>
14.		<i>Haemonchus sp.</i>
15.		<i>Oesophagostomum sp.</i>
16.		<i>Ostertagia sp.</i>
17.		<i>Strongyloids sp.</i>
18.		<i>Trichostongylus sp.</i>
19.		<i>Trichuris sp.</i>
20.		<i>Oxyuris sp.</i>
21.		<i>Necator sp.</i>

Prevalence of nematode genera

S. No.	Name of the genera	Total samples examined	Positive samples	
			Nos.	%
1.	<i>Strongyloides</i>	262	11	4.19
2.	<i>Trichostrongylus</i>	262	5	1.90
3.	<i>Toxocara</i>	262	60	22.90
4.	<i>Ascaris</i>	262	18	6.87
5.	<i>Chabertia</i>	262	1	0.38
6.	<i>Trichuris</i>	262	6	2.29
7.	<i>Dictyocaulus</i>	262	2	0.76
8.	<i>Oesophagostomum</i>	262	2	0.76
9.	<i>Capillaria</i>	262	1	0.38
10.	<i>Haemonchus</i>	262	3	1.14
11.	<i>Ostertagia</i>	262	4	1.52
12.	<i>Cooperia</i>	262	2	0.76

Class-wise prevalence of helminthes parasite

S. No.	Class	Identified helminthes (species)
1.	Trematode	<i>Fasciola hepatica</i>
2.		<i>Paramphistomum cervi</i>
3.		<i>Schistosoma spindalis</i>
		<i>Schistosoma japonicum</i>
		<i>Schistosoma bovis</i>
4.		<i>Dicrocoelium lanceatum</i>
5.		<i>Gastrothylax crumenifer</i>
6.		<i>Fischoederius elongatus</i>
7.	<i>Ornithobilharzia turkestanicum</i>	
8.	<i>Skrjabinema ovis</i>	
1.	Cestode	<i>Moniezia expansa</i>
		<i>Moniezia benedeni</i>
1.	Nematode	<i>Strongyloides papillosus</i>
2.		<i>Trichostrongylus axei</i>
		<i>Trichostrongylus colubriformis</i>
3.		<i>Toxocara vitulorum</i>
		<i>Toxascaris leonina</i>
		<i>Toxocara canis</i>
4.		<i>Ascaris vitulorum</i>
5.		<i>Chabertia ovina</i>
6.		<i>Oesophagostomum radiatum</i>
7.		<i>Haemonchus contortus</i>
8.		<i>Ostertagia sp.</i>
9.		<i>Cooperia sp.</i>
10.	<i>Dictyocaulus sp.</i>	
11.	<i>Trichuris ovis</i>	
12.	<i>Capillaria sp.</i>	

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, BIRATNAGAR (EASTERN REGION)

Regional Veterinary Laboratory (RVL) has been situated in sub-metropolitan city, Biratnagar-17, of eastern Nepal and was established in the fiscal year 1988/1989 AD. But until 1990/1991, the laboratory was not functional and could not perform its activities as per objectives due to lack of manpower, necessary equipments and frequent changes in organizational structure. From fiscal year 1991/1992, the RVL has its separate identity. There was provision of manpower and other logistics. The programme was launched as per objectives.

The working area of this RVL is all districts of Eastern Development Region (EDR). In this eastern region, there are three zones (Mechi, Koshi and Sagarmatha) and 16 districts. Geographically, the region is divided into three eco-zones (high hills, mid hills and terai).

High hills:

This eco-zones lies in the northern part of the region covering Taplejung district of Mechi zone, Sankhuwasabha district of Koshi zone and Solukhumbu district of Sagarmatha zone. Livestock rearing is the main occupation of the farmers in this region. Yak/Nak, chauri, sheep and goat are being reared in this region.

Mid-hills:

This region falls between high hills on its north and tarai at the south. Panchthar, Illam, Dhankuta, Terathum, Bhojpur, Okhaladhunga, Khotang, and Udaypur districts are under this eco-zone. Farmers follow mixed farming system and agro-based livestock industries are their main occupation. Cattle, buffalo, swine, goat are being reared in this region. Poultry and rabbit farming are also popular among the farmers

Terai:

Jhapa, Morang, Sunsari, Saptari and Siraha districts of Nepal are under Terai eco-zone. Though traditional system of livestock rearing is followed in this region, in recent years, poultry, dairy industries and piggery are being commercialized especially in east-west highway corridor.

Objectives of Regional Veterinary Laboratory

- ❖ To provide prompt and efficient disease diagnostic services to the farmers of the region.
- ❖ To investigate and diagnose the epidemics in the region.
- ❖ To assist and support DLSOs in disease diagnosis and epidemic control.
- ❖ To supervise and assist in diagnostic services to basic and primary laboratories situated in DLSOs of the region.
- ❖ To collect, analyze and predict the animal diseases prevailing in the region.
- ❖ To develop human resources for the field level veterinary services.
- ❖ To co-ordinate and support national animal disease control and eradication programme.
- ❖ To support and facilitate the national veterinary regulatory services.
- ❖ To participate actively in collaborative and coordinated research program in animal health and production in the region.

Annual Progress Report (2064/2065)

S.N.	Programme	Unit	Annual target	Annual Progress	Progress%
1	Laboratory services				
1.1	Parasitological examination	No.	1500	2181	100%
1.2	Microbiological examination	"	800	854	100%
1.3	Pathological examination	"	400	445	100%
1.4	Serological examination	"	500	631	100%
1.5	Haematological examination	"	250	261	100%
1.6	Biochemical examination	"	500	1099	100%
1.7	Sample sent to other lab.	"	600	1301	100%
2	Investigation and Surveillance Program				
2.1	Infectious Bovine Rhinotrachitis	Times	6	6	100%
2.2	Epidemic investigation	"	6	6	100%
3	Supervision and monitoring of DLSOs	"	9	9	100%
4	Veterinary Disease Investigation Workshop	"	1	1	100%
5	Publication and epidemic reporting				
5.1	Publication of Six monthly epidemiological bulletin	"	2	2	100%
5.2	Publication of annual epidemiological bulletin	"	1	1	100%
5.2	Publication of annual technical report	"	1	1	100%
5.3	Annual technical bulletin publication	„	1	1	100%
6	Purchasing of Books	„	1	1	100%

Laboratory Services:

The routine laboratory works of RVL, Biratnagar, involve examination of fecal samples, CMT and MWT tests of milk samples. Cultural examination of mastitis positive milk samples are done to isolate and identify the bacteria responsible for this disease. Blood samples are received here, particularly for Hb, PCV, TC, DLC tests, total protein and blood protozoa identification. Serum samples are used to estimate Ca and P level in the blood of animal. Similarly, serological test is done to screen brucella affected animal and salmonella affected poultry in this region. Hypersensitivity test (tuberculin test) is done to isolate tuberculosis affected animal. Examination of skin scraping and urine samples is frequently done in RVL, Biratnagar.

Parasitological examination

In this examination, both internal and external parasites are identified from the samples. For internal parasites, fecal examination of different animals is done routinely. The fecal samples are received mainly from farmers, DLSOs and also collected from field during survey and investigation programs. Most frequently the fecal examination is done by sedimentation and floatation techniques to identify the gastro-intestinal parasites. However, in certain cases, Mc Master Technique is followed to quantify the eggs per gram (EPG) in feces.

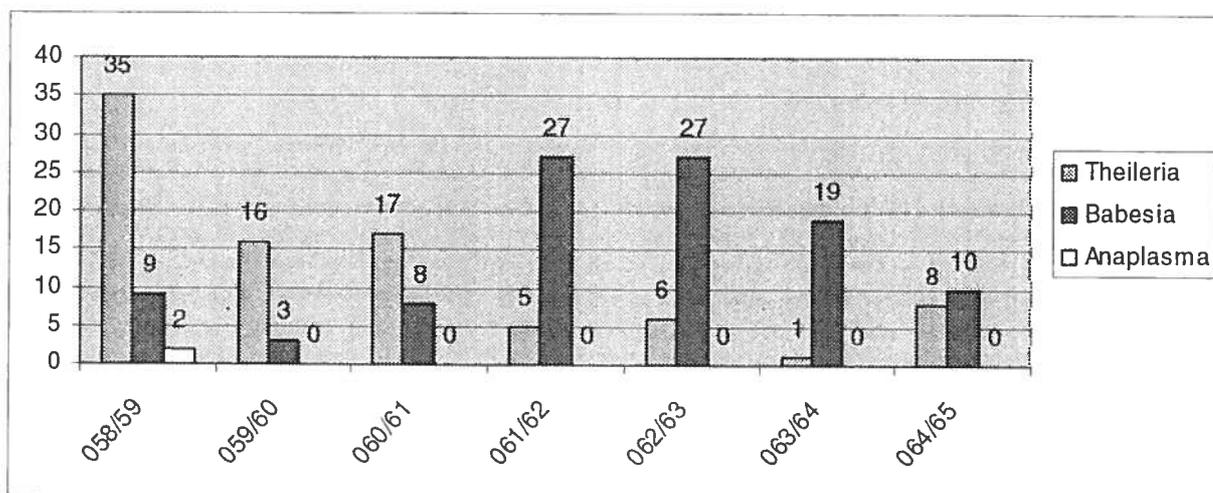
In the fiscal year 2064/065, altogether 1906 fecal samples from different species of animal were received and examined. Among 1906 samples, 1517 samples (79.59%) were positive and 389 samples (24.4 %) showed negative result. The result of fecal test revealed that fasciolosis (62 %) is the most prevalent parasitic infestation followed by paramphitomiasis (22 %) and nematodiasis (14%), the least.

Haematological examination:

Under haematological examination, TLC, TEC, DLC, PCV and Hb tests are done in this lab. Hb estimation is done by Sahli's haemoglobinometer, PCV by microhaematocrit method, total count of RBC and WBC by haemocytometer. For DLC, blood samples are stained with Giemsa.

Blood samples received from different districts of eastern region were examined for blood parasites. A total number of 261 samples were examined for blood parasites. Out of them, 18 samples were positive in which babesiosis was dominant having 10 positive samples. Rest 8 samples were of theileriosis.

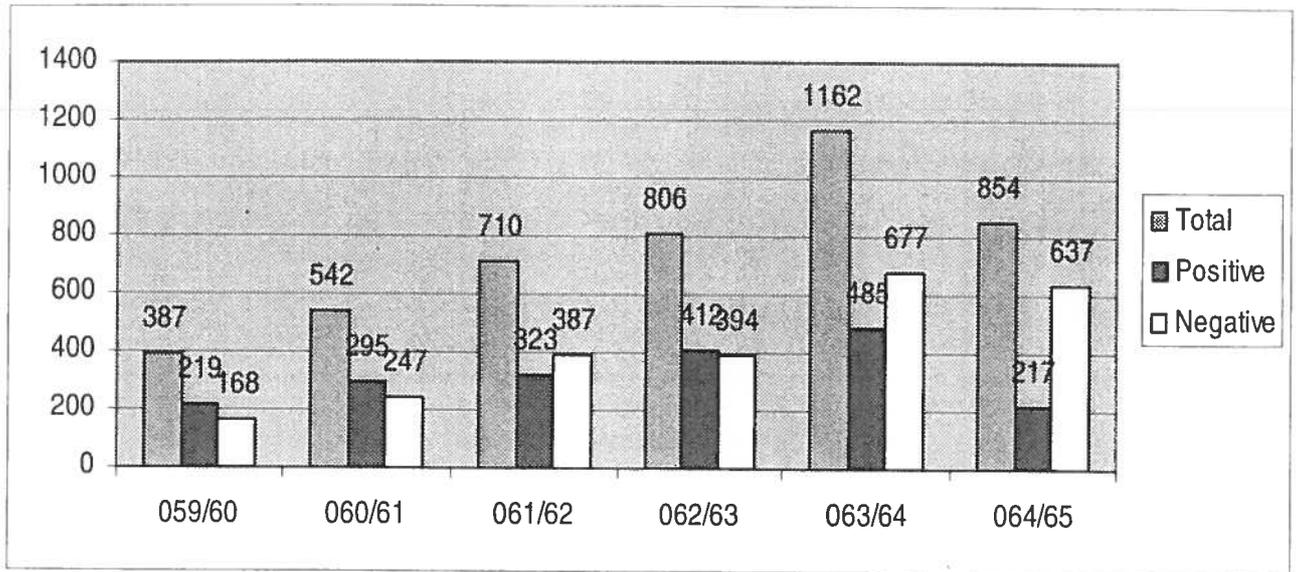
Comparative chart of positive blood samples (f/y: 058/59 to 064/65)



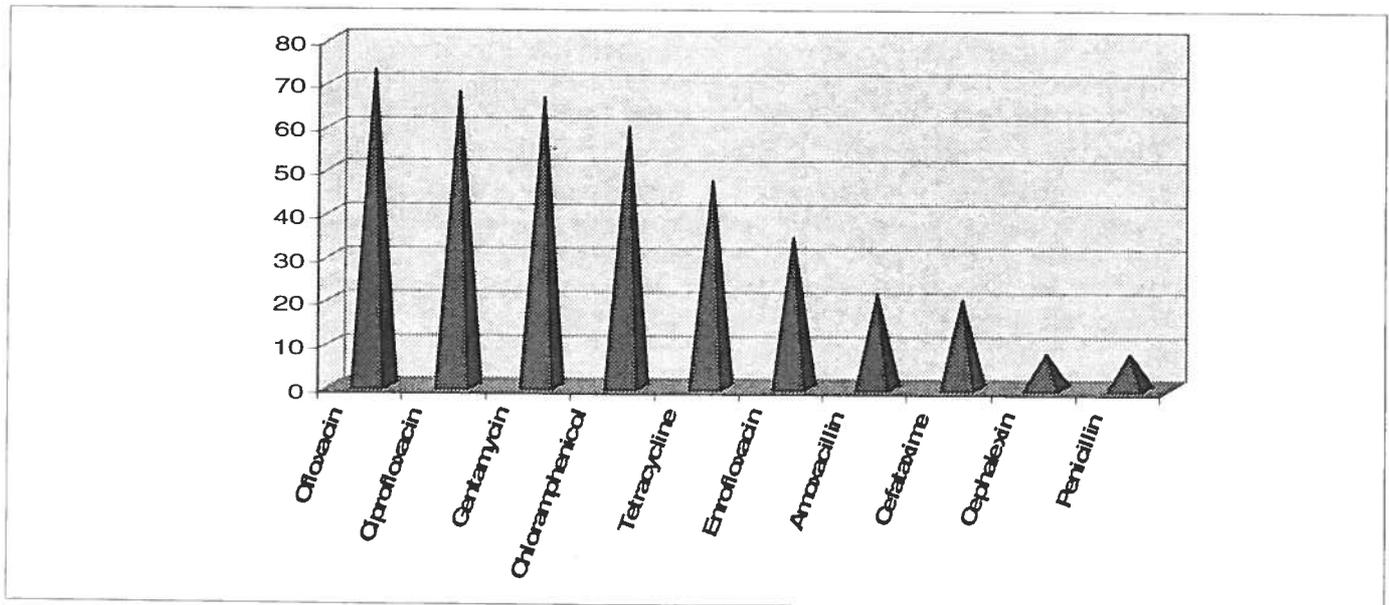
Microbiological examination:

Altogether 854 milk samples were registered in this laboratory in the fiscal year 2064/065. Out of them 217 samples were positive for CMT and MWT tests. The most prevalent bacteria isolated from these positive milk samples were staphylococcus, streptococcus, E. coli, klebsiella, pseudomonas, enterobacter etc.

Milk test from F/Y: 2059/60 to 064/65:

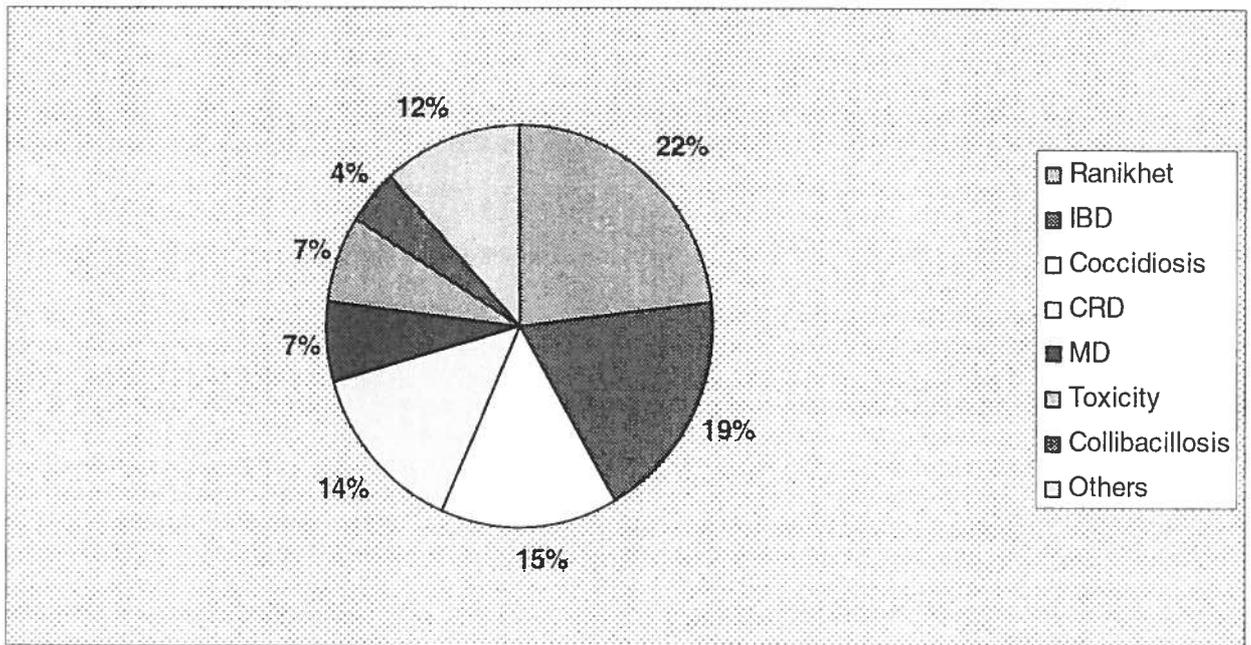


Antibiotic sensitivity Test:



Pathological examination:

Mostly postmortem examinations of dead birds and occasionally of dead animals are done in the laboratory. During PM examination impression smears, swab, tissues are collected for required tests. Altogether 445 dead birds were received to the lab. On the basis of PM examination and lab tests, diagnosis is done. Out of 445 samples, ND had higher incidence followed by IBD and Coccidiosis.



Biochemical examination:

Examination of urine and analysis of blood is routinely done to assess the different conditions of urine and blood constituents. Serum samples are collected from farmers, sites of investigation program, etc. Altogether 1099 serum samples were collected and analyzed in the fiscal year 2064/065 for the estimation of total protein, glucose, phosphorus, using specific kits. Urine samples were tested by using dipsticks (multisticks) as well as biochemical methods. Examination of urine was done for specific gravity, PH, sugar, albumin, ketone bodies, urobilinogen etc. Mostly Rothera's test and Robert's test were done to detect ketone bodies and protein respectively.

Serological Examination:

Serological examination is done mainly for two diseases (salmonellosis and brucellosis) in this laboratory. In fiscal year 2064/065 altogether 46 samples were tested for pullorum disease by Plate agglutination method (PAT). All samples were found to be negative. Similarly, Rose Bengal plate test (RBPT) is done for screening the brucella positive animals. This test was done in 585 animals (cattle and goat) and all the samples were negative for this test.

Sample sent to CVL, Kathmandu in F/Y 2064/065:

As the laboratory is not well equipped with the modern equipments, the samples are sent to CVL. Sometimes, the samples have to be sent to CVL for reconfirmation of the diagnosis.

Bird serum	151
Bird swab	122
DEAD BIRD	14
SERUM FOR IBR	13
Serum for PPR	1001
TOTAL	1301

PPR Vaccine Distribution:

At the start of F/Y 2063/64, total dose: **206,550**

Vaccine distributed dose: **42450**

.N	District	Vaccine dose
1	Udaypur	3000
2	Sankhuwa:	3000
3	Bhojpur	2100
4	Terhathum:	2100
5	Saptari:	4500
6	Siraha:	1500
8	Khotang	8150

**National PPR Programme
Sero-monitoring
F/Y-2064/065**

DLSOs	Udaypur	Sunsari	Morang	Siraha	Saptari	Dhankutta	Illam	Jhapa	Total
Vaccination to be done (No.)	18000	25000	25000	25000	25000	18000	18000	25000	179000
Serum to be collected (No.)	90	125	125	125	125	90	90	125	895
Collected Serum (No.)	94	125	130	127	133	90	94	127	920

Epidemiological Reporting Of EDR

Total no. of animals treated, EDR F/Y 2064/65					
S.N.	District	No. of cases where animal examined	No. of cases where no animal seen but sample examined	No. of cases where no animal seen and no sample examined	Total
1	Morang	7506	7728	7779	23013
2	Sunsari	10271	6808	7352	24431
3	Jhapa	6414	4723	4280	15417
4	Siraha	12232	2218	7518	21968
5	Saptari	6441	504	6964	13909
Total		42864	21981	33893	98738
6	Tehrathu	3359	1340	7182	11881
7	Dhankuta	4045	2769	12572	19766
8	Bhojpur	4414	2655	10813	17882
9	Ilam	7257	5634	5901	18792
10	Panchtha	1627	2401	3902	7930
11	Udayapu	2820	1662	7100	11582
12	Okhaldhu	2269	2323	4448	9040
13	Khotang	3314	4357	14282	21953
Total		29105	23141	66200	118486
14	Solu	1265	1237	2101	4603
15	Taplejun	3875	1410	7369	12654
16	Sankhuw	2410	3556	16078	22044
Total		7550	6203	25548	17477
Grand Total		79519	51325	125641	256485
Percentage		31 %	20.01 %	48.99 %	

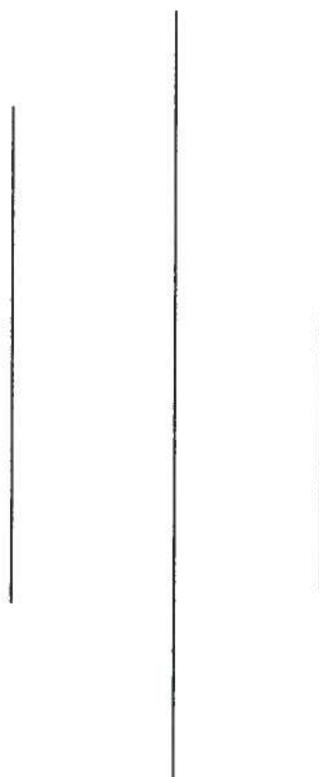
Vaccination done in EDR f/y 2064/065 (Based on epidemiological report)

District	B.Q.	H.S.	FMD	S. fever	Rabis	Total
Morang	2850	2850			119	5819
Sunsari	34878	38673				73551
Jhapa	1685	6468	703	132	112	9100
Dhankutta	27030	17678		20	85	44813
Bhojpur	5386	5386		67	330	11169
Terhathum	1127	1444				2571
Sankhuwa	5234	5234		301	94	10863
Ilam	----	-----				----
Panchthar	2000	2000			601	4601
Taplejung	6769	6769			179	13717
Solu	-----	----				----
Okhaldhunga	-----	---				----
Khotang	5695	5695			08	11398
Udayapur	5421	5421		40	80	10962
Siraha	2001	2001			36	4038
Saptari	8602	8602			--	17204
Total	108678	106421	703	560	1644	218006

Government of Nepal
Ministry of Agriculture & Cooperatives
Department of Livestock Services
Directorate of Animal Health

Regional Veterinary Laboratory

Janakpur
2064/065



Regional Veterinary Laboratory

Janakpur

Introduction:-

Regional Veterinary Laboratory of the central development region is situated in Janakpur. It provides diagnostic services to all the 19 Districts of central development region. Various diseases diagnosed at the Laboratory units i.e. Pathology, Parasitology, Microbiology, Haematology & Biochemistry. Serological & Histopathological Laboratory test result are obtained by dispatching the relevant specimens to CVL, as these diagnostic facilities are not available in RVL Janakpur at present.

The Working areas of this RVL is all districts of Central region but mainly focused on these districts that is Dhanusha, Mohottary, Bara, Parsa, Rautahat, and Sindhuli. In this Central Region there are 3 zones and 19 districts geographically the region is divided in to 3 Eco-zones, Mountain, Hill & Terai.

Mountain :-

This ecozones lies in the northern part of the region covering Rasawa, Sindhupalchok of Bagmati zone. Livestock rearing in the main occupation of the farmers in this region Yak, Nak, chauri, sheep & goat are being reared in this region.

Hills :-

Kathmandu, Bhaktapur, Lalitpur, Dolkha, Dhading, Makawanpur, Sindhuli are under this region. Farmers follow mixed farming system and agro-based livestock industries are their main occupation Cattle Buffalo, pig, poultry & goat are being reared in this region. Poultry farming are also popular among the farmers.

Terai :-

Dhanusha, Mohottary, Sarlahi, Bara, Parsa, Rautahat & Chitwan are under this Terai region. Through traditional system of livestock farming in followed in this region poultry, dairy & piggy are being Commercialized especially in east west high way and its vicinity.

Objectives of RVL Janakpur

- To Provides disease diagnostic service to the Farmers.
- To Investigate & diagnose the epidemics in the Region.
- To assist & support DLSOs in disease diagnosis and epidemic Control.
- To Support animal health and Infertility camps in the region.
- To supervise basic laboratories runs in DLSOs of the region.

Staff of RVL Janakpur (At the end of F/Y 2064/065)

S.N.	Name of Staff	Post	Starting Date	Work of Responsible Unit
1.	Dr. Mahantha Yadav	S.V.O.	2064.05.19	Chief
2.	Mr. Mahandra Prasad Sah	V.O.	2064.01.16	Pathology, Monitoring
3.	Mr. Krishna Bhushan Mahato	V.O.	2063.09.18	Planning, Heamatology, Bird flu Programme
4.	Mr. Anirudra Sah	V.T.	2061.03.17	Microbiology, Serology
5.	Mr. Pradip Yadav	V.T.	2064.06.01	Parasitology
6.	Mr. Vishnu Kant Jha	J.V.T.	2060.01.14	Biochemistry, Help desk
7.	Mr. Phul Narayan Yadav	J.V.T.	2058.04.09	Sterilization
8.	Mr. Shiv Narayan Yadav	Kharidar	2052.12.16	Administration, Store
9.	Mr. Rudra Prasad Ghimire	Accountant	2061.12.09	Financial

10.	Mr. Kula Nand Jha	Driver	2055.08.18	Driving
11.	Mr.	Computer Operator		Vacant
12.	Mr. Gogal Raut	Office assistant	2057.09.03	Office guard
13.	Mr. Dinesh Datta	Office assistant	2054.03.08	Office Attendant

Annual Progress Report (2064/065)

S.N	Programmers	Unit	Annual Target	Annual Progress	Annual Weightage	Progress %
A.	Laboratory Service					
1.	Parasitological Examination	Nos.	1500	2802	7.5	100
2.	Microbiological Examination	Nos.	800	5	7.2	0.6
3.	Pathological Examination	Nos.	250	272	4.9	100
4.	Serological Examination	Nos.	400	771	6.2	100
5.	Hematological Examination	Nos.	350	350	6.5	100
6.	Biochemical Examination	Nos.	400	405	6.2	100
7.	Sample Send to other Lab.	Nos.	600	715	7.2	100
B.	Investigation & Surveillances Programs					
1.	Study on Infertility	Times	6	6	14.0	100
2.	Epidemic Investigation	Times	6	6	15.0	100
C.	Supervision Programmed					
1.	DLSOs Lab. Management Follow up	Times	9	9	8.5	100
D.	Work Shop Programme					
1.	Veterinary disease investigation workshop.	Times	1	1	2.1	100
E.	Publication					
1.	Six Monthly Epidemiological bulletin	Times	2	2	1.6	100
2.	Annual Technical Report	Times	1	1	1.1	100
3.	Annual Technical Report	Times	1	1	0.6	100
F.	Book Purchase					
		Times	2	2	2.1	100
G.	Laboratory animal management					
		Times	12	12	1.6	100

Laboratory Services

The routine Laboratory Works of RVL Janakpur, mainly involves examination of faecal Samples CMT test of milk samples and cultural examination of mastitis. Positive milk samples are done to isolate and identify the bacteria responsible for this disease. Blood samples bought here through DLSOs Particularly for Hb, PCV, TC, DLC, total protein and blood protozoa identification. Examination of skin scraping & urine test frequently has been done in RVL Janakpur.

Parasitological Examination

Parasitological examination (internal & external), faecal examination of different animals has been done routinely. The faecal samples are received mainly from farmers referred by DLSOs and also collected from Dhanusha, Mahottary, Sarlahi, Sinnduli, Rauthat, and Bara, field area during surveillance and investigation programme. The examination has been done by sedimentation and floatation techniques to identify the gastro intestinal parasites. Mc'master technique is followed to quantity the eggs per gram (EPG) in faeces.

In the F/y 2064/065 total 2,802 faecal sample from different species of animals were received & examined. Among these samples 2,611 samples (93.17%) were positive result and 190 samples (6.83%) showed negative results. The results of faecal test revealed that fasciola (36.06%) paramphistomum (42.62%) remaining (24.52%) stronggles, ascaris & other parasites.

Figures of faecal samples examined and types of parasites in this test are followed

Month	No.of samples	Fasciola	Param Phistomum	Stronggles	Ascaris	Other	-VE
Srawan	53	12	22	7	4	5	3
Bhadra	373	102	184	32	18	13	24
Asoj	875	212	349	85	39	98	92
Kartik	560	118	254	49	64	55	20
Mensir	426	132	187	36	32	28	11
Poush	202	67	82	17	12	10	14
Magh	20	5	8	3	—	2	2
Falgun	72	18	28	12	5	4	5
Chaitra	30	8	9	3	4	2	4
Baisakh	17	3	8	—	3	1	2
Jestha	63	15	17	12	8	6	5
Asad	110	38	46	9	7	2	8
Total	2801	730	1194	265	196	226	190

Haematological examination

Haematological examination TLC, TEC, DLC, PCV, & Hb test are done in this laboratory. Total count of RBC, WBC by haemocytometer for DLC blood samples are stained with Giemsa blood samples received from different districts of central region.

Total 350 blood samples were examined for different blood parameters as well as for blood parasites. Out of 350 samples 258 samples were found negative for any blood parasites & rest 93 were found positive for different blood parasites.

Details of blood sample examination

Month	Total sample	Anaplasma	Babesia	Theileria	Trypa	-VE
Shrawan	4	—	—	2	—	2
Bhadra	5	—	1	2	—	3
Asoj	24	1	4	6	—	13
Kartik	7	—	3	2	—	2
Marg	9	1	2	—	—	7
Poush	15	1	1	5	—	8

Magh	2	–	–	–	–	2
Falgun	25	–	2	3	–	20
Chaitra	63	–	2	8	2	51
Baisakh	7	–	–	1	1	4
Jestha	44	1	4	–	10	29
Asad	146	2	7	16	4	117
Total	350	6	25	45	17	258

Pathological Examination

The pathological examination includes mostly post mortem examination of the dead birds received from commercial poultry farms. 99% cases bought from Janakpur municipality area & periphery the rural area of the Dhanusha district for pathological test. A total 272 cases of post mortem examination were presented during the F/Y 2064/065 all the cases received was birds. No cases of large & small ruminants and other species of animal were received. The status of poultry disease in the area is shown in table.

Trend of disease occurrence in poultry

S.N.	Tentative diagnosis	total cases	
		Number	Percent
1.	Coccidiosis	112	41.17
2.	IBD	78	28.67
3.	CRD	53	19.48
4.	Salmonellosis	13	4.77
5.	W.B.D	9	3.30
6.	Micotoxicosis	7	2.57

Microbiological Examination

Total 405 milk samples were taken in this laboratory in the F/Y 2064/065 out of them 362 were positive for CMT test. the most prevalent bacterial isolated from these positive milk samples staphylococcus, streptococcus, E.coli etc.

Milk test month wise F/Y 2064/065

Month	No. of samples	CMT positive	CMT negative	% of positive
Srawan	31	28	3	6.91
Bhadra	38	62	6	15.30
Asoj	42	38	4	9.38
Kartik	26	24	2	5.92
Mansir	30	28	2	6.91
Poush	42	38	4	9.38
Magh	20	16	4	3.95
Falgun	32	30	2	7.40
Chaitra	19	16	3	3.95
Baisakh	17	14	3	3.45
Jestha	24	19	5	4.69
Asad	54	49	5	12.09
Total	405	362	43	89.38

Antibiotic Sensitivity test results

Antibiotics used	Percent efficacy
Enrofloxacin	82%
Ciproflaxacin	75%
Gentamycin	67%
Cloxacillin	65%
Oxytetracyclin	55%
Amoxicillin	48%
Penicillin	25%

Biochemical examination :-

Examination of urine is routinely done to assess different conditions of urine. Serum samples are collected from farmers sites of investigation under the programmes of sero surveillances & sero monitoring. Urine samples tested in this laboratory by using multi-stick as well as biochemical methods. Examination of urine was done for specific gravity, pH, Sugar, albumin, ketone & protein etc. altogether 35 urine samples were tested & 715 serum samples collected & sent to CVL for PPR disease diagnosis.

Serology :-

In this unit the serum samples are collected from different districts during disease investigation & surveillances programmes. Most of the serum samples collected from livestock and poultry were dispatched to CVL and referred laboratories for diagnosis serum monitoring of vaccinated goats and poultry were also done with the help of CVL.

Details of the serological test result

S. N.	Districts	Animal species	No. of samples	Test requested	Results		Remarks
					+ve	-ve	
1.	Dhanusha	cattle	11	H.S.	2	9	
2.	Mahottary	buffalo	12	H.S.	2	10	
			9	F.M.D.	4	5	
3.	Sarlahi	goat	91	PPR	9	82	
4.	Bara	cow, buffalo	16	H.S.		16	
5.	Parsa	—	—	—	—	—	
6.	Sinduhali	goat	46	PPR	—	—	
7.	Rauthat	—					
8.	Makwanpur	—	—	—	—	—	
9.	Kathmandu	goat	76	PPR	4	72	

Sample send to CVL for further Investigation in F/Y 2064/065

Regional veterinary laboratory Janakpur is not well equipped with the modern equipments. The samples are sent to CVL the samples have to be sent to CVL for diagnosis of the disease & Sometimes reconfirmation of the different types of disease diagnosis. FMD suspected samples sent to FMD laboratory Budhanilkant, Kathmandu.

S.N.	Types of Samples	Number
1.	Bird serum	5
2.	Bird swab	15
3.	Serum for PPR	697
4.	FMD	13
5.	Total	715

PPR Vaccine Distribution

S.N.	Districts	Vaccine dose
1.	Dhanusha	3500
2.	Mahottary	7000
3.	Sarlahi	10000
4.	Sindhuli	5000
5.	Bara	5000
6.	Parsa	5000
7.	Rautahat	5000
	Total	40500

Note :-

At the start of the F/Y 2064\065
 Total PPR dose-----83500
 Vaccine distributed dose ----40500
 Remaining dose - - - - -40000

National PPR Programme Sero-Monitoring F/Y 2064\065

Name of DLSOs	Dhanusha	Mahottary	Sarlahi	Sindhuli	Bara	Parsa	Rautahat
Vaccination to be done (no.)	3500	7000	10000	5000	5000	5000	5000
Serum to be collected (no.)	63	82	46	73	-	-	81

National PPR Programme Sero-Surveillance F/Y2064\065

Name of DLSOs	Dhanusha	Mahottary	Sarlahi	Sindhuli	Bara	Parsa	Rautahat
Serum to be collected (no.)	173	182	146	64	-	-	63

Bird flu Surveillance in Central development region, RVL Janakpur (F/Y 2064/065)

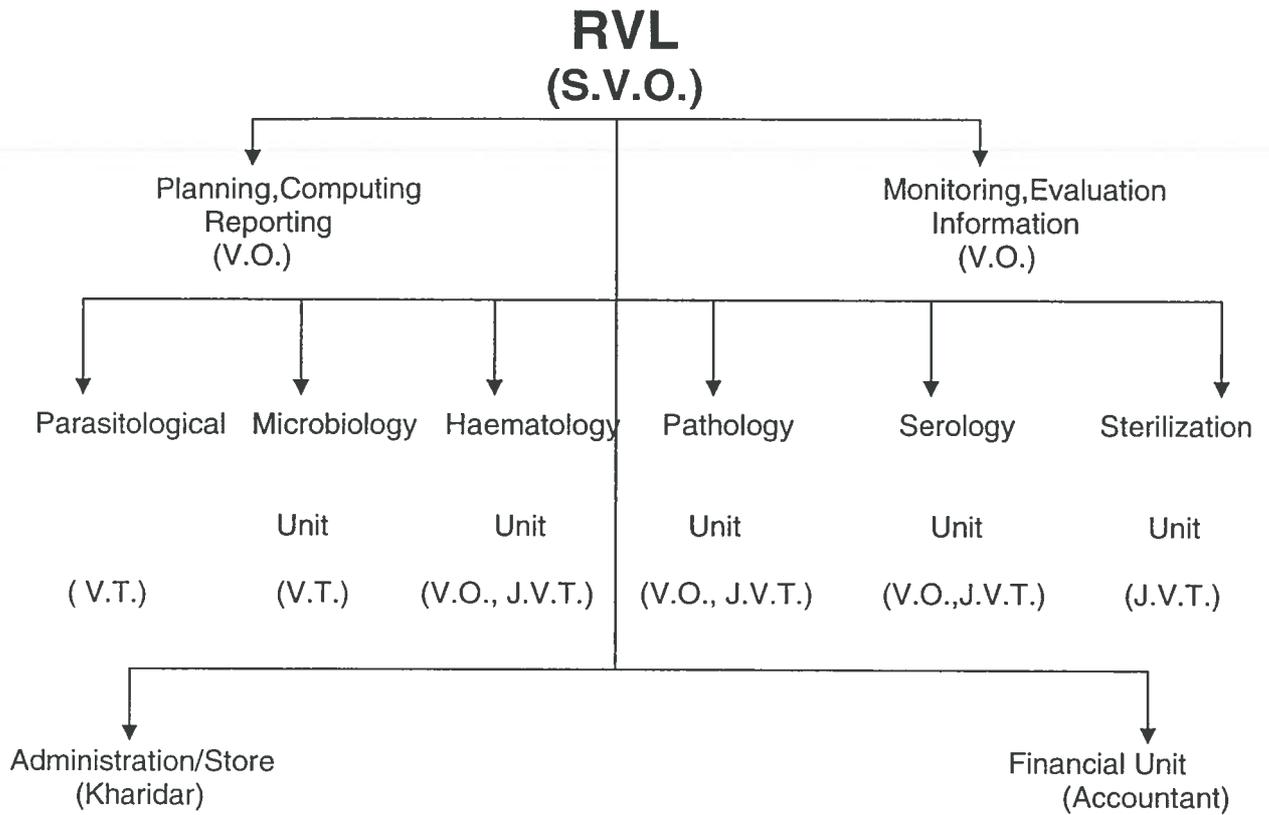
S.N	Districts	Types of birds	Types of forming	Types of samples collected		Total	Test result
				TS	CS		
1.	Dhanusha	Poultry	CB	110	110	220	-ve
		Duck	BD	5	17	22	-ve
2.	Mahottary	Poultry	CB	96	67	163	-ve
3.	Sarlahi	Poultry	CB	150	140	290	-ve
4.	Sindhuli	Poultry	CB	67	47	114	-ve
5.	Bara	Poultry	CB	87	44	131	-ve
6.	Parsa	Poultry	CB	76	52	128	-ve
7.	Rauthat	Poultry	CB	86	96	182	-ve
	Total			677	573	1250	

Note :-

- CB :- Commercial Bird
- BD :- Backyard Duck
- BP :- Backyard Poultry

Epidemiological Reporting of Central development Region 2064/065

s.n	districts	no. of cases where sick animal examined	no. of case where no animal examined but samples examined	no. of case no animals seen no sample examined	total
1.	Dhanusha	19296	1716	264	21276
2.	Mohottary	8402	3312	175	17064
3.	Sarlahi	9724	4413	185	14322
4.	Sundhuli	4616	2884	4872	12372
5.	Bara	11352	2712	2964	17028
6.	Parsa	3480	4008	7380	14868
7.	Rauthat	8320	3406	180	14906
8.	Makwenpur	5640	5196	4272	15108
9.	Chitwan	8784	10620	7776	27180
10.	Dhading	5892	5664	5736	17292
11.	Kathmandu	3636	1512	4344	9492
12.	Bhaktapur	3564	2352	636	6552
13.	Lalitpur	8220	2772	5400	16392
14.	Karvre	7024	1927	6224	15175
15.	Rasawa	600	372	12684	13665
16.	Nawakot	492	7992	12660	21144
17.	Ramechap	3828	9660	2784	16272
18.	Dolkha	3267	1644	5267	11822
19.	Sindhupalchock	4702	1203	2805	10712



Note :-

- S.V.O :- Senior Veterinary officer
- V.O. :- Veterinary officer
- V.T. :- Veterinary Technician
- J.V.T.:- Junior Veterinary Technician

National Avian Laboratory Chitwan .

National Avian laboratory was established on first of Baisakh, 2061 in Bharatpur, Chitwan by then government of Nepal to meet the demand of the poultry industry and entrepreneurs. By its nature this laboratory is purely specific i.e. only for birds.

Newly established laboratory has many lacunas due to insufficient resources. Evolution of poultry industry nationwide and mainly in Chitwan created the situation for establishment of Avian laboratory immediately which was proposed in 9th five year plan.

Newly emerged fast growing poultry industry singly hold the 4 % National GDP and about more than Rs.16000 million has been invested in this sector .Opportunity of employment of great number of veterinarians, para-vets, farm entrepreneurs and establishment of new industry related to poultry farming are other important factors. Its future objective is to analytic services for vaccine titration and feed analysis. But now a days activities like sample collection, poultry disease diagnosis, sero-surveillance for avian influenza.

Construction work of this Laboratory building was started in 055/056 initially but up to now it is not fulfilled according to our needs like lab animal house , biological pit , road , drainage , store , staff quarters second storey office building .

Beside there is lack of vehicle which creates great problems during sample collection , sero- surveillance , monitoring of neighboring districts farm and high risk area investigation programme.

Objectives

Objective of National Avian laboratory are classified into three categories according to immediate need of NAL and nation's plan.

A .Immediate objectives

- i. To develop facilities to make available accurate and reliable diagnostic services for avian diseases of epidemiological importance inside the country.
- iii. Surveillance of avian diseases of zoonotic importance to formulate effective control strategy.

B .Mid-term objectives

- Isolation, characterization and preservation of different bacteria, virus, fungi and protozoa causing diseases in poultry.
- Analysis of feed for nutrients and detection of different toxins.
- Study of environment in poultry farming

C. Long-term objectives

- To develop and recommend effective vaccination schedule based on epidemiological evidences and sero-monitoring of the flock.
- To develop different types of antigens, anti-sera antibodies from field isolates.
- To develop different types of test kits for poultry diseases.
- To assure easy and steady supply of biological needs for poultry disease diagnosis.
- To develop vaccines based on local strains.
- To develop it as center of research and development for all aspects of poultry industry.

Location Detail of NAL Chitwan ,Bharatpur

1. Geographical location:

Bharatpur,
District headquarter of Chitwan District
On the Eastern bank of River Narayani
Elevation: 2109Mt
(Longitude & latitude of Chitwan district)
Longitude: 83 degree 35 minute -84 degree 84 minute East
Latitude: 27 degree 21 minute -27 degree 46 minute North

2. Link to Road:

On the way of Mugling to Bharatpur (Besides of by-pass road)
180k.m.south west from Capital City Kathmandu
150k.m. north west from Birganj, the gateway city of Nepal
About 200 k.m North East from Lumbini, the birth place of Lord Buddha
About 170k.m south east from Pokhara the famous tourist destination
Only 15k.m.away from Royal Chitwan National Park

3. Link to air route:

The near by Bharatpur airport only about 500 m away from the main office.

4. Climate

Subtropical monsoon climate with maximum temperature in July



Pathological Examination of carcasses

Clinical sign and symptoms are similar in many diseases & for differential diagnosis post mortem is the first and for most task in lab. Now a day, well established and equipped Laboratories have different section for proper diagnosis like Bacteriology, Virology, Histology, Serology, Bio-chemistry etc. For Postmortem fresh sample should be registered because decaying, decomposed due to delay collection samples would give false positive or false negative report.

In our Laboratory (NAL) bacteriology, virology & histology sections get samples from PM room, that's why sample should be fresh.

Diseases Suspected from PM findings

S.N.	Disease names	Month												Total
		Shrawan	Bhadra	Aswin	Kartik	Mansir	Pous	Magh	Phalgun	Chaitr	Baishak	Jestha	Ashar	
1.	IBD	0	21	0	5	4	0	1	20	9	4	3	10	77
2.	Ascariasis	10	13	11	0	2	2	4	6	3	1	12	11	75
3.	NAD	5	5	0	6	1	3	1	14	1	9	2	4	51
4.	Gout	0	1	2	1	5	8	1	13	2	10	5	0	48
5.	Coccidiosis	10	0	0	2	14	2	0	10	1	1	2	1	43
6.	Ascitis	10	0	0	0	0	0	0	0	13	1	9	10	43
7.	Mycotoxin	0	12	10	0	1	1	2	1	1	4	3	1	36
8.	E.Coli	0	1	1	0	2	2	4	2	3	9	4	0	28
9.	Tape worm	12	4	3	1	0	0	0	0	0	1	0	5	26
10.	Salmonella	0	1	8	0	0	1	1	0	3	2	1	0	17
11.	CRD	0	1	1	0	0	0	0	1	9	1	2	1	16
12.	Heat stress	2	1	3	0	0	0	0	0	0	7	1	1	15
13.	Marek's Disease	0	1	1	1	0	1	3	0	1	5	2	0	15
14.	Metabolic disease	0	2	1	1	1	0	0	0	0	1	2	0	8
15.	Fowl cholera	1	1	1	2	0	0	0	2	0	0	1	0	8
16.	Omphalitis	0	1	4	0	0	0	0	0	0	0	0	0	5
17.	IB	0	0	0	0	0	1	0	0	2	1	0	0	4
18.	ND	0	0	0	0	1	0	0	0	0	1	0	1	3
19.	Pericarditis	0	1	1	0	0	0	0	0	0	0	0	0	2
20.	Fatty liver syndrome	0	1	0	0	1	0	0	0	0	0	0	0	2
21.	Leechi heart disease	0	0	0	0	0	0	0	0	0	1	0	0	1
22.	Peritonitis	1	0	0	0	0	0	0	0	0	0	0	0	1
23.	Pigeon pox	0	0	0	0	0	0	0	0	0	1	0	0	1
	Total	51	67	47	19	32	21	17	69	48	60	49	45	525

Note: CRD-Chronic respiratory Disease ,I BD : Infectious Bursa Disease , ND-New castle Disease, IB-Infectious Bronchitis ,NAD-Not actual

Month wise treated Birds

S.No.	Disease name	Month												Total
		Shra wan	Bha dra	Aswe en	Katric	Mans hir	Poush	Magh	Phal gun	Chaitra	Bais hak	Jestha	Asha dha	
1	Metabolic	1500	0	6000	0	0	0	0	1200	100	0	1800	0	10600
2	Toxin	2300	0	0	500	0	500	900	3100	0	0	6000	1350	14650
3	Omphalitis	1400	0	2000	500	0	501	0	0	2000	0	1300	11500	19201
4	ND	2000	0	1100	18000	0	7	0	400	3801	0	3500	0	28808
5	Coccidiosis	1400	0	1500	1	500	520	900	6000	0	0	500	2400	13721
6	Pasteurellosis	0	2600	0	0	0	0	0	0	0	0	0	0	2600
7	MD	0	1200	2500	2000	0	0	2500	31500	1000	0	7000	2500	50200
8	Leucosis	0	0	0	1000	0	0	0	0	1800	0	0	0	2800
9	Worm	1500	0	0	0	0	0	0	0	1000	0	0	14	2514
10	IBD	2000	1000	675	400	1500	400	400	2500	0	3200	5300	7850	25225
11	Fowl Cholera	1500	0	0	0	0	0	0	0	0	0	5000	0	6500
12	Stress	0	2000	1100	0	0	0	1000	0	1000	0	0	0	5100
13	Gout	0	1700	0	2000	3000	0	0	1000	0	0	0	3000	10700
14	E.coli	0	4900	2000	5800	2000	0	900	0	1500	6800	3000	2870	29770
15	NA	0	9650	5910	10001	5400	8701	4950	5150	10050	13551	16525	10520	100408
16	Salmonella	0	500	0	0	0	0	0	0	0	0	0	0	500
17	CRD	0	0	0	4000	500	0	0	0	1830	2000	15400	0	23730
18	Bp	0	0	0	0	0	0	900	0	0	0	0	0	900
19	Ascitis	0	0	0	0	0	0	0	1000	0	0	0	0	1000
20	IB	0	0	0	0	0	0	0	0	7000	0	2500	500	10000
21	Fowl Typhoid	0	0	0	0	0	0	0	0	2600	0	2800	500	5900
	Total	13600	23550	22785	44202	12900	10629	12450	51850	33681	25551	70625	43004	364827

1. Microbiological test at NAL

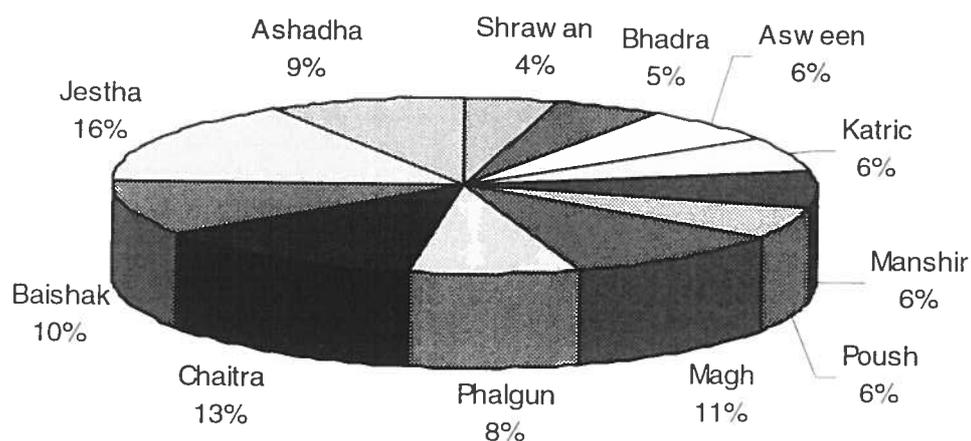
This unit prepared several media like Nutrient Agar, Mac Conkey Agar, Salmonella Agar, EMB Agar, XLD Agar, Blood agar & SDA. Beyond that culture of bacteria and fungus is another vital function of this unit. In fiscal year 2064/065 218 cases were referred to this unit. Among them majority are for culture of bacteria like salmonella, E. coli, streptococcus and Fungus etc.

1. Bacteriological unit

Monthly received sample in Bacteriology unit

S.no.	Month	Samples types				Total
		Liver	Heart	Lungs	Spleen	
1	Shrawan	6	6	0	0	12
2	Bhadra	7	4	0	0	11
3	Aswin	9	5	0	0	14
4	Kartik	9	5	0	0	14
5	Marg	9	2	0	0	11
6	Poush	8	7	2	5	22
7	Magh	15	5	2	1	23
8	Phalgun	11	3	0	0	14
9	Chaitra	18	11	0	1	30
10	Baishak	14	13	0	1	28
11	Jestha	22	4	0	0	26
12	Asar	13	0	0	0	13
Total		141	65	4	8	218

Sample received /Month in Microbiology lab at NAL 064/065

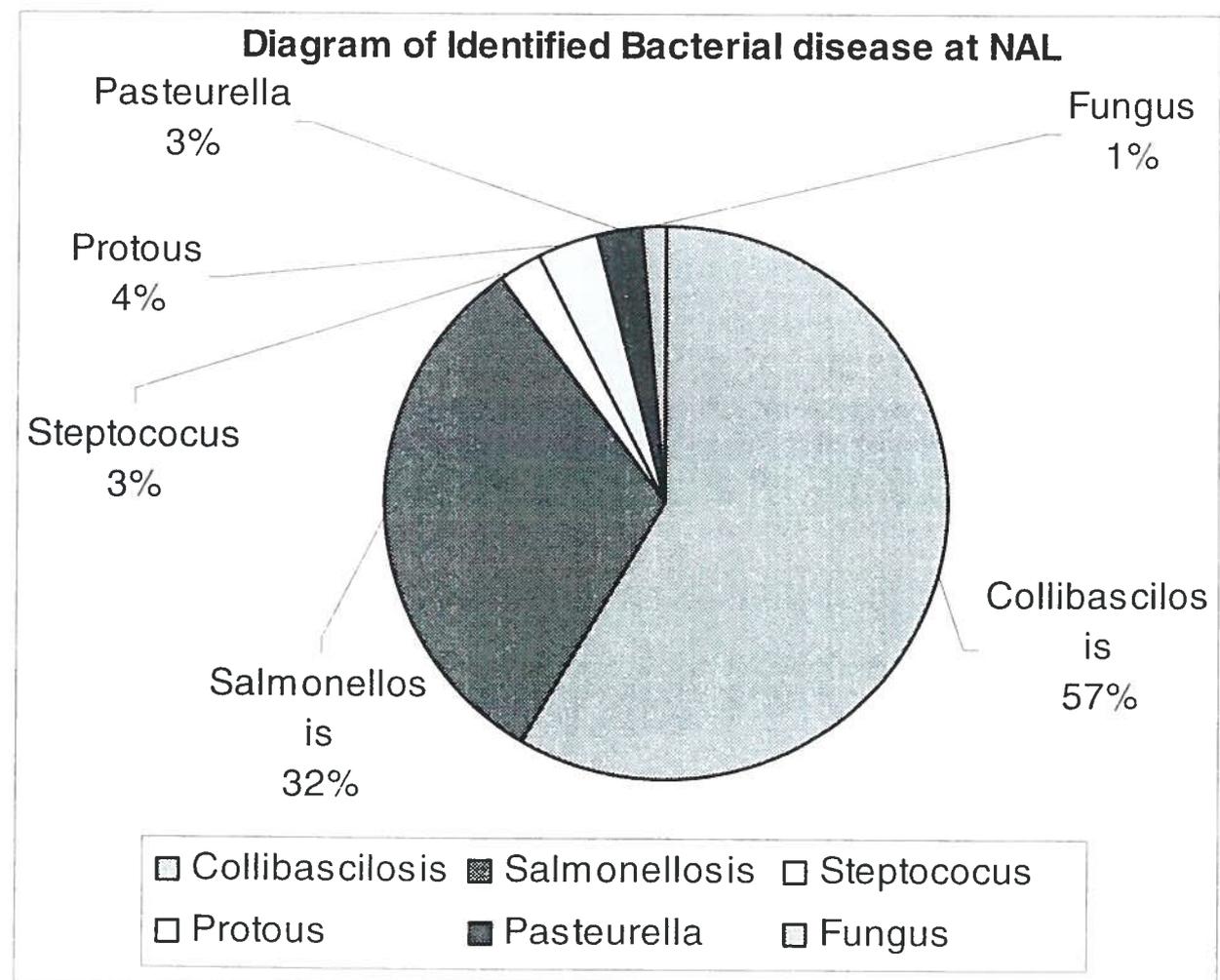


Shrawan Bhadra Asween Katric Manshir Poush Magh
 Phalgun Chaitra Baishak Jestha Ashadha

Bacteria found in Bacteriology unit monthly

S.no.	Month	Bacterial Types						Total
		Collibacilla	Salm -onella	Stepto -coccus	Proteus	Pasteurella	Fungus	
1	Shrawan	1	0	0	0	0	0	1
2	Bhadra	1	1	0	0	0	0	2
3	Aswin	1	0	1	1	1	0	4
4	Kartik	1	1	1	0	0	0	3
5	Marg	3	2	0	1	0	0	6
6	Poush	5	6	0	0	1	0	12
7	Magha	4	1	0	1	0	1	7
8	Phalgun	10	4	0	0	0	0	14
9	Chaitra	5	0	0	0	0	0	5
10	Baishak	5	3	0	0	0	0	8
11	Jestha	6	4	0	0	0	0	10
12	Asar	4	3	0	0	0	0	7
Total		46	25	2	3	2	1	79

Diagram of Identified Bacterial disease at NAL



2. Histopathological process at NAL

Introduction:-

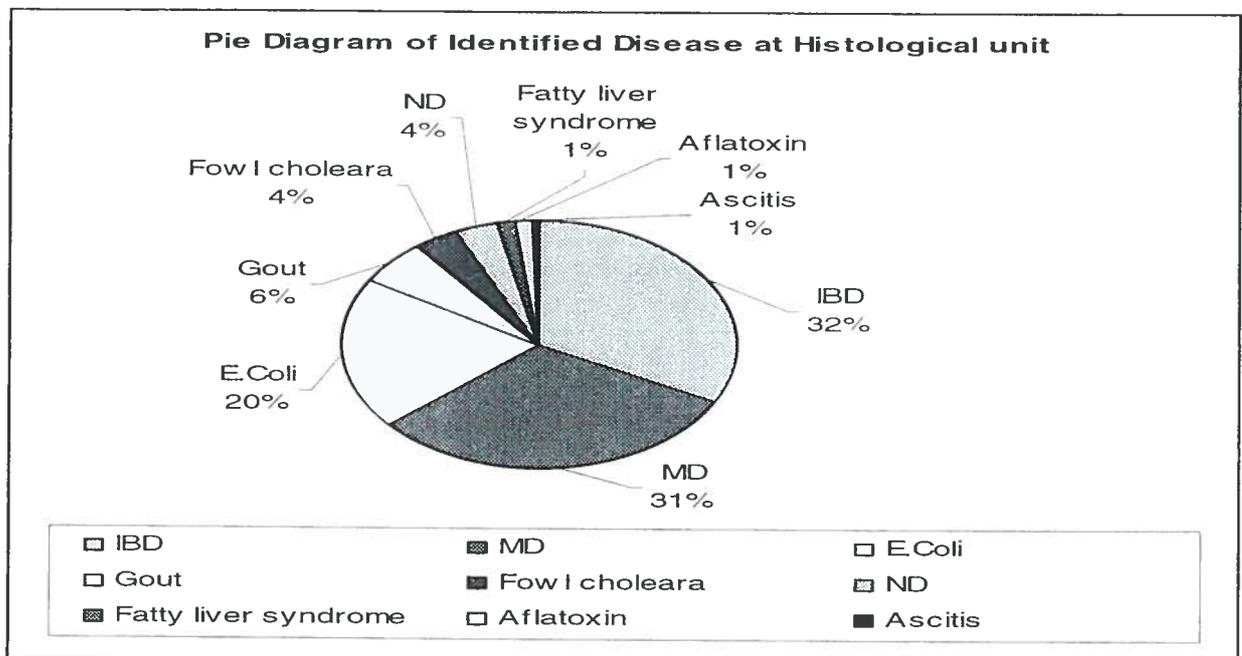
This unit is well equipped than of other unit of NAL .Result delivery is comparatively delay by it's nature but disease diagnosis through Histopathological unit is equally important to others .In this unit majority of works (tissue processing , staining) are doing manually. Sometimes if the samples are in large number then Automatic Machines are in use.

Manual Method of Histopathological Unit

- **Sample collection:-**We collect samples in 10%Buffered formalin and keep them 72 hrs for fixation.
-
- **Dehydration:-** 70% Alcohol--- for 2hrs
95%%Alcohol--- for 2hrs
100% Alcohol --- 1 for 2hrs
100% Alcohol ---11 for 2 hr
- **Clearing:-** Xylene is used for clearing at over night
- **Impregnation:-** Wax 1 for 2hrs
Wax 11 for 2hrs
Wax 111 for 1hr
- **Blocking:** - We use L size frame for making block.
- **Sectioning:** - We use Microtome for trimming tissue samples in 3-5 Micrometer..
- **Staining method:-**
Xylene -1 for 5 minute
Xylene- 11 for 5 minute
Xylene- 111 for 2 minute
100% Alcohol -1 for 2 minute
100% Alcohol-11 for 2 minute
95% Alcohol for 2 minute
80% Alcohol for 2 minute
70% Alcohol for 2 minute
Wash with tap water 1-2 dips
Haematoxylene solution
Tap water 1-2 dips
Eosin 5 minutes
70% alcohol-- 1 minute
80% alcohol—1 minute
90% alcohol ---1minute
95% alcohol ---1 minute
100% alcohol ---1 for 1 minute
100% alcohol ---11 for 1minute
Xylene ----1 for 5 minute
Xylene ----11 for 5 minutes
Xylene ----111 for 5 minutes
Histopathological Identification

Among them following diseases were found, after the interpretation of preparing slides.

S.N.	Disease name	Positive test	Percentage	Remarks
1.	IBD	119	32.62%	
2.	MD	113	30.95%	
3.	E. Coli	73	20%	
4.	Gout	21	5.75%	
5.	Fowl Cholera	14	3.83%	
6.	ND	13	3.56%	
7.	Fatty liver syndrome	5	1.36%	
8.	Aflatoxin	5	1.36%	
9.	Ascitis	2	0.54%	
10.	Total positive test	365	100%	



VIROLOGY & SEROLOGY UNIT

This unit is involved in the diagnosis of the mainly viral diseases and some bacterial through serological tests eg. Salmonella, mycoplasma etc. This unit has no tissue culture facility and other modern facilities, it is doing isolation of virus in embryonated eggs, HA-HI tests, PAT, Rapid Antigen tests and supportive biochemical's analysis tests. This unit is equipped with AGID test and ELISA Reader. Unit receives samples mainly from PM of NAL and from field.

ON-GOING DAY TO DAY ACTIVITIES:

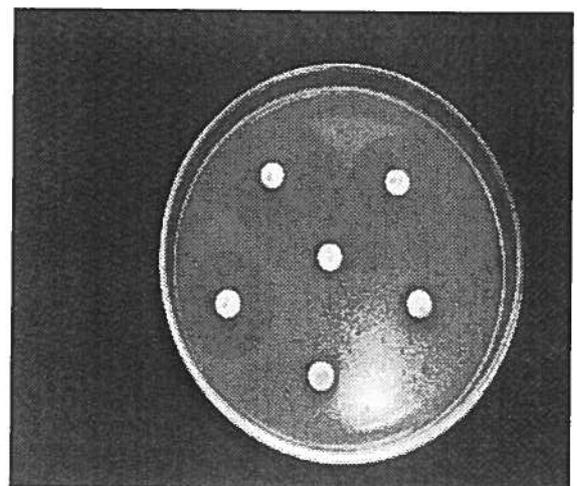
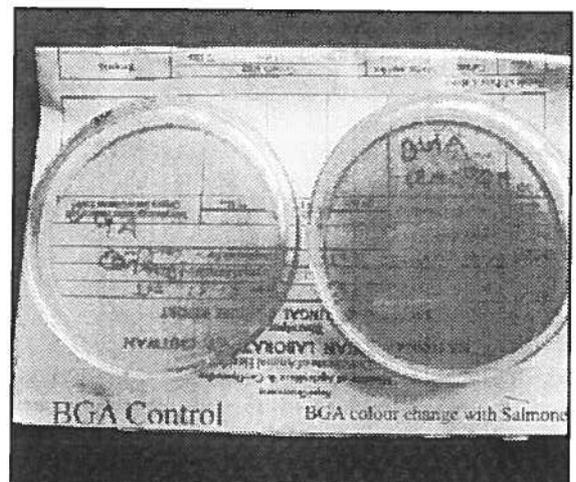
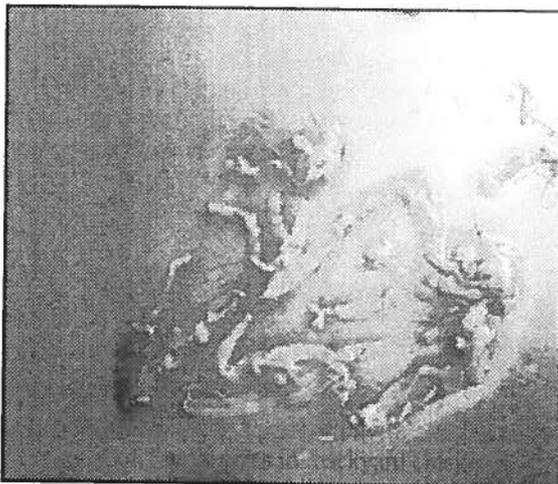
- Egg Inoculation
- HA-HI tests
- Plate / Slide Agglutination tests
- Rapid Antigen tests
- Biochemical analysis (Calcium, Phosphorus & Total protein)
- Sample collection & Dispatch

PROGRESS:

SN	ACTIVITIES/TESTS	NUMBER	REMARKS
1	VIROLOGICAL EXAMINATION	602	
2	SEROLOGICAL EXAMINATION	722	
3	BIOCHEMICAL ANALYSIS	925	

MAJOR ACTIVITIES / TESTS AND RESULTS:

SN	ACTIVITIES/TESTS	TOTAL	+VE	-VE	REM
1	Egg Inoculation	248	-	-	
2	HA-HI	278	16	262	
3	Rapid Antigen test	141	5	136	
4	PAT- Salmonella p.	300	8	292	
5	PAT- Mycoplasma	302	16	286	
6	Biochemical Analysis				
7	Calcium	313	-	-	
8	Phosphorus	311	-	-	
9	Total Protein	288	-	-	
10	Sample collection & Dispatch	290	-	-	



NATIONAL AVIAN LABORATORY [NAL] CHITWAN, BHARATPUR

Laboratory Investigation Report (Year :2008)

S. N.	Disease/Activities	Animal species	No. of samples received	District	No. of samples tested	Diagnostic test method used	Test result		Remarks
							Ve+	Ve-	
1	Post-mortem (PM) exam.	P, Pig, Val	604	Chit, Np, Mak	593	Pathological observation and sample collection.	555	38	Out of 593 PM examined dead birds suspected diseases are as MD, ND, Ascitis, IBD, E. coli, Gout, Enteritis, Salmonella, Mycoplasmosis, Brooder Pneumonia, Internal parasite, Typhoid & Metabolic observed.
2	Histo-pathological identification	P, Pig, Val	234	"	456	Histo-pathological exam.			Lab test conducted by NAL.
3	Microbiological identification	P, Val	295	"	295	Culture+Biochemica	112	183	E.Coli-66, Salmonella-35, Shella-2, Enterobacters-2, E. Coli+Salmonella-2, Fungus-2, Pseudomonas-1, Klepsella-1, & Pasteurella-1
4	Anti-microbial sensitivity	"	83	"	83	Antibiotic Sensitivity Test			Ciprofloxacin, Enrofloxacin, Colistin, Tetracycline, Gentamicin, Doxycycline, Nitrofurantoin, Cephalixin & Co-Trimoxazole.
5	Virological identification	P, Pig, Val	615	"	491	Egg Inoculation, HA/HI, Rapid Ag Test.	34	457	IBD-11, ND-23.
6	Salmonella Screening	"	301	"	279	PAT	12	267	Salmonella pullurum plate test antigen used.
7	Mycoplasma Screening	P, Pig	307	"	279	"	0	279	Mycoplasma synoviae plate test antigen used.
8	Biochemical analysis								
8.1	Calcium	P, Pig	24	"	24	Spectrophotometer			Calcium Kit used .
8.2	Total protein	"	20	"	20	"			Total protein Kit used .
8.3	Phosphorus	"	15	"	15	"			Phosphorus Kit used .
9	Sample dispatch to CVL for further investigation								

Sl. No.	Sample Type	P, D	213	ॐ ॐ	NA	Requested for HPAI Isolation & Reconfirmation.
9.1	Serum	P, D	213			
9.2	Tracheal swab	"	76	"	"	"
9.3	Cloacal swab	"	69	"	"	"
9.4	Harvested fluid	"	4	"	"	"
9.5	Bacterial seed	"	21	"	"	Requested for Bacteria Isolation & Reconfirmation
9.6	Death Bird	P	2	Chit	"	Requested for Isolation of Avian encephalomyelitis.

Abbreviations used:

District or Office code: Chit- Chitwan, Mak-Makawanpur, Np- Nawiparasi, kap-Kapilbastu, Lab-Laboratory, CVL-Central Veterinary Laboratory, No.- Number.

Disease name: E.Coli-Escherichia coli bacteriosis, IBD-Infectious burcial disease, ND-Newcastle disease, MD-Marek's Disease

Animal Species :- P-Poultry, Pig-Pigeon, Val-Valture, D-Duck, NA- Not Available, ve+-Positive, Ve- -Negative.

Test method used: Ag-Agglutination, PAT-Plate Agglutination Test, HA- Haemagglutination Test, HI-Haemagglutination inhibition, # - Slide preparation, OD-Optical Density.

Prepared by:
Mr.Shailendra Bhandari
Veterinary Technician

Checked by:
Dr.L.B.Dhakal
Veterinary Officer

Approved by:
Dr. T. R. Neupane
Senior Veterinary Officer

Annual Programme & Progress of NAL

B.T.No 40-3-500/40-4-500 (2064/065)

S.N.	Activity	unit	target	Budget allocated	progress %
1.					
1.1	Compound wall fencing	Times	1	300000.00	100
1.2	Pathology	Number	2400	58000.00	100
1.3	Histopathology	„	600	65000.00	100
1.4	Parasitology	„	300	45000.00	100
1.5	Hematology	„	300	54000.00	100
1.6	Microbiology	„	900	59000.00	100
1.7	Biochemical	„	900	47000.00	100
1.7	Virology	„	600	82000.00	100
1.9	Serology	„	600	84000.00	100
2	Sample Collection & Dispatch		600	48000.00	100
3	Test Confirmation	„	150	50000.00	26
4	Laboratory maintenance				
4.1	Lab. water Supply system	Times	1	30000.00	100
4.2	Lab. Animal	„	12	15000.00	100
5	Investigation & surveillance of Avian Disease				
5.1	Epidemic Investigation	„	6	107000.00	100
5.2	Monitoring & surveillance of wild/Migratory birds	„	3	59000.00	100
6	Reporting & Publication				
6.1	Browser publication	„	1	10000.00	100
6.2	Preparation of Annual Technical Report	„	1	10000.00	100
6.3	Epidemiological & Zoonosis Reporting	„	24	1200.00	100

Man Power Situation of National Avian Laboratory, Bharatpur. (F/Y 064-065)

S.N.	Type of the post	Class	Number	Fulfilled	Vacant	Remarks
A.	Technical					
1.	Senior Veterinary Officer	G II	1	1	-	
2.	Veterinary Officer	G III	2	2	-	
3.	Veterinary Technician	NG I	2	2	-	
4.	Junior Vet. Technician	NG II	2	2	-	
Total Technical			7	7	-	
B.	Administration/Account					
1.	Asst. Accountant	NG.II	1	1	-	
2.	Kharidar	NG.II	1	1	-	
3.	Office Assistant	-	2	2	-	
Total Administration			4	4	-	
Grand Total			11	11	-	

**Staff of National Avian Laboratory, Bharatpur
(At the end of F/Y 2064-065)**

S.N.	Designation	Post	Class	Remarks
1	Dr. Tika Ram Neupane	SVO	G II	
2	Dr. Laxman Bahadur Dhakal	VO	G III	
3	Dr. Peetambar S. Kushwaha	VO	G III	
4	Mr. Endu Ray Yadav	VT	NG I	
5	Mr. Shailendra Bhandari	VT	NG I	
6.	Mr. Ram Prasad Chaudhari	VT	NG I	
7	Mr. Ganga Prasad Yadav	JVT	NG II	
Administration/Account				
8	Mr. Bishwo Nath Adhikari	Accountant	NG I	
9	Mr. Rishhee Ram Acharya	Kharidar	NG II	
10	Mr. Bhanu Bhakta Sapkota	Office Assitant		
11	Mr. Purna Prasad Sapkota	Office Assitant		

Regional Veterinary Laboratory, Pokhara

Introduction

Western Development Region (WDR) is situated between 82° 30' to 85° 15' east longitude and from 27° 15' to 29° 30' north latitude. It occupies about 20% (29355 Sq. Km.) of total areas of Nepal. The region shares boundaries with Uttar Pradesh of India in the south and Tibet of China in the north. The region is bulging between Central and Mid-western development regions of Nepal in the east and west respectively. Geographically, WDR is divided into the following three main domains:

Himalayan region

Himalayan region is located in the northern part of the WDR, covering Mustang, Manang and upper belt of Gorkha districts. Yak/Nak, sheep, alpine goats (Chyangra) and mule rearing formed the way of life of the people in this region.

Hilly region

Hilly region lies in between the Himalayan and Terai regions. This region comprised of Arghakhanchi, Gulmi, Palpa, Shyanga, Kaski, Tanahu, Lamjung, and lower belt of Gorkha, Parwat, Baglung and Myagdi districts. People of divergent ethnic groups, casts and cultures share their common way of living. Agro-based livestock industry in this region is the main source of income of the people. Poultry farming, goat rearing and dairy industries are becoming familiar near the cities/towns and in the areas where market is accessible.

Terai region

Terai region covers Nawalparasi, Rupandehi and Kapilbastu districts. This plain extends from east to west of the region and stretched from 15 to 40 Kilometers in width. Sediments and silt are main constituents of soil deposited by rivers making it more fertile and this belt supplies the food and fibers to other regions of the country. Compared to mountains and hilly regions, this region has relatively better infrastructure and market accessibility. People of this region are motivated to adopt livestock farming in commercial scale.

There are about 1.45 million cattle, 1.16 million buffalo, 1.48 million goats, 0.23 million sheep, 0.21 million pigs, 2.8 million poultry and few thousands horses, mules, ducks, rabbits, ass and yak/nak in western development region. The population of livestock in the region is very high as compared to their production. Though many factors are contributory, the health of animal plays a vital role to increase the production and productivity of animal. Every year, several diseases and parasitic problems attribute a considerable amount of economic loss to livestock rearing farmers of the country warranting switching on a massive disease control program. Major economically important diseases of cattle and buffaloes are Foot and mouth disease (FMD), Haemorrhagic septicemia (HS), Helminthiosis, infertility, mastitis and blood protozoan diseases whereas PPR, Gastrointestinal nematodosis and Clostridial diseases in sheep and goats. Swine fever and FMD in pigs and New Castle disease (ND), Infectious bursal disease (IBD), Coccidiosis, Hemorrhagic Enteritis and Mycotoxicosis in chickens are major disease problems.

Mission of the Regional Veterinary Laboratory, Pokhara

The mission of the Regional Veterinary Laboratory, Pokhara is to promote the health of livestock, poultry and companion animals and to insure safe animal products for the consumer by assisting District Livestock Development Offices, veterinarians, clients, and others responsible for animal health in the detection and prevention of disease by conducting responsible investigation on animal diseases and providing accessible, accountable, timely, and accurate diagnostic services.

Objectives of Regional Veterinary Laboratory

- ⇒ To provide accessible, timely and accurate diagnostic services to the livestock and poultry farmers and to veterinarians, veterinary technicians and their owners in the region.
- ⇒ To conduct diagnostic examinations, record results, report information, and assist in the interpretation of results to submitting DLSOs, Veterinarians, and veterinary technicians.
- ⇒ To investigate the animal disease epidemics in the region and assist, advice and support DLSOs to control them.
- ⇒ To prepare epidemiological profile of livestock and poultry diseases and maintain and disseminate the regional epidemiological information database on animal health in the regional as well as in the national networks.
- ⇒ To investigate relatively important livestock diseases in the region and formulate control measures for the same with wider consultation to the experts.
- ⇒ To monitor and report the incidence and threat of animal diseases, as well as diseases that are transmissible from animal to humans.
- ⇒ To supervise and assist in diagnostic services to basic and primary laboratories based at DLSO's of the region.
- ⇒ To conduct and support the laboratory and animal health related training programs for the Para vets in the region.
- ⇒ To coordinate national disease control and eradication programs in the region.

These objectives are accomplished by the application of different diagnostic assays, interpretation of diagnostic procedures, consultation with animal health professionals of the Animal health directorate and Department of Livestock Services and training and continuing education of persons responsible for delivering animal health care services.

Major Laboratory Tests Facilities of Regional Veterinary Laboratory, Pokhara

Regional Veterinary Laboratory, Pokhara, located at Ramghat, the centre of Pokhara city, provides diversified veterinary Laboratory test facilities for the farmers, private veterinary practitioners and district Livestock Service Office of this region. It mainly tests the following categories of the samples:

1. Parasitology Unit:

Parasitology unit tests faecal samples of various species using direct smear method, sedimentation method, floatation techniques where necessary. In parasitology unit it not only identifies the parasites involved in the infestation but also quantify the parasitic burden as EPG for the nematode, Trematode and cestodes and OPG for the coccidial oocysts.

It recently added the nematode larval culture technique to identify the nematode larvae. For external parasites the parasitology unit performs microscopic examination of skin scrappings for the identification of mange mite species.

It conducts blood parasite test using blood smear examination and for the blood filaria examination using Knot's method.

2. Microbiological Unit:

Microbiology unit tests diversified samples like milk, tissues, blood, aspirated fluids and tissues etc. Both aerobic and anaerobic culture facilities are available. It also perform identification of the Bacterial and fungal organisms using various biochemical tests, staining, morphology etc. The laboratory is capable of handling Mycobacterium and Mycoplasma species for culture. The microbiology unit also performs antibiotic susceptibility test and advice for the appropriate antibiotic for the treatments.

In virology the laboratory is capable for the isolation of NewCastle Disease and Infectious Bronchitis virus using egg inoculation method.

3. Pathology Unit:

Pathology unit mainly perform Post mortem examination on various species of animals and collect appropriate samples for the histopathological examination and despatched to the histopathology unit of Central Veterinary Laboratory for the examination. The unit perform annual necropsy of about 900 animals and birds mostly poultry birds.

It performs various types of cytological studies for the disease diagnosis.

4. Serology Unit:

Serology unit of Regional Veterinary Laboratory, Pokhara mainly perform Brucellosis test using RBPT antigen, Mycoplasmosis and Pullorum disease of poultry using Mycoplasma gallisepticum and Mycoplasma synovae antigen by Plate agglutination test. New Castle disease and Infectious Bronchitis Disease is diagnosed using their respective antigen and antisera by HA/HI and AGID tests.

In the near future the unit is going to introduce ELISA test for the diagnosis of various animal diseases. Regional Veterinary Laboratory has one ELISA Reader in hand. It is performing Agar gel diffusion test for the diagnosis of Swine fever in pigs.

5. Biochemistry Unit:

Biochemistry unit analyse mainly serum for the estimation of Calcium, Phosphorus, Magnesium and total proteins at present of cattle, buffalo, goats and dogs. In the near future it is going to analyse other serum biochemical parameters like Zinc, glucose, BUN, Creatinine, Liver function test with other enzymes etc.

It is performing the Urine tests by estimating Albumine, Bilirubin, and Urobilinogen using dipstik test kit.

6. Hematology Unit:

The Hematology Unit of Regional Veterinary Laboratory is providing routine hematological parameters of all the animals and Poultry.

Table 1: Annual work Program and summary of achievements of Regional Veterinary Laboratory, Pokhara (for fiscal year 2064/65 & Budget No: 40-4-500/40-3-500)

S. N.	Programmes and Activities	Annual Targets			Annual Progress	Weigh tage (%)	Remarks
		Units	Targets	Weigh tage (%)			
A	Capital Expenditure						
	Purchase of Computer Scanner	Nos.	1	1.27	1	1.27	
C	Running Expenditure						
1	Laboratory Services						
1.1	Parasitological Examinations	Nos.	800	6.21	1862	6.21	
1.2	Microbiological Examinations	Nos.	400	7.48	420	7.48	
1.3	Pathological Examinations	Nos.	700	6.21	981	6.21	
1.4	Serological Examinations	Nos.	400	6.59	411	6.59	
1.5	Hematological Examinations	Nos.	150	3.30	154	3.30	
1.6	Biochemical Examinations	Nos.	200	5.96	200	5.96	
1.7	Sample collection and dispatch	Nos.	600	10.01	1517	10.01	
2	Disease Investigation and Surveillance Program						
2.1	Investigation of Nematodiasis in Goats	Times	12	16.86	12	16.86	
2.2	Investigation of Epidemic	Times	15	16.48	15	16.48	
3	Monitoring and Supervision						
3.1	Monitoring and Supervision of district based Laboratories	Times	10	8.24	10	8.24	
4	Animal Disease Investigation Interaction Workshop	Times	1	3.80	1	3.80	
5	Publication Program						

5.1	Half Yearly Epidemiologic Bulletin publication program	Times	2	2.53	2	2.53	
5.2	Annual Technical Report Publication	Times	1	1.27	1	1.27	
6	Purchase of Scientific books	Nos.	5	2.53	0	0	
7	Laboratory Animal Management	Times	12	1.27	12	1.27	

1. Laboratory Services

1.1 Parasitological Examination

Faecal samples were examined adopting both qualitative and quantitative methods. In the fiscal year 2064/65 altogether 1862 faecal samples from different species of animals such as cattle, buffalo, sheep, goats, dogs and poultry were examined. Out of 1862 faecal samples examined 803 samples were found to be positive for various internal parasites. Total number of faecal samples of goats examined for EPG count was 487. Upto 1300 EPG of GI nematodes were found in the goats of Syanja and Tanahu districts. Fasciola, Paramphistomum, Coccidia, Strongyloides, Strongylus, Trichuris and Monezia were major internal parasites identified. Two hundred seventy three faecal samples were negative for internal parasites. All together 981 intestinal and caecal scrapping of poultry during necropsy examination revealed that 65 cases were found positive for coccidiosis.

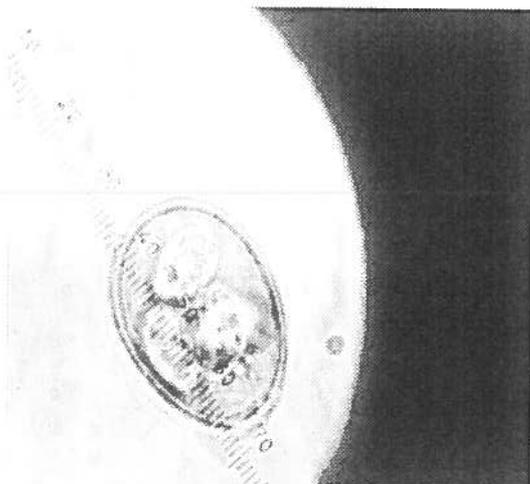
The results of monthly examinations of faecal samples are presented in Table 2.

Five skin scrapings from rabbit, pigs and dogs were received for the examination and identification of mites. All Five samples were positive for mites.

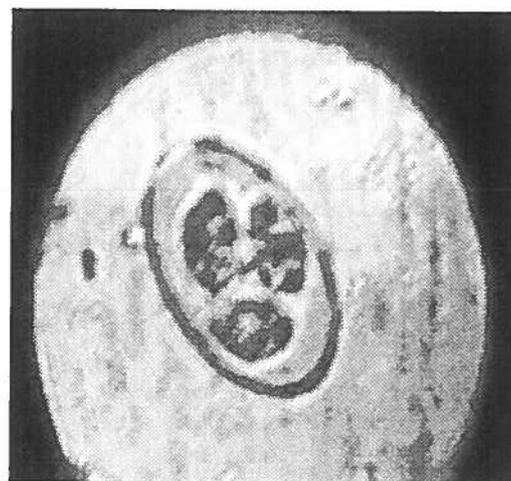
Study of Coccidiosis in goats

All together 798 fecal samples from goats were examined for the fecal coccidial load (OPG Counts) in which 351 samples were positive for different kinds of coccidia. In this study the average oocyst per gram (OPG) was found to be 3290 and in 13 samples the oocyst cannot be counted due to the densly presence of the oocysts. From the positive about 60 samples were cultured in 2% potassium dichromate solution for the identification of the oocyst. In this following species of coccidia were identified.

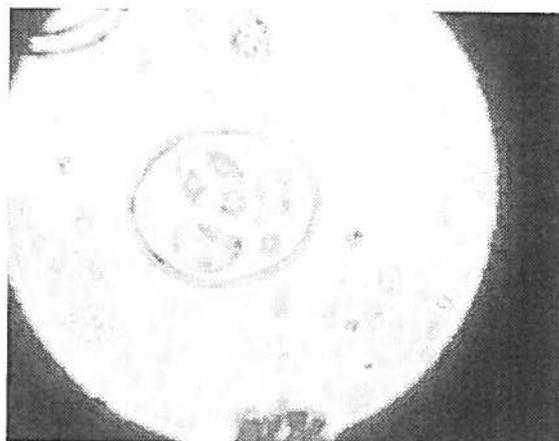
- *E. arloingi*,
- *E. hirci*,
- *E. ninakohlyakimovae*
- *E. caprina*,
- *E. aspheronica*,
- *E. caprina*,
- *E. jolchijevi*,
- *E. caprovina*,
- *E. christenseni*



E. christensani 10×100x



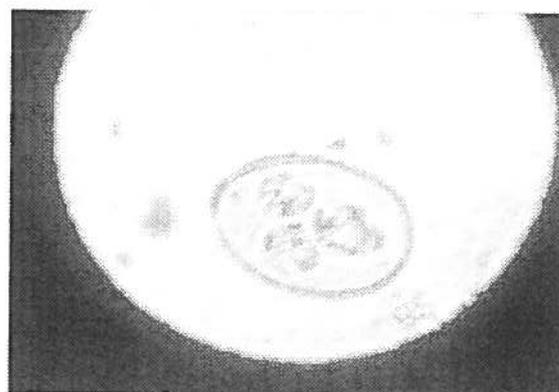
E. caprina 10×100x



E. ninakohlyakimovae 10×100x



E. apsheronica 10×100x



E. arlongi 10×100x

Table 3: Monthly Fecal Examination Result during 2064/65.

Parasites	Months												Total	Total %
	Sharawan	Bhadra	Aswin	Kartik	Mangsir	Poush	Magha	Falgun	Chaitra	Baisakh	Jestha	Asar		
Fasciola	3	2	4	3	1	0	5	3	0	2	0	1	24	2.98
Paramphistome	3	4	2	1	3	1	0	2	1	0	3	4	24	2.98
Ascaris	0	1	3	1	0	2	3	1	0	0	1	3	15	1.86
Strongyle	5	0	29	2	7	32	0	14	0	0	0	8	97	12.07
Strongyloides	1	3	59	18	33	17	0	5	25	11	6	9	187	23.28
Trichuris	0	0	1	0	0	4	0	0	3	0	1	0	9	1.12
Monezia	0	0	10	3	0	4	0	0	1	0	0	0	18	2.24
Coccidia	3	16	92	31	67	122	17	8	7	16	23	14	416	51.80
Others (B.coli)	0	1	0	3	0	5	0	0	0	4	0	0	13	1.61
Toatal Positive	15	27	200	62	111	187	25	33	37	33	34	39	803	100

1.2 Microbiological Examinations

Microbiological examinations include the isolation and identification of bacteria and fungi from the pathological samples received in the laboratory. Bacteriological culture and antibiotic sensitivity tests were performed of the samples received for microbiological investigation. During 2064/65 a total of 420 samples were examined in microbiology unit of the laboratory. Out of them 232 sample were milk for the test of mastitis. These entire 232 samples were subjected for the Sodium Lauryl Sulphate Test (SLST) screening test for detecting mastitis in the animals. In SLST test of milk sample about 151 samples of animals were found to be positive and then these 151 samples were subjected for the microbiological culture. In the culture 99 samples of milk showed bacterial growth which is summerized in the following table.

Table 3. Bacterial isolates and their percentage in the mastitic milk samples.

Serial No.	Name of the Bacterial Isolates	No. of samples positive	Percentage of Positives
1	<i>E.coli</i>	42	27.81
2	<i>Streptococcus spp.</i>	17	11.25
3	<i>Proteus spp.</i>	4	2.64
4	<i>Staphylococcus spp.</i>	6	3.97
5	<i>E.coli + Streptococcus spp.</i>	5	3.31
6	<i>E.coli + Staphylococcus spp.</i>	2	1.32
7	<i>No. growth</i>	52	34.43
	Total	151	100

For culturing the milk sample the laboratory used Nutrient Agar, Blood Agar and MacConkey Agar. After the growth the bacteria were identified by colony morphology, Gram characteristics, and Certain Biochemical characteristics like, Oxidase test, Catalase test etc.

The following is the compilation of Bacterial isolates performed in the RVL, Pokhara from the year 2056/57 to 2064/65

Table No. 4: Major Bacteria isolated from the milk sample in RVL, Pokhara from Fiscal Year 2056/57 to 2064/65.

Bacterial Isolates	Number of isolates (No. in paranthesis are percentage of isolates)								
	FY 056/57	FY 057/58	FY 058/59	FY 059/60	FY 060/61	FY 061/62	FY 062/63	FY 063/64	FY 064/65
<i>E. coli</i>	34 (18.88)	34 (19.31)	33 (18.43)	26 (19.26)	34 (18.78)	33 (22.97)	54 (33.13)	73 (47.40)	59 (60.82)

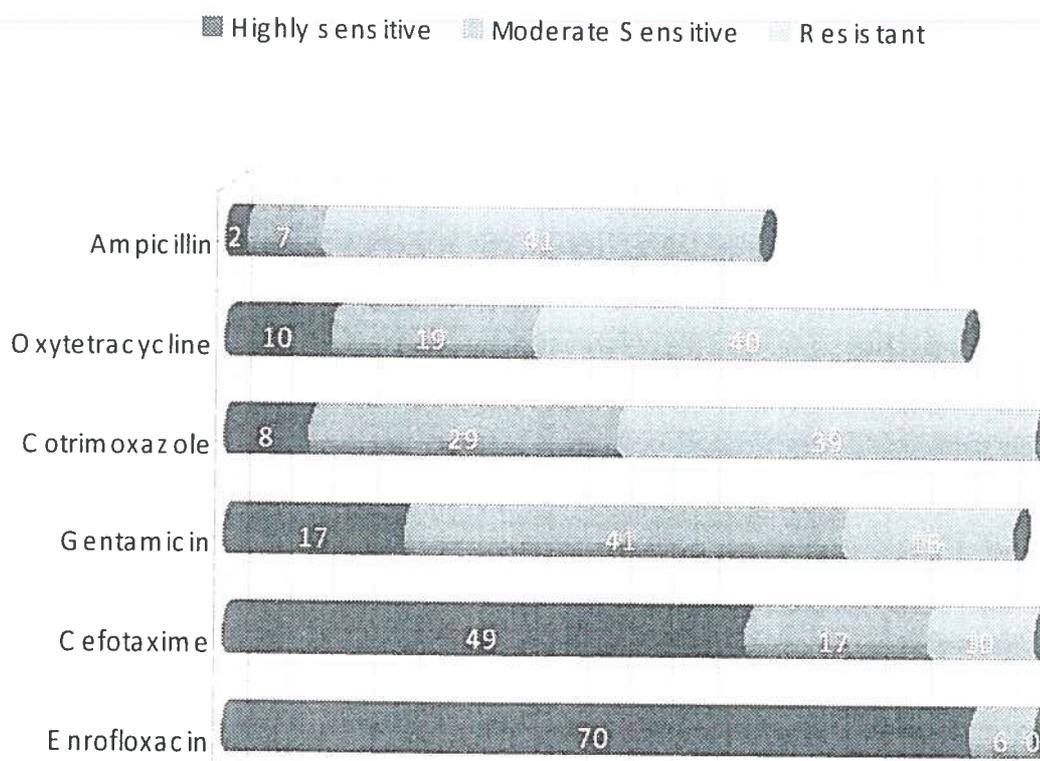
<i>Streptococcus spp.</i>	35 (19.44)	35 (19.88)	36 (20.11)	16 (11.85)	21 (11.60)	17 (11.48)	17 (10.43)	29 (18.83)	22 (22.68)
<i>Staphylococcus spp.</i>	45 (25.00)	42 (23.86)	41 (22.90)	30 (22.22)	47 (25.96)	28 (18.92)	38 (23.31)	12 (7.80)	12 (12.37)
<i>Bacillus spp.</i>	-	-	-	28 (20.74)	35 (19.33)	31 (20.94)	28 (17.17)	4 (2.60)	-
<i>Proteus spp.</i>	21 (11.66)	19 (10.80)	18 (10.05)	13 (9.63)	14 (7.73)	7 (4.73)	9 (5.52)	8 (5.19)	4 (4.13)
Other isolates	45 (25.00)	46 (26.13)	51 (28.50)	22 (16.30)	30 (9.40)	19 (12.83)	17 (10.43)	-	-
Total	180	176	179	135	181	148	163	154	97

The entire milk sample showing the growth is then subjected for antibiotic sensitivity testing. The antibiotics used for the sensitivity test were Ampicillin, Chloramphenicol, Cephotoxime, Cotrimoxazole, Enrofloxacin, Gentamicine and Tetracycline. Following is the table which shows the result of in vitro Antibiotic sensitivity test conducted for the mastitic milk samples.

Table 5: In-vitro Antibiotic sensitivity of mastitic milk samples.

S.N.	Antibiotic Disc Used	Highly Sensitive		Intermediately Sensitive		Resistance	
		No.	%	No.	%	No.	%
1	Enrofloxacin	70	92.10	6	7.89	0	0
2	Cephotoxime	49	64.47	17	22.37	10	13.15
3	Gentamicine	17	22.97	41	55.40	16	21.62
4	Cotrimoxazole	8	10.52	29	38.15	39	51.31
5	Oxytetracycline/Tetracycline	10	14.49	19	27.54	40	57.97
6	Ampicillin/Amoxycillin	2	3.92	7	13.72	41	80.39

Antibiogram of the milk samples



The above antibiotic sensitivity pattern clearly states that almost all organisms are sensitive to Enrofloxacin antibiotic with very small number of Intermediate sensitivity and resistant. Similarly, Ampicillin is highly resistant antibiotic found with only 9.80 % highly sensitive.

In chronic cases of mastitis, milk samples were also cultured on Saboraud's Dextrose Agar for the fungus culture and identification. One milk samples was found to be positive for *Candida* spp.

Apart from these other pathological and clinical cases of poultry and other animals the tissue, swabs and tissue fluids were subjected to bacteriological culture. In this the laboratory conducted about 188 such sample. The result and the isolate were given in the following table.

Table 7: Bacterial species isolated from other pathological Samples

Bacterial Species	Number isolated from other pathological samples
<i>Staphylococcus</i> spp.	18
<i>Streptococcus</i> spp.	11
<i>Bacillus</i> spp.	29

<i>Pasturella multocida</i>	2
<i>Proteus spp.</i>	8
<i>Micrococcus spp.</i>	3
<i>Enterobacter spp.</i>	16
<i>Salmonella spp</i>	34
<i>Escherichia coli</i>	66
<i>Pseudomonas spp.</i>	1

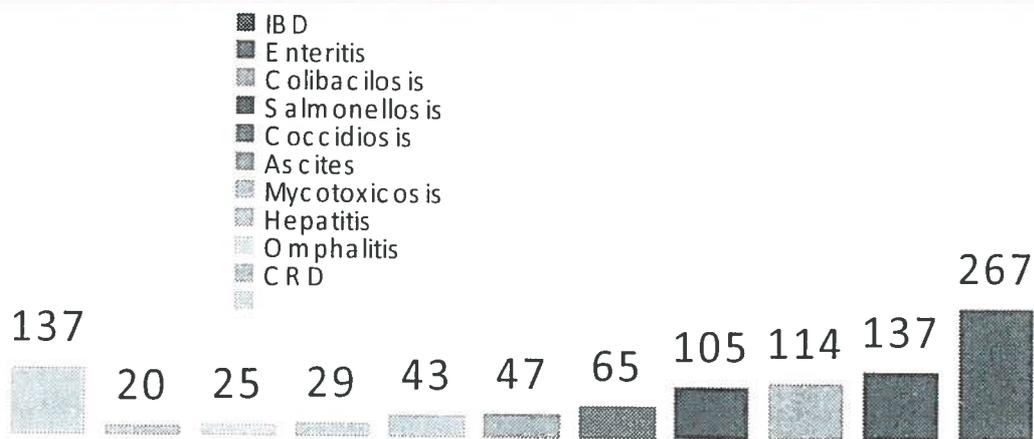
1.3 Pathological Examinations

Pathological examinations mostly consisted of necropsy examination of animals and poultry. Nine hundred eighty one poultry, 8 pigs and piglets, 3 goats, 1 sheep, 1 dog and 1 ducks were brought for the postmortem examination. In the pathology unit, the cause of death of chickens presented was generally drawn on the basis of both the post mortem lesions observed and laboratory investigation of samples collected during necropsy examinations. Some times samples collected during necropsy were sent to Central Veterinary Laboratory, Tripureswor for the confirmatory diagnosis. Diseases of chickens diagnosed on the FY 26064/65 are summarized in Table 8.

Table 8: Diseases of chickens diagnosed by necropsy and lab examinations

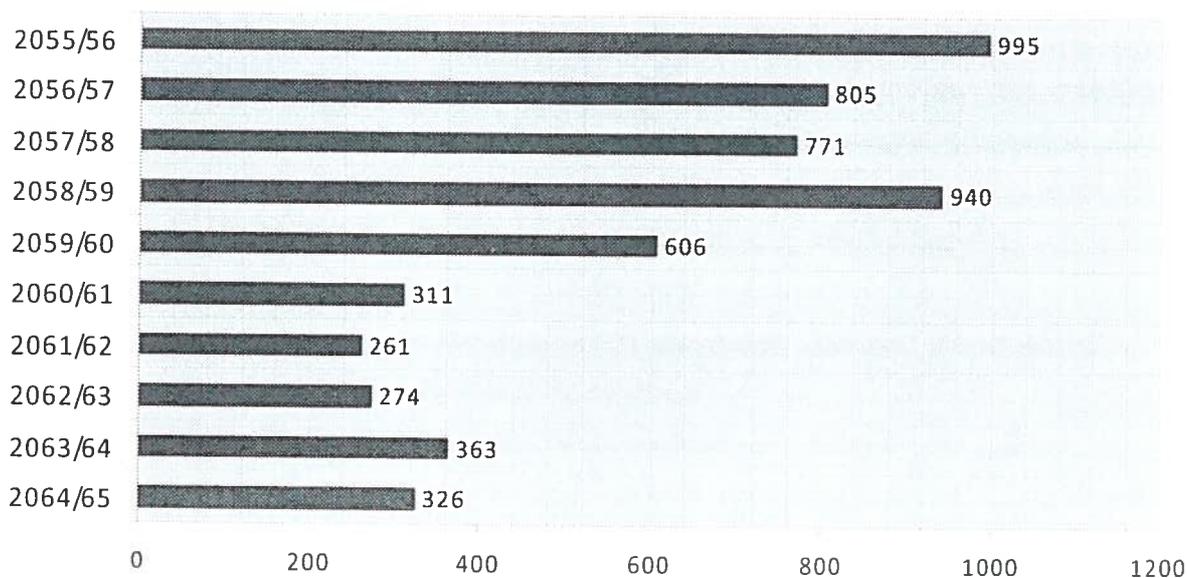
S.N.	Disease diagnosed	Number of Cases	Percentage of Cases
1	Infectious Bursal Disease/Gumboro	267	26.99
2	Colibacillosis	139	14.04
3	Hemorrhagic Enteritis	137	13.85
4	Salmonellosis	105	10.61
5	Coccidiosis	65	6.57
6	Ascitis	47	4.75
7	Micotoxicosis	43	4.34
8	Hepatitis	29	2.93
9	C.R.D.	20	2.02
10	Pneumonitis	17	1.71
11	Nutritional Deficiency	16	1.61
12	Nephritis	13	1.31
13	Ascariasis	11	1.11
14	Visceral Gout	9	0.91
15	Ranikhet Disease/NewCastle Disease	3	0.30
16	Other Diseases	68	6.87
Total		989	100

Diseases of chickens diagnosed by necropsy and lab examinations

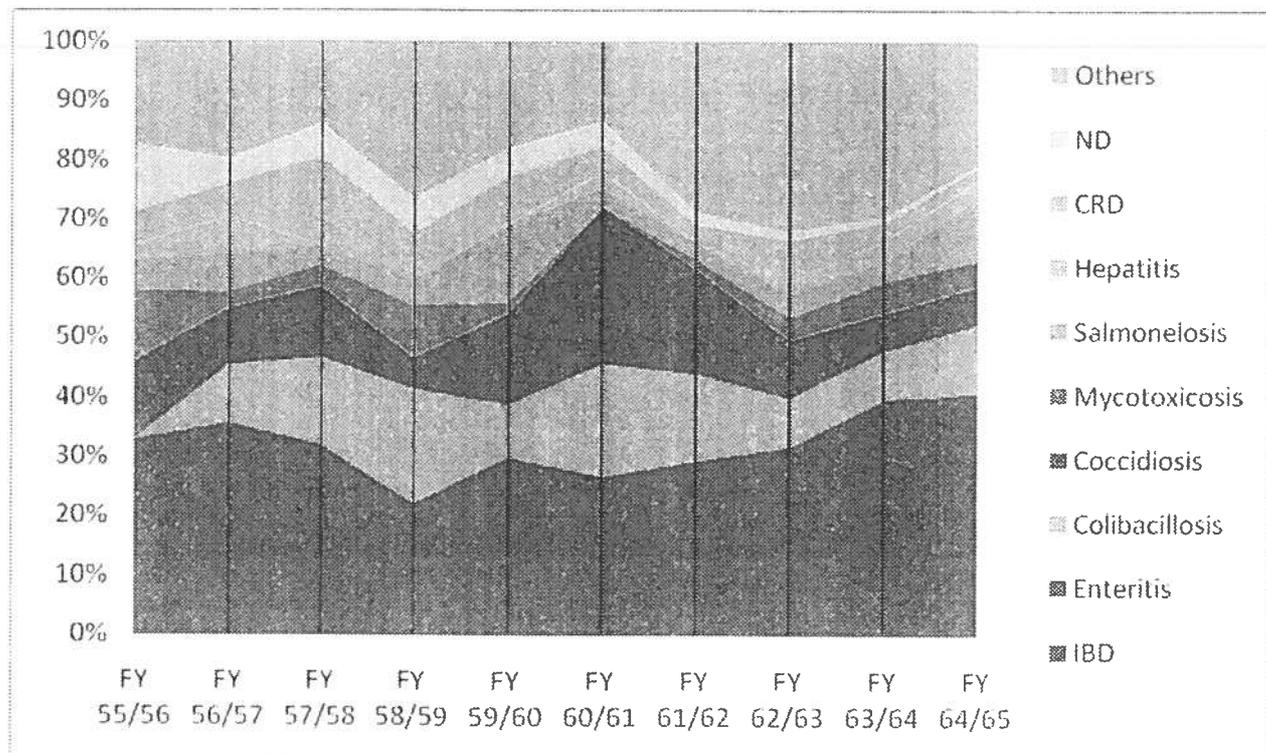


Pathology unit of RVL, Pokhara is now getting a load for disease diagnosis. The annual case load in the poultry sector since FY 2055/56 to FY 2064/65 is presented in the following diagram and the most important poultry diseases diagnosed during that period is shown in the following table.

Caseload of necropsy examination of RVL, Pokhara from FY 2055/56 to FY 2064/65



Percentage of Disease Occurance in different FY in Poultry (PM Diagnosis Based)



It is clear from the above graphical presentation the necropsy examination is in increasing trend. This may be due to the reliability of diagnosis and treatment, prompt services and trust of the farmer to the laboratory. From the year 2060/61 the examination is two fold increased and in following year the increase further increased by 55% but in the 2062/63 it reduced by 18%. This reduction is due to the houx of bird flu which lead the reduction of poultry keeping by the farmers. But in the year 2063/64 it increased by 4.5 % again.

Again in the year 2064/65 the necropsy examination was increase further reaching the case load up to around 1000. The poultry in pokhara valley mainly the broiler population and backyard poultry farming is in increasing trend that is also reflected by this number of necropsy examination.

Table No. 9: Poultry Diseases Diagnosed in Different Fiscal Years from 2055/56 to 2064/65

S.N.	Poultry Diseases Diagnosed through Necropsy Examination	No. of cases in Different Fiscal Year (No. in paranthesis are % of Cases)									
		FY 2055/56	FY 2056/57	FY 2057/58	FY 2058/59	FY 2059/60	FY 2060/61	FY 2061/62	FY 2062/63	FY 2063/64	FY 2064/65
1	Gumboro Disease (IBD)	78 (24.00)	75 (20.67)	55 (20.00)	34 (13.03)	62 (19.93)	121 (19.96)	206 (21.90)	176 (22.25)	206 (25.60)	267 (26.99)
2	Haemorrhagic Enteritis	30 (9.30)	55 (15.16)	33 (12.04)	24 (9.20)	33 (10.61)	44 (7.26)	78 (8.29)	88 (11.12)	124 (15.40)	137 (13.85)

3	Colibacillosis	-	35 (9.64)	40 (14.61)	50 (19.20)	28 (9.00)	117 (19.30)	141 (15.00)	68 (8.59)	68 (8.44)	139 (14.04)
4	Coccidiosis	43 (13.30)	35 (9.64)	33 (12.04)	14 (5.40)	49 (15.75)	164 (27.06)	172 (18.30)	82 (10.36)	53 (6.58)	65 (6.57)
5	Ascites	-	-	-	-	10 (3.21)	9 (1.48)	-	29 (3.66)	51 (6.33)	47 (4.75)
6	Mycotoxycosis	39 (12.00)	10 (2.75)	10 (3.65)	23 (8.82)	6 (1.92)	-	16 (1.70)	32 (4.04)	45 (5.60)	43 (4.34)
7	Hepatitis	8 (2.40)	23 (6.34)	-	-	-	16 (2.64)	-	18 (2.27)	29 (3.60)	29 (2.93)
8	Salmonellosis /Pullorum Disease/BWD	17 (5.200)	23 (6.34)	9 (3.28)	11 (4.22)	42 (13.50)	20 (2.30)	27 (2.90)	33 (4.17)	28 (3.48)	105 (10.61)
9	CRD	18 (5.20)	20 (5.50)	41 (15.00)	21 (8.05)	25 (8.03)	27 (4.45)	29 (3.01)	58 (7.33)	26 (3.23)	20 (2.02)
10	Intestinal Helminthiasis	11 (3.40)	15 (4.13)	16 (5.83)	10 (3.84)	2 (0.64)	8 (1.31)	13 (1.40)	8 (1.01)	8 (0.99)	11 (1.11)
11	New Castle Disease	37 (11.30)	15 (4.13)	15 (5.50)	15 (4.82)	15 (4.82)	25 (4.12)	19 (2.00)	11 (1.39)	7 (0.87)	3 (0.30)
12	Marek's Disease	5 (1.50)	20 (5.50)	-	5 (1.92)	2 (0.64)	-	1 (0.10)	4 (0.50)	5 (0.62)	-
13	Visceral Gout	-	4 (1.10)	-	-	-	-	-	36 (4.55)	3 (0.37)	9 (0.91)
14	Litchi Heart Disease (IBH- HP)	-	-	-	-	-	14 (2.31)	79 (8.40)	34 (4.29)	-	-
15	Other Poultry Diseases	41 (12.57)	33 (9.09)	22 (8.03)	54 (20.70)	43 (13.82)	25 (4.12)	186 (19.81)	155 (20.00)	169 (20.99)	114 (11.50)
Total		326	363	274	261	311	606	940	771	805	989

1.4 Serological examinations

Serological examinations mainly consisted of plate agglutination test of chicken serum to detect antibody against *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Salmonella pullorum* organisms. Similarly, serum samples from cattle, buffalo, sheep and goats were tested for brucella antibodies using Rose Bengal Plate Agglutination Test (RBPT). During the fiscal year 2064/65, the serum samples tested and their results are presented in table 10.

Animal/Bird Species	Number of Serum Tested	Serum Tested For	Test Method Applied	Results	Percentage of Positives
Cattle	40	Brucellosis	RBPT	All Negative	0
Buffalo	20	Brucellosis	RBPT	All Negative	0
Goats	43	Brucellosis	RBPT	All Negative	0
Poultry	308	Salmonellosis	PAT	122 +ve	39.61
Poultry	88	Mycoplasmosis	PAT	17 +ve	19.31
Total Serum Sample Tested in the Serology Unit = 486					

1.5 Haematological Examinations

Hematological unit of the laboratory is well equipped to determine a range of hematological parameters such as Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC), Differential Leucocytes Counts (DLC), Erythrocyte Sedimentation Rate (ESR), determination of hemoglobin (HB) and Packed Cell Volume (PCV) and staining of blood smears for blood protozoa and bacteria. A total of 154 blood samples from animals were examined for different hematological parameters. The results of the blood smear examinations revealed 1 *Theileria* spp in a Cattle bull.

1.6 Biochemical examinations

Biochemical examinations included biochemistry of serum and routine and microscopic examination of urine. Multistick strip was used for routine urine analysis. Microscopic examination of urine was done after centrifugation of the urine samples. Using spectrophotometer and commercially available biochemical kits biochemical parameters of serum samples were determined. A total of 200 samples were examined in biochemistry unit including 98 urine samples. Out of 98 urine samples examined, 27 were diagnosed to be haematuria, 9 proteinuria and 26 miscellaneous cases.

1.7 Sample Collection and Dispatch

During 2064/65, a total of 1517 serum, blood and tissue samples of different animal species and poultry were collected from the disease outbreak investigation sites. 1122 various samples were subjected for the laboratory investigation at RVL, Pokhara. A total of 392 various samples were dispatched to Central Veterinary Laboratory, Kathmandu and 3 samples to National FMD and TADs laboratory, Kathmandu for confirmatory disease diagnosis.

2. Disease Investigation and surveillance Program

2.1 Investigation of Epidemics:

Various disease outbreaks of animal and poultry were investigated during fiscal year 2063/64. Whenever request for investigation of an outbreak was received from the district to the RVL, a veterinarian or a technician or a team of technicians with necessary sampling kit visited to the site of epidemic, collected epidemiological information and appropriate pathological samples. In the laboratory, pathological samples collected from the field were processed to find out the etiology of the outbreak. Epidemiological information gathered from the site of an outbreak was used to decide the test to be performed in the laboratory and to assist in the confirmation of disease diagnosis. Samples, not possible to process in this laboratory were referred to CVL, Kathmandu.

A total of 15 epidemics were investigated during 2064/65. Out of 15 outbreaks investigated, some were confirmed by laboratory while others confirmations were based on clinical signs and postmortem findings. The outbreaks findings are presented in Table 12.

Table no: 12 Disease Outbreak Investigated during 2064/65.

S.N.	Disease Investigated	Months	Species Affected	District
1	Rabies	Shrawan	Dogs (Pig ?)	Kaski
2	Ranikhet Disease	Bhadra	Poultry Broiler	Kaski
3	Fern Poisoning	Aswin	Goats	Tanahun
4	Infectious Bronchitis	Kartik	Poultry Broiler	Kaski
5	Abortion (Brucellosis)	Kartik	Cattle	Kaski
6	Salmonellosis	Mangsir	Poultry Layers	Kaski
7	Managemental Problem	Poush	Broiler	Baglung
8	Ranikhet Disease	Magha	Poultry Broiler	Kaski
9	Enteritis in Swine	Falgun	Swine	Kaski
10	Salmonellosis	Falgun	Poultry Broiler	Kaski
11	Enteritis in Goats	Chaitra	Goats	Tanahun
12	Ranikhet	Baisakha	Poultry Broiler	Kaski
13	Magnesium Tetani	Jestha	Cattle and Buffalo	Tanahun
14	Infertility	Jestha	Cattle	Syangja
15	Colibacillosis	Asar	Swine	Kaski

Mycobacterial Disease Investigation

Single Intradermal Tuberculin test of 11 bulls and 6 buffalo bulls of National Animal Breeding Centre, Pokhara were carried out. These test revealed all negative for bovine tuberculosis.

Bird flu surveillance and sample collection

Although bird flu has not yet appeared in Nepal, the Regional Veterinary laboratory (RVL) Pokhara was involved in bird flu surveillance and sample collection activities. Fewa Lake, Rupa Lake of kaski district was visited and an interview with the people living near lakeside was carried out. According to the interviewee mainly Bakula, Water wild Ducks, Dangre, Chibe chara and Gaunthali are the birds generally found in the lakes. No any sick or dead birds were found during the visit. The veterinarians from RVL, Pokhara visited the commercial and village poultry flocks of Tanahu, Gorkha, Gulmi, Baglung, Parvat and Kaski districts. The data on number of birds in the farms, birds' age group, disease situation and bio-security measures adopted were collected. Clinical and necropsy examinations of the flocks were carried out. No any symptoms resembling bird flu were noticed. However in three poultry farms some of the sick birds were found to be suffering from Gumboro. The diagnosis was made on the basis of postmortem examination. In some of the poultry farms of Kaski district were found to have died due to Colibacillosis, Gumboro and Coccidiosis. Poultry farmers were made aware about the bird flu disease and its symptoms and advised on adoption of bio-security measures in the farms.

For laboratory surveillance of bird flu different types of samples were collected from different high risk districts of this region from commercial and village poultry, and ducks which are presented in the following table.

Sample collection as a laboratory surveillance form different high risk district of Western Region.						
Districts	Cs	Ts	Fs	Ss	Unidentified	Total
Kaski	143	89			83	315
Rupandehi	100	506	3			609
Nawalparasi	46	40		31		117
Kapilbastu	40	100		43		183
Total	329	735	3	74	83	1224

A team from the laboratory conducted the live market surveillance on Avian Influenza in Lekhanath Municipality of Kaski and Siddharthnagar of Rupandehi District. From where various samples were also collected and tested this gave negative result for Avian Influenza.

Sero-surveillance of PPR vaccination Programme

During the fiscal year 2064/65 sero-surveillance of PPR vaccination in the sheep and goats programme in the western region was carried out. A total of 300 serum samples from Tanahu, Shyanja, and Parvat were collected from the vaccinated sheep and goats. All the collected serum samples were despatched to Central Veterinary Laboratory, Tripureswor, Kathmandu for the examination of antibody against PPR vaccination.

Technical Report of 2008, RVL Surkhet

Introduction:

Regional Veterinary Laboratory Surkhet performs various diagnostic activity through its several laboratory units. They are parasitology, Pathology, Microbiology, Biochemistry, Hematology, Serology, washing and sterilization units.

Regional Veterinary Laboratory Service receives various sample (Specimen) from different sources. Majority of the specimen are received from D.L.S.O, individual farmers and also specimen are collected by the R.V.L itself during the surveillance and epidemic investigation.

Laboratory Services:

Parasitology Unit:- This unit involves the examination of fecals and skin scraping generally trematodes, Nematodes, Cestodes and Mites are identified by this unit. F/Y 2008 (Jan- Dec) Total 1500 Fecal Sample From Different Species That are Cattle, Buffalo, Goat were received. Among them 1351 sample (90%) were positive. Where as 149 sample (10%) were Neg. The result of the Fecal Examination shows Fasciolosis is the most commonly occurring parasitic infestation.

Result of the Faecal Examination.

Table:

Month	Sample Test		Fasciola	Paraphistom	Tri	Hem	Asca	Other	Total
	+ve	-ve							
July	111	20	60	15	20	6	5	5	131
August	100	25	55	10	12	15	5	3	125
September	105	15	52	12	10	20	4	7	120
October	103	10	40	11	15	20	8	9	113
November	103	12	45	12	14	24	4	4	115
December	125	10	69	16	12	16	6	6	135
January	96	8	56	15	10	10	3	2	104
February	93	14	40	14	15	15	5	4	107
March	131	10	89	15	10	10	4	3	141
April	155	10	99	14	15	15	6	6	165
May	124	9	75	12	14	12	9	2	133
June	105	6	53	12	21	12	5	2	111
Total	1351	149	733	158	168	175	64	53	1500
Percentage	90%	10%	54%	12%	12%	13%	5%	4%	

Skin Scraping

Skin scraping are collected from animal suffering from skin disease and examine for the identification of the mites. The total 15 samples from the different spp. of animals were received and examine which revealed the presence of Sarcoptic-5 psoroptic-3 and Neg. -7 were found.

Hematology unit :-

Hematology unit deals with all hematological Examination.in this unit

T.L.C.,D.L.C.,estimation of P.C.V.and H.B.estimation

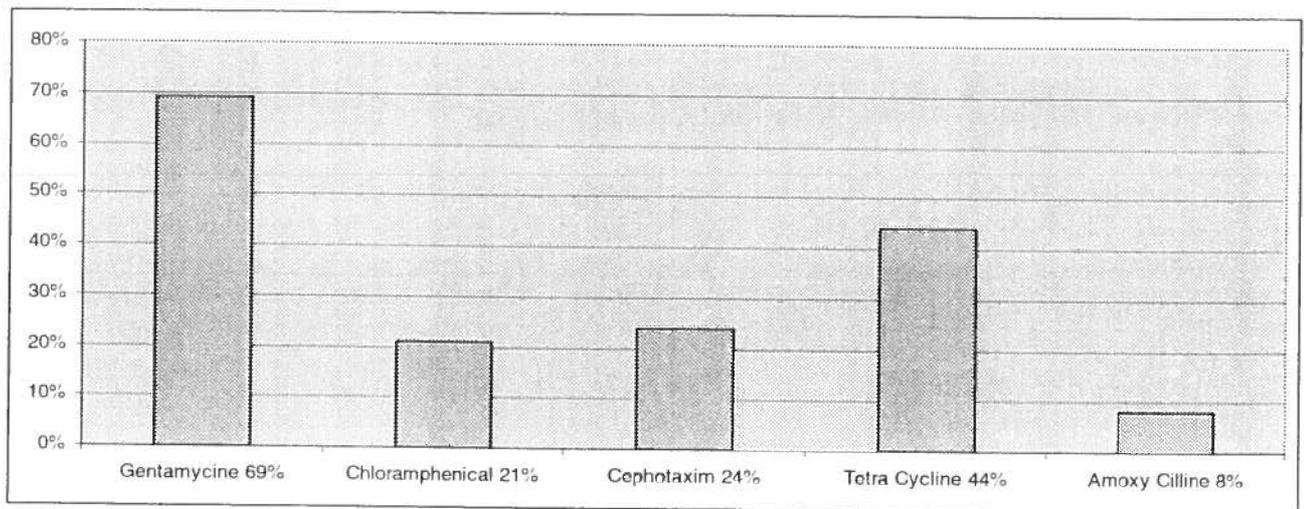
Where as 381 blood samplewere Examins during the Fly 2008

Microbiology Unit:

Milk Sample from the suspected cases of mastitis also collected from laboratory unit. The milk sample screened as positive for the occurrence of the mastitis through California mastitis test. These samples are put for the bacterial culture and the isolates are suspected to antibiotic sensitivity test to study the efficacy of the commonly used antibiotics against the various bacteria.

The total 158 milk sample were received in microbiology laboratory unit during the Fs2008 the most common bacterial isolates were streptococcus, staphylococcus and e-coli. The result of the CMT test has been presented in table below.

Microbiology				
Result of C.M.T.				
Month	Total Sample	Positive	Negative	Positive %
July	20	10	10	50%
August	16	9	7	56%
September	9	5	4	56%
October	6	5	1	83%
November	7	4	3	57%
December	8	3	5	38%
January	12	8	4	67%
February	10	6	4	60%
March	12	7	5	58%
April	14	8	6	57%
May	18	12	6	67%
June	26	12	14	46%
Total	158	89	69	56%



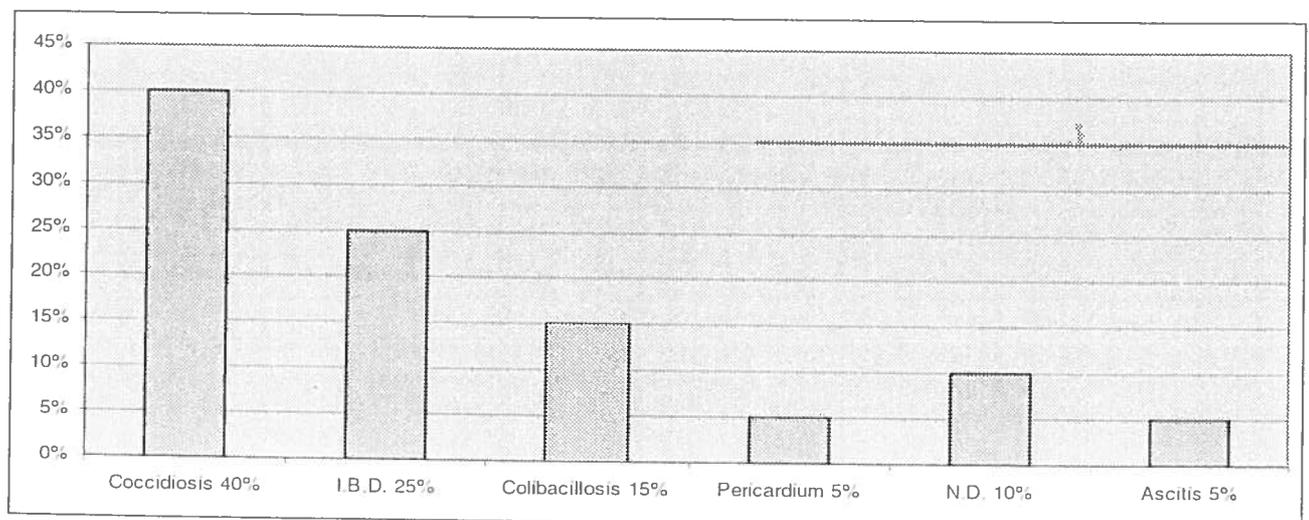
Biochemistry Unit:

This unit involves the routine examination of urine and analysis of the blood serum to analyse the various biochemical constituents. Analysis of the blood serum is done for the estimation of the total protein glucose phosphorus using commercial kits. Urine sample as tested by using multisticks as well as traditional biochemical methods. Examinations of urine is done for specific gravity, pH and to explore the bodies, urobilogen and blood.

Pathology Unit:

Mostly postmortem examination of the dead birds and occasionally of dead animal are done in the laboratory or field condition. During postmortem examination specimen intended for smears, swab and tissues are collected and tested accordingly. Specimens intended for microscopic pathology are sent to the central veterinary. As the facility is not available in R.V.L pathology unit.

Among the 450 cases of poultry birds received from the P.M. examination coccidiosis was found to be the most common disease followed by coccidiosis, colibacillosis, hydropericardium and Ranikhet disease. The status of various diseases diagnosed during the F/Y was given in the graph below.



Serological Unit:

In this unit various types of the serum sample were tested for the identification of brucellosis of goat and isolation of the salmonella in poultry disease.

SEROLOGY

Sample	Suspect	Test Method	Positive	Negative	Total Sample	Sample Send to CVL
Goat Serum	Brucella	P.A.T & Elisa	7	33	40	7 Sample Sent to CVL
Goat Saliva	Rabies	Rapid Test	+1 Re Confirm CVL	1	1	1 Sample Sent to CVL
Serum (Sayers)	N.D.	N.D. Antigen		-5	5	
Swab (Poultry)	Bird Flue A.I.	A.I. Rabies Test	-	-43	43	
Serum (Poultry)	Salmonella	P.A.T.	6	-	6	

REGIONAL VETERINARY LABORATORY, SURKHET ANNUAL LABORATORY INVESTIGATION REPORT-2008

YEAR 2008

DURATION:- JANUARY TO DECEMBER

S. N.	Disease	Animal Species	No. of Sample received	District	No. of Sample Test	Diagnostic Test Method Used	Test Result		Remarks
							+VE	-VE	
1.	Fecal Examination	B, C, G, SW, Poultry.	1500	Surkhet	1500	Sedimentation Method.	1351	149	Fas-512, Para-181, Tri-178, Nema.-101, Asca-219, Cocci-90, Other-46
2.	Skin Scraping	S, D, G.	15	Surkhet	15	Microscopic Examination	8	7	Sarcoptic & psoroptic
3.	Rabies	Dog	2	Surkhet	2	F.A.T. Test	1	1	Positive
4.	P.P.R.	Goat	231	Dang., Surkhet, Bir diya, Daitlekh, jark of,	231	P.A.T.	2	-	Test Controlled by C.V.L.
5.	Drug Sensitivity Test	C, B, G. Poultry	93	Surkhet	93	Neutrient Agar	93	-	Genta 60% Chloroph-15, Cipro-25%
6.	C.M.T. Test	C, B	158	Surkhet	158	Used of C.M.T.	89	69	89 Case Positive
7.	Blood Test (P.C.V., T.L.C., D.L.C.)	B, C, G, S.	381	Surkhet Bankey Bardiya	381	Microscopic Examination	381	-	Normal Value
8.	Post Martum Examination	Poultry, Dog	450	Surkhet	450	Postmortum	450	-	I.B.D. 40%, N.D.-5%, Ecoli-15%, Cocci-25% Other-15
9.	Salmonella Test	Poultry	195	Surkhet	195	P.A.T., Culture	95	100	
10.	New Castal Disease	Poultry	50	Surkhet	50	N.D. Kit	5	-	
11.	Sample Dispatch.	Poultry, Du.	231	Surkhet	-	-	-	-	Sent to C.V.L.

Abbreviation- B = Buffalo, G = Goat, D = Dog, Du = Duck, C = Cattle

Regional Veterinary Laboratory, Dhangadhi, Far Western Region

Introduction:

Far Western Development region consisting of nine districts only and divided into two zones viz. Seti & Mahakali is the smallest region among the five development regions. The region shares borders with the Tibetan China to the north and with the Indian states of Uttaranchal and Uttar Pradesh to the south west.

Geographically the region is divided into three parts namely, Mountains, Hills and Terai. The mountainous districts of the region comprises of Bajura, Bhajhang, and Darchula, where livestock rearing mainly consisting of migratory flocks of sheep and goats is the way of life, since the steep slopes of the mountains is not suitable for other agricultural practices. Cattle, Yak, Nak, Chauri, and Changra are the important livestock raised by the people in these districts.

The four hill districts of the region viz. Baitadi, Dadeldhura, Doti and Accham keep livestock mainly for milk and milk products like ghee which forms the main source of income to the farmers. Accham district is also important with regards to the availability of smallest indigenous cattle- Acchami cow which is classified as endangered breed of cattle as its population is rapidly decreasing due to crossbreeding. If conservation measures are not taken immediately, we will lose the pure gene of this breed within a few years from now.

Kailali and Kanchanpur are the only terai districts of the Far western region, but they have a high potentiality for the development of livestock industry. Almost all kinds of livestock viz cattle, buffalo, sheep, goat, pig, poultry, duck, etc. are raised by the farmers of these two districts. Pig farming is more common among the tharu communities who are the ethnic group of people of the terai districts of the country. On the other hand goat and poultry farming in general is steadily inclined towards commercialization, but particularly poultry rearing principally broiler farming is rapidly gaining popularity among the farming communities of the two districts, with growing number of the ethnic groups of the tharu community also taking interest towards broiler farming practices.

There are a number of infectious diseases which are constrain to the development of the livestock sector of the region, apart from management, nutritional and other factors. The major being the FMD, PPR, Swine Fever, HS, and parasitic diseases like Liverfluke, Strongylus, Strongyloides, Haemonchus, Paramphistome etc. Darchula district which was so far free of PPR, an important disease of sheep and goats has recorded its first outbreak of the disease during Jestha-Asar of 2063 following distribution of breeding goats there by an NGO working in livestock sector in the district. The source of those goats distributed to farmers community in Darchula is identified to be Jhalari v.d.c. of Kanchapur district.

Apart from the above mentioned disease conditions few of the zoonotic diseases of major public health interest like Rabies and Japanese Encephalitis have been creating havoc in the human health which indirectly affects the livestock sector development. While Rabies is found to occur sporadically throughout the year affecting livestock and humans, Japanese Encephalitis is more commonly found to occur during the months of Shrawan and Bhadra affecting large number of human population each year. In the recent outbreak of Japanese Encephalitis more than 65 people have been admitted to different hospitals in Kailali and Kanchanpur districts, out of which 12 have already died due to this disease. Mostly children below the age of 14 years are said to have been affected. This statistics of J.E is lower compared to last year which recorded over 200 cases and death of about 65 persons which, can be attributed to the mass vaccination campaign of human population and swine population prior to the onset of monsoon. The future of pig farming is at stake due to

consecutive outbreak of this disease year after year, since pig is considered to be the reservoir host of the virus causing the disease. The disease spreads to humans when an infected culex mosquito accidentally feeds on the human host after having fed on a carrier host i.e. pig or duck.

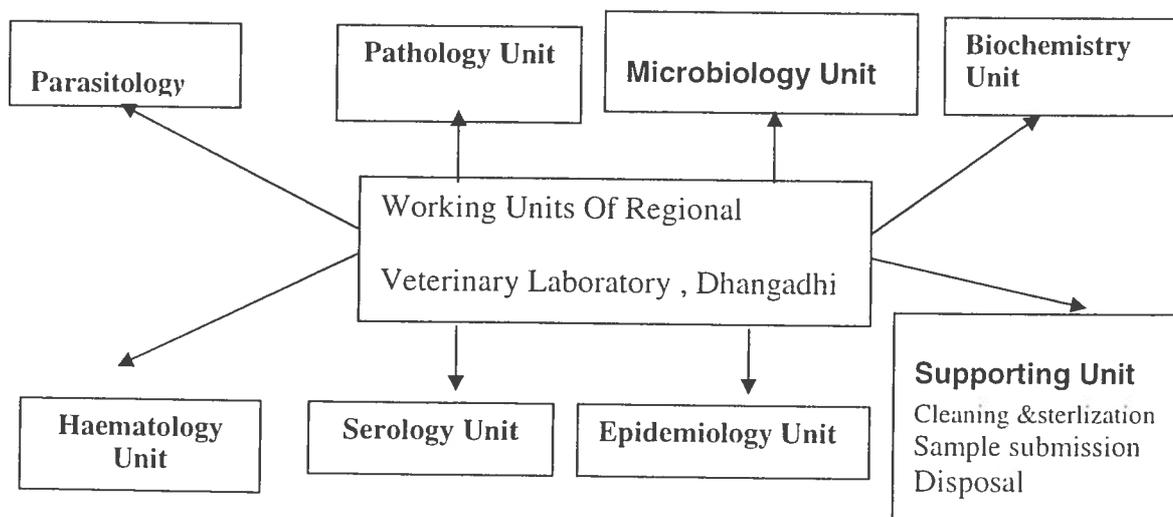
Although, Far western region is still lagging behind in its development efforts of the poultry industry as a business entrepreneur. Broiler farming is carried out in small commercial scale in Kailali and Kanchanpur districts. Recently some farmers of Dadeldhura and Doti are showing interest in poultry farming, and they have started to keep poultry in number of few hundreds spontaneously. A few economically important diseases of poultry in this region are New Castle, Infectious Bursal Disease, Coccidiosis, Chronic Respiratory Disease (CRD), Inclusion Body Hepatitis (IBH) also called Leechi Heart Disease, Colibacillosis, Mycotoxicosis etc. The disease like IBH is basically introduced from India due to open border and the rampant problem of bringing Indian chicks through illegal route for farming. Due to presence of open border with India and having many illegal routes of entry our quarantine system is unable to control free movement of livestock and their products.

However, the disease diagnosis is still not based fully on the laboratory findings. It is most of the times based on the history, statements put forward by the farmers, and the clinical findings of the animal on examination. Although it's been more than a decade has elapsed since the Regional Veterinary Laboratory, Dhangadhi was established in 2049/050, it has not been able to provide diagnostic services to all the districts of the region. The main constrain evident for this is the insufficient flow of samples from the districts livestock services. Never the less there has been considerable improvement in recent years with comparison to earlier years as some of the DLSO's and the quarantine offices are now submitting increasing number of samples for examination to the laboratory.

Activities of Regional Veterinary Laboratory, Dhangadhi

Regional Veterinary Laboratory, is located in the heart of Dhangadhi municipality of Kailali district. This laboratory is established as the reference laboratory of the region with its service area covering the nine districts of the Far western Region.

The laboratory has several units to perform its routine laboratory work and work of investigation programme under its mandate. The details of the units of the laboratory is presented below :



3. Annual Work Program of RVL, Dhangadhi 2064/2065

3.1 Approved Annual Work Program of fiscal year 2064/65 and Summary of Achievement of RVL, Dhangadhi is presented in Table

S. N.	Programs and Activities	Unit	Annual Target		Annual Progress	Progress Percentage	
			Quantity	Weightage			
1.	Laboratory Service programme-						
1.1	Parasitological Examination	No.	1500	6.30	2466	100%	
1.2	Microbiological Examination	No.	250	6.30	258	100%	
1.3	Pathological Examination	No.	300	5.60	315	100%	
1.4	Serological Examination	No.	300	4.90	308	100%	
1.5	Hematological Examination	No.	200	5.60	224	100%	
1.6	Biochemical Examination	No.	200	6.30	250	100%	
1.7	Dispatch of samples (CVL and other Lab	No.	250	6.44	738	100%	
2.	Disease Investigation and Surveillance Programme						
2.1	Investigation and surveillance of Khari Disease.	Time	6	26.61	6	100%	
2.2	Investigation of Epidemic Diseases	Time	6	13.59	6	100%	
3.	Inspection and Supervision Programme						
3.1	Inspection and Supervision of District Labs.	Time	6	6.44	6	100%	
4	Annual workshop on Animal Disease Investigation)	Time	1	3.5	1	100%	

5	Publication Programme:						
5.1	Haf Yearly Epidemiological bulletin publication	Time	2	2.1	2	100%	
5.2	Annual Technical Book Publication	Time	1	1.4	1	100%	
6.	Purchase Programme						
6.1	Purchase of scientific Books and Journals	Time	1	2.8	1	100%	
7	Lab Animal Management	Time	12	2.1	1	100%	

Avain Influenza Contol Progm 064/65

S. N.	Programmes and Activities	Unit	Annual Target		Annual Progress	Progress Percentag	
			Quantity	Weightage			
I.	Semple Collection and Exam	No	1200		1310	100%	

3.2 Annual Work Programme of RVL, Dhangadhi 2063/2064

3.2.1 Approved Annual Work Program of fiscal year 2063/64 and Summary of Achievement of RVL, Dhangadhi is presented in Table

S.N .	Programs and Activities	Unit	Annual Target		Annual Progress	Progress Percentage	
			Quantity	Weightage			
1.	Laboratory Service programme-						
1.1	Parasitological Examination	No.	1000	3.89	2345	100%	
1.2	Microbiological Examination	No.	250	4.77	301	100%	
1.3	Pathological Examination	No.	300	4.02	392	100%	
1.4	Serological Examination	No.	350	4.14	442	100%	

1.5	Hematological Examination	No.	200	3.64	211	100%	
1.6	Biochemical Examination	No.	200	3.64	217	100%	
1.7	Dispatch of samples (CVL and other Lab	No.	250	9.38	443	100%	
2.	Disease Investigation and Surveillance Programme						
2.1	Investigation and surveillance of Khari Disease.	Time	12	22.71	12	100%	
2.2	Investigation of Epidemic Diseases	Time	12	19.95	12	100%	
3.	Inspection and Supervision Programme						
3.1	Inspection and Supervision of District Labs.	Time	10	9.79	10	100%	
4	Training Programme:						
4.2	Computer	Person	2	1.25	2	100%	
5.	Annual workshop on Animal Disease Investigation)	Time	1	4.14	1	100%	
6.	Publication Programme:						
6.1	Quarterly Epidemiological bulletin publication	Time	4	4.02	4	100%	
6.2	Annual Technical Book Publication	Time	1	2.51	1	100%	
7.	Purchase Programme						
7.1	Purchase of scientific Books and Journals	Time	1	1.88	1	100%	

3.3 PPR seromonitoring Programme:

District	No. of Vaccinated Goat and sheep		Collection of Serum samples (Number)		Remarks
	Target	Progress	Target	Progress	
Dadeldhura	14000	14,000	70	70	Decrease in the budget allocation every year for the purpose of sero-surveillance is a big problem. If the trend continues to next year it will be difficult to work.
Kanchanpur	18,000	18,000	90	90	
Kailali	18,000	18,000	90	90	
Bajura	12,000	12,000	60	60	
Bajhang	12,000	12,000	60	60	
Achham	14,000	14,000	70	70	
Baitadi	14,000	14,000	70	70	
Doti	14,000	14,000	70	70	

Percentage of Progress during f/y 2064/65

Animal Health Services Programme: 100%

Weight age of progress: 100%

4. Laboratory Services

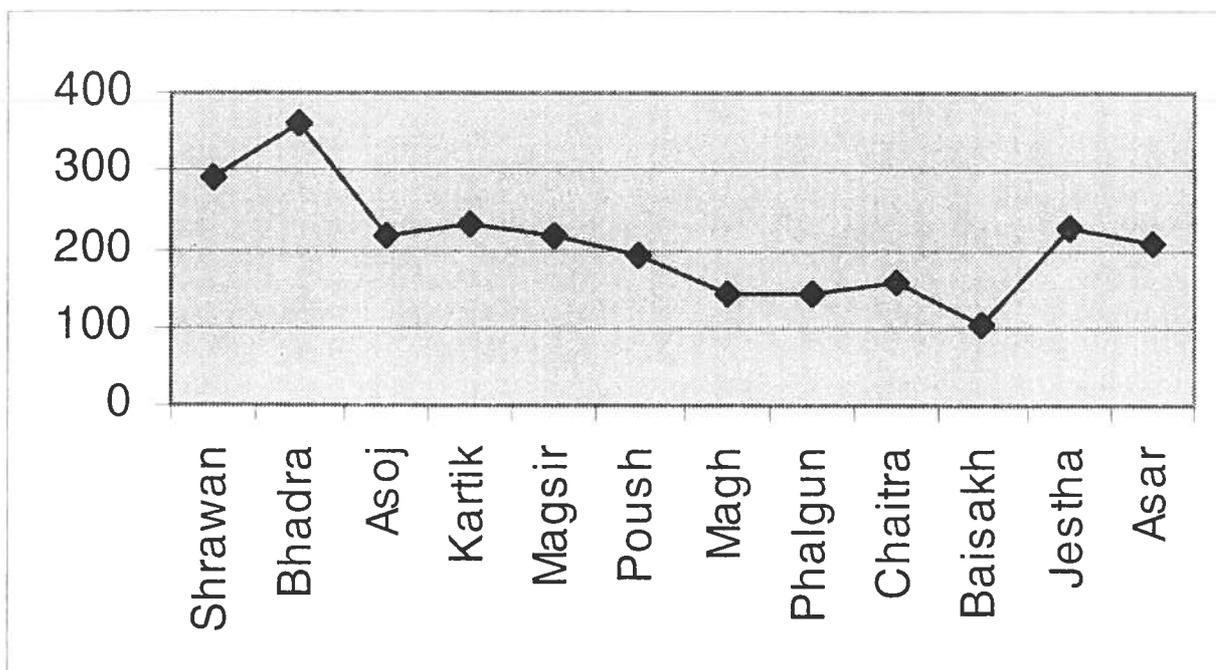
4.1 Parasitological Examination :

Altogether 2340 samples were tested for different parasitic conditions of livestock and poultry populations. Samples for examination mainly consisted of the regular faecal samples submitted to the district livestock service office, Kailali. Apart from this samples were also collected from field during epidemic disease investigation and the investigation programme in our set annual programme. The most common helminthic parasite identified during faecal examination was Fasciola followed by other internal parasites of nematode group, viz. Strongylus, Strongyloids, Ascaris, Trichuris, Coccidiosis, Moniezia, etc. The prevalence of internal parasites in livestock population is presented in the bar diagram below. As it is evident from the graph that most of the faecal samples examined at the RVL are positive for one or the other internal parasites. Out of 2340 samples tested 1830 (78.20%) were positive and only 510 (21.79%) were negative for any parasites.

This clearly reveals the economic loss that the farmers are incurring due to lowered production and productivity of their animals arising from the prevalence of internal parasites in their animal. It also indicates the lower degree of awareness and ignorance on the health and productivity of the animals on the part of the farmers.

The high prevalence of Fasciola in the livestock population of the region, particularly the two terai districts namely Kailali and Kanchanpur invite great problems during the months of summer and monsoon. It is suspected to cause a wide spread outbreak of black's disease in the goat population of these two districts causing heavy economic loss to the farmer due to high morbidity and mortality that usually follows the infection every year.

Month wise parasite load in livestock population.



Note from above graph that the parasitic load begins to increase from Jestha onwards reaching the highest during Bhadra. There is some what stable parasitic load from Asoj to Baisakh. A greater parasitic problem is significant during summer and rainy seasons, which drops with the onset of autumn and remains relatively constant until the end of spring season. Below is a table showing the prevalence of different parasites against various months.

Month wise Prevalence of Different internal Parasites.

	Shara.	Bhad	Asoj	Kar.	Marg	Pou.	Magh	Phal	Chai	Bai	Jes	Asar
Fas.	29	60	60	24	32	33	23	47	40	32	61	28
Prm	56	158	64	38	44	40	28	40	51	57	47	62
S.lus	26	59	24	16	14	14	21	13	26	16	17	13
S.loid	4	11	9	12	1	3	1	5	6	1	0	14
Ascar	0	7	4	7	1	2	1	3	2	2	2	8
Cocci	13	13	15	7	5	12	4	3	7	2	0	1
Others	23	73	48	62	43	38	52	72	85	62	61	70
Total	151	392	230	166	131	142	130	183	217	172	188	196

4.2 Serology:

A total of 442 serological tests of different types were performed against the target of 350 during the fiscal year 2063/64. Most of the serum samples collected were from goats for various diagnostic tests viz. PPR, Mycoplasma, Brucellosis and other disease conditions responsible for causing abortion in these animals, the samples so obtained were forwarded to CVL. The detail of serological tests performed on serum of various animals is presented in the table below along with the results. Poultry serum was mainly used as diagnostic aid of two major disease conditions namely Salmonella and Mycoplasma.

Serum samples collected from bovine population were mainly under the Khari disease investigation program. These samples were obtained from Baitadi and Darchula and forwarded to CVL for necessary biochemical test of the serum apart from performing the regular screening test of Brucellosis using Rose Bengal Plate Agglutination Test.

Result of Serological examination performed at the RVL, Dhangadhi

S.No.	Animal sp.	Number of Serum samples	Type of test performed								Samples sent to CVL
			Salmonella		Mycoplasma		Brucellosis		Tuberculin		
			No. +ve	No. -ve	No. +ve	No. -ve	No. +ve	No. -ve	No. +ve	No. -ve	
1	Bovine	62					0	33			62
2	Sheep/goat	172					0	87			172
3	Poultry	81	7	56	9	76					5
4	Human	12									12
5	Pigs	20									20

The serums of humans and pigs were collected for investigation of Japanese Encephalitis (J.E.) program running under CVL. Human serums were collected from suspected cases of J.E. brought to Seti Zonal Hospital, Dhangadhi.

Result of Serum samples sent to CVL

S.No.	District	Animal spp.	No. of sample	Date	Test requested	Result		Remarks
						+ve	-ve	
1	Kanchanpur, AQO	Goat	22	2064/5/4	PPR	8	14	
2	Kailali, Godavari	Goat	11	2064/5/27	PPR	1	10	
3	Kanchanpur (Zonal Adm. Office)	Goat	5	2064/7/8	PPR			Not received
4	Dadeldhura	Goat	30	2064/9/21	PPR	26	4	
5	Kanchanpur AQO	Goat	23	064/10/10	PPR			Not received

6	Krishnapur, Kanchanpur	Goat	13	064/11/14	PPR	5	8	
7	Banbedha, Kailali	Goat	10	065/3/8	PPR	10		
8	Chaumala, Kailali	Goat	2		PPR	2		
9	Mahendranagar, Kanchanpur	Goat	10		PPR	10		
10	Jhalari, Kanchanpur	Goat	6		PPR	3	3	
11	Darchula	Goat	17	2065/3/9	PPR	14	3	
12	Kanchanpur	Poultry	5		Avian flu		5	
13	Bhajan	Cattle	7		FMD	3	4	Otype
14	Achham	Cattle	5		FMD	1	4	OType
15	Dadeldhura	Cattle	3		FMD		3	

4.3 Haematology:

Haematological examination at the RVL, Dhangadhi included TLC, DLC, TEC, PCV, Hb, ESR, and examination for presence of blood parasites. Blood samples were mainly collected from livestock during outbreak of epidemic, cases referred by the DLSO Kailali, and from animals selected for the specific investigation programme of the laboratory.

Table: Haematological tests conducted at the RVL, Dhangadhi.

Type of Test	Number	Remarks
TLC	4	
TEC	12	
DLC	86	1 recorded 54.6% eosinophil (Allergic reaction to intramammary infused penicillin i.e. Pendistrin HS)
PCV	200	
Hb	200	Generally low in Khari diseased animals
Blood parasites	80	2 positive, Babesia,

On our examination of haematological parameters of Khari diseased animals, they invariably showed low Hb. concentration. Hb. value in these animals ranged from low of 5.4 to a high of 7.6 g./dl.

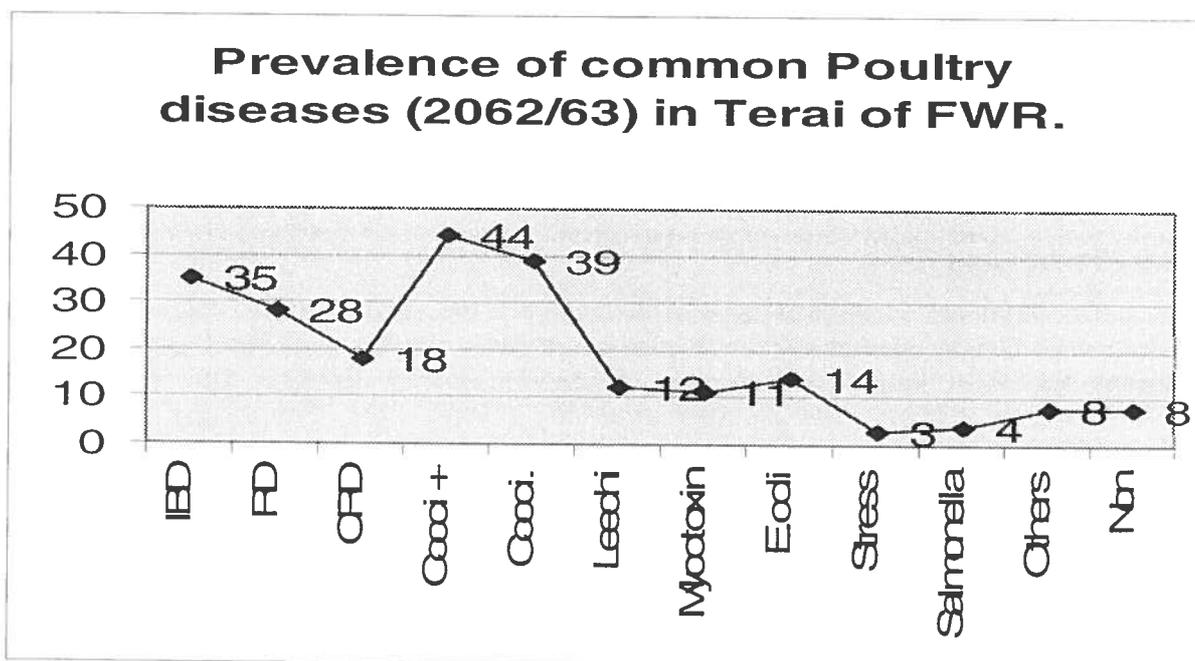
Shown above is the udder and the inner thigh of a milking buffalo which was affected with mastitis and received intra-mammary infusion of pendistrin HS that incited a generalized allergic reactions with formation of rashes all over the body. The udder, teat, inner thigh and lower abdomen were severely affected with intense rash formation and appearance of erosive wounds as is seen in the picture above. Differential leukocyte count of the above animal recorded an excessively high eosinophil count i.e. 54.6%.

Out of 163 blood smears examined for blood protozoan 2 were found positive for babesia.

4.4 Pathology:

This unit of the laboratory mostly receives poultry carcass for necropsy study and disease diagnosis. However, dead bodies of other animal species is also received occasionally, especially during disease outbreak and in cases of veterolegal importance. Out of the total of 458 postmortems performed during the fiscal year 2064/65 only 12 cases of goats and 2 of wild pigs from Kailali, Dadeldhura, Kanchanpur and the rest were of poultry species. Since most of the pathological samples comprised of poultry, it is important to present the major diseases diagnosed, based on findings of postmortem lesions.

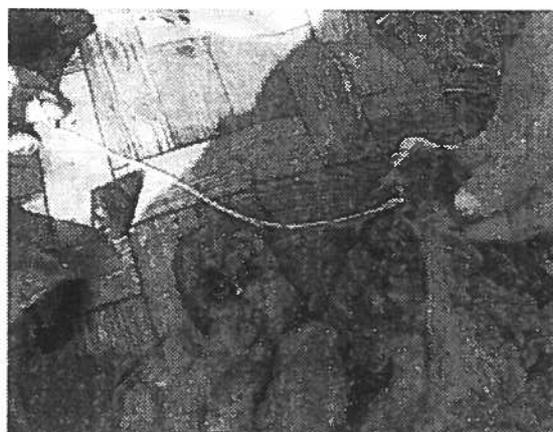
Table: Common Poultry Diseases of Far Western Region



Cocci + as used in the graph indicates diseases when coccidiosis was diagnosed with other common diseases. Coccidiosis was identified as the commonest disease of the poultry of this region, followed by Infectious Bursal Disease, New Castle Disease, Chronic Respiratory Disease, Collibacillosis and Leechi heart. Less commonly occurring but important diseases like stress, Gout, Bacillary White Diarrhoea, Salmonella and Ascitis were also diagnosed at the laboratory.

Apart from poultry post mortem examination of 6 goats and 2 wild pigs was also performed. The diseases diagnosed for goats were Pneumonia, Jaundice,

Kumri and Parasitic infestations respectively and that for Pig Haemorrhagic Septicaemia and parasitic infestation with *Macrocanthorhincus hirudinaceus*. The picture below shows the *M. hirudinaceus* worm attached to the intestinal wall with typical granulation formation at the site of attachment on the wall. It can be observed in the second picture that the penetration of the intestinal was has incited peritonitis, which causes a fatal inflammation of the peritoneum in the affected animal.



Additionally one brain sample was collected from a yearling buffalo she calf from Amarbasti of Kanchapur and sent to the CVL for confirming the suspected Rabies case. The report received from the CVL confirmed Rabies in the sample sent and the farmer was accordingly advised for necessary action.

4.5 Microbiology:

The samples subjected to microbiological examination at the Regional Veterinary Laboratory, Dhangadhi constitutes of milk, nasal swab, vaginal swab, and swab from visceral organs like liver, lungs, intestine etc. of various animal species. The media used for microbiological culture were Nutrient agar, Mc conkey agar, Blood agar, and Saboroud Dextrose agar. Bacteria and fungi were identified on the basis of colony characteristics, Gram's staining property and the structure of the organism as seen under the microscope. Due to limitation of the facility in the laboratory biochemical tests for identification of bacteria could not be performed with exception of a few like the catalase test. The result of microbiological test is presented in the table as below:

Animal	Type of sample	Number	Major bacteria identified	Remarks
Cattle	Milk	42	Strep., Staph., Cory.,Pseudomonas, Enterobacter	
Buffalo	Milk	106	" "	
Goat	Nasal, vaginal swab	44	Strep., Staph., Bacillus	
Poultry	Liver	22	E. coli, Strep., Staph., Coryne.	
	Lungs	22	" "	
	Intestinal swab	8	" "	
	Egg	16	Negative	

The milk samples positive for California mastitis were tested for antibiotic sensitivity test following preliminary culture in order to choose right antibiotic for the treatment of mastitis. The result of antibiotic test is given in the table bellow:

Table: Result of Antibiotic Sensitivity test for Milk Samples.

Antibiotic	Total No. of tests	Sensitive (%)	Resistant (%)	Intermediate (%)
Gentamycin	21	34 (74)	8 (17)	4 (9)
Cepro.	21	30 (71.4)	4 (9.52)	8 (19)

Tetracyclin	21	6 (13)	38 (82.6)	2 (4)
Cloxacillin	21	2 (4.3)	44 (95.6)	0
Ampicillin	21	2 (4.3)	44 (95.6)	0

4.6 Biochemical:

Biochemical test of milk, urine, and serum were performed at the laboratory with the optimum use of the limited facility of the laboratory. Altogether 27 urine samples were examined using multistix for detection of urobilinogen, protein, pH, blood, specific gravity, ketone bodies, glucose, bilirubin etc. Microscopic examinations were carried out only if the case history and urine sample was suggestive of presence of infection in the urinary tract or cast cells in the urine.

The total number of biochemical tests performed on milk, serum and urine samples along with the method used with their respective results is given in the table below:

Sample type	Number	Test type	No. +ve	No. -ve	Remarks
Milk	148	CMT	53	95	
Urine	19	Multistix			16 large, 3 small moderate ketone
Serum	62	biochemical for Ca			Unreliable result

Biochemical examination of serum was performed with the serum sample of buffaloes suffering from Khari disease mainly for estimation of serum calcium level. However, result was not reliable. The test was repeated two three times on the same sample which gave a different result every time the test was repeated. However, the Calcium content of the serum was recorded from a low of 3 to a high of 18mg/dl.

Epidemic Outbreak investigation :

The number and the epidemic outbreak investigation carried out by the laboratory is given in the table below :

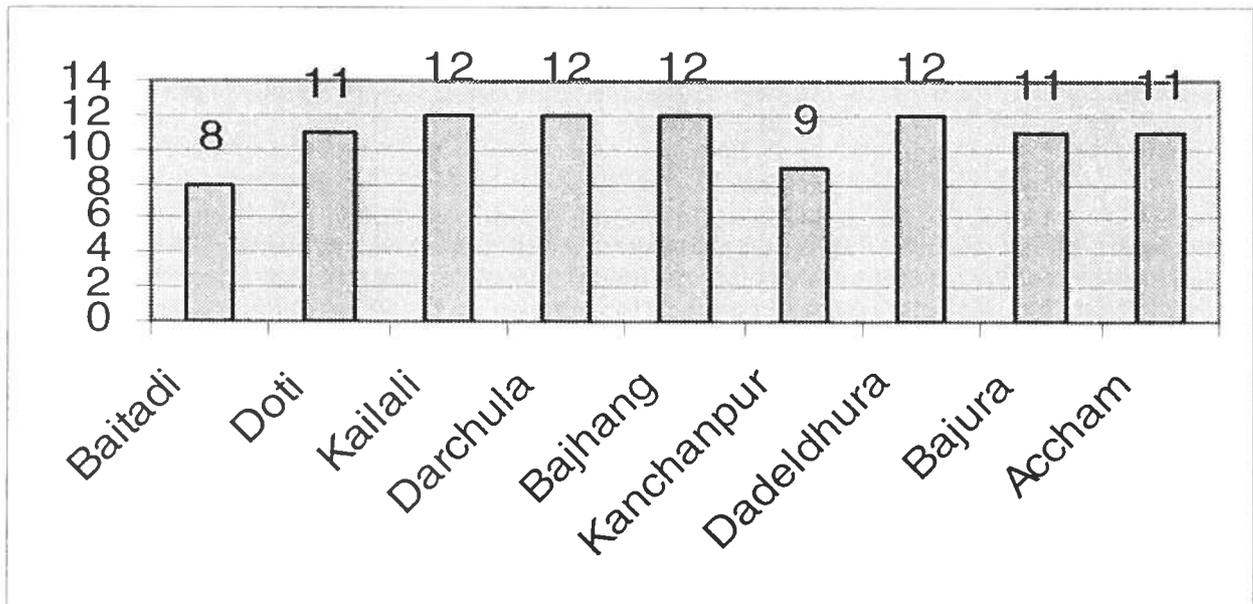
Table: Disease outbreak in the far western region during the fiscal year 2063/064

Month	Year	District	VDC	Species affected	Disease
Bhadra	2064	Dadeldhura	Jogbuda	Cattle	Ergot poisoning
Magsir	2064	Kailali	Chaumala	Goat	Kumri
Phalgun	2064	Kailali	Godavari	Goat	PPR
Chaitra	2064	Kailali	Sahajpur	Goat	Babesiosis

Baisakh	2064	Kailali	Ghorsuwa	Goat	PPR
Jestha	2064	Dadeldhura	Jogbuda	Goat	PPR
Asar	2064	Darchula		Goat	PPR

The monthly epidemiological reporting from the districts is satisfactory. However it is quite regretful to mention that the kind of response which Kanchanpur has shown is very discouraging. It has reported only for 9 months and even more discouraging is that it sent disease report of six months in a bundle towards the end of fiscal year. The graph below illustrates the number of epidemiological reports received from different districts.

Monthly epidemiological reporting received from different districts



5.1 Publication of Quarterly Epidemiological Bulletin:

Quarterly Epidemiological bulletin of the Far Western Region is published by this laboratory with the objective of disseminating the information on the animal disease situation of the region to the Veterinarians, Technicians, other related individuals and organizations. The information incorporated in the bulletin are obtained from the respective DLSSO's of the region and the Quarantine Office. It is mainly done with a view to collect and maintains a reliable epidemiological data within the country so as to enable us to establish a strong and reliable disease information exchange system within and outside the country. This is a prerequisite for OIE member country to take part in the livestock related trade in the world market. The final report which is published from the Veterinary Epidemiology Center at Tripureshwor, Kathmandu consists of the disease situation of all the five development regions and according to the geographical distribution of the country for the period of one year. Since The quarterly and annual bulletin comprise the detail of disease existence of this region, it will only be the repetition of the same thing to present it here.

Bird Flu related Activities of the RVL, Dhangadhi

The news of outbreak of avian influenza in China and Maharashtra state of India both neighboring countries of Nepal created a situation of high alert in the country. Due to its high sensitivity we had to re-orient our scheduled annual activities and plan our work giving greater preference to the Avian influenza surveillance program. Although it was difficult to accomplish the task given without additional financial resources, still we managed to perform the surveillance program for avian influenza (both passive and active) by partially using the budget allocated for other activities.

We had virtually performed both passive and active surveillance program for avian influenza during the fiscal year 2062/063. Under passive surveillance over 46 commercial farms were inspected in different districts and observation of Suklaphanta wild life reserve in Kanchapur district along with important natural lakes like Ghodaghodi located in Sandepani v.d.c. of Kailali district was carried out frequently. These wild life reserves and natural lakes were visited 3 times during the program period of less than 6 months.

Active surveillance was performed frequently and samples from commercial poultry as well as local poultry raised in backyards of houses in different districts were collected and sent to the CVL for test. During the period over 115 cloacal and tracheal swabs, 15 poultry serum, and 6 dead birds and poultry were collected and dispatched to the CVL for test.

INVESTIGATION OF KID MORTALITY IN GOATS OF THE FAR WESTERN REGION

Background:

Goat rearing is an important component of livestock production which in turn is an integral part of Nepalese agricultural practices. Since few years goat rearing is fast gaining popularity in districts of far western region of Nepal. The present goat population of the region is projected as 616115 (Statistical information on Nepalese Agriculture, 2002/2003, MOAC), which constitutes 9% of the total goat population of the country. Goat is the second largest source of meat in our country standing next only to buffalo and contributes about 19.47% of the total meat production in the country (Statistical Information on Agriculture, 2002/2003). At the present rate of growth of goat population which happens to 7% per annum, around 40000 goats are added to the existing population every year. However, together with its fast growth goat rearing is facing newer challenges in management, health and disease control.

Among the various constraints advocated as responsible for the decreased productivity in livestock sector in this country, disease is generally agreed as one of the most important factors. With the progress in goat farming in the region and steady shift in farming system from traditional subsistence goat farming practices toward commercialization has come many problems. And one of the problems that have emerged as relatively important among others is the increased mortality of kids below six months of age. In the past also the problem of kid mortality had been reported from various farms – small to big of different districts, and also from the goat development farm of Budhitola. A study on Goat and sheep health with particular reference to kid mortality (M.P. Aryal et.al, NARC 2001) showed that the problem of kid mortality was positively correlated with the number of kids born to the dam at a time rather than to the parity of the dam. In order to scientifically address this problem an investigation on kid mortality was initiated in the two far western districts of the region namely Kailali and Kanchanpur.

Objectives:

Long Term:

To identify the major causes of kid mortality and disseminate the information generated to the field veterinarians, technicians and needy farmers.

Short Term:

- To assess the prevalence of infectious and non infectious causes of kid mortality.
- To study the epidemiological pattern of kid mortality so that effective control measures could be suggested.
- To strengthen the diagnostic capability of the laboratory.

Methodology:

Goat rearing farmers for the investigation program from Kailali and Kanchanpur were selected by an interaction program organised separately for the two districts. A total of 10 farmers Six from Kailali and four from Kanchanpur were selected and were distributed with the questionnaire format developed by the RVL, Dhangadhi to be filled on monthly basis. Timely visit by the RVL staff was made to monitor the programme and collect desired samples from the dead of sick kids for laboratory investigation.

Result and Discussion

The information obtained through the questionnaire survey over two years of the investigation period with the selected goat raising farmers revealed that on an average 34.8

kids were born per farm in a year. Mortality of female kids during pre-weaning period was found greater than the male kids (table 1). This is contrary to the observation made by Neopane (1996), Singh et al (1990), Gebrelul et al (1994) and Aryal et. al (2001) that reported higher survival rate of female kids. However, Mittal (1976), Majumdar et al (1980) and Sharma et al (1981) reported the non significant effect of sex on survival rate of kids during pre-weaning period.

Table 1 : Pattern of Kid Mortality

S.No.	Pattern	Number
1	Number of kids born	622
2	Number of Male kids born	328
3	Number of female kids born	294
4	No. of male kids death	46
5	No. of female kids death	58

The pre-weaning death of kids was found to be related with the type of birth. Highest percent of death was recorded in the kids which were born as triplets followed by twins. Least number of mortality was recorded with kids born as single (table 2). The finding is in agreement with the report of Aryal et al (2001), Sharma et al (1981), Prasad (1983) and Singh et al (1990) who reported that singles had higher survival rate than the twins or the triplets. Mortality rate of 33.33% presented here, which appears to be exaggeration of the finding may be due to small size of the triplets observed, nevertheless justifies the increased mortality in triplets compared to twins and singles due to limited nursing opportunity with the increased litter size. Still it requires further investigation to reflect the exact or the reliable percentage of the deaths occurring in the kids born as triplets. However, the overall percentage of kid mortality was observed to be well below 10 percent which is expected in well managed farms.

Table 2: Kid Mortality in Association with the Type of birth

S.No.	Type of birth	No. born	No. death	Percentage
1	Single	276	11	3.98%
2	Twins	310	37	11.93%
3	Triplet	36	12	33.33%
Total		622	60	9.64%

As we can make out from the table above that the overall rate of kid mortality is recorded to be 9.64%

Rate of kid mortality in relation to the season also appeared to differ with the season, with highest mortality recorded during the summer/rainy season, followed by winter compared to lower mortality observed during the spring and the autumn seasons. This finding is in agreement with the report of Aryal et. al (2001). The reason for higher mortality during rainy and winter season could be attributed to the adverse weather condition imposing stress upon the kids rather than the occurrence of any specific disease condition. It is also likely that greater number of kids is born during these two seasons compared to other two seasons which witnesses lower birth rate.

Table 3: Kid Mortality in Relation to Season

S.No.	Season	Number of death	Percent
1	Winter (Marg.- Magh)	17	28.3%
2	Spring (Phalgun – Baisakh)	8	13.33%

3	Summer/Rainy (Jestha – Shrawan)	29	48.33%
4	Autumn (Bhadra - Kartik)	6	10%

Mortality was observed highest during the period of 15 days to 1 month of age in the kids. Next was the period between 1 to 3 months of age (table 4). This observation is contrary to the findings of Aryal et al (2001) that reported highest mortality in kids less than 15 days of age revealing a decreasing trend in the mortality with the increasing age of kids.

Table 4: Mortality associated with the age of kids

S.No.	Age of Kid	Number dead
1	Less than 15 days	11
2	16 to 30 days	24
3	1 to 3 months	16
4	Above 3 months	9

Laboratory Findings:

During the investigation period, samples collected from the farm were examined, and the result so obtained on parasitological, Haematological, and Microbiological examination is listed below.

Parasitology:

Most of the faecal samples collected from the kids under investigation were found infested with one or the other kind of nematodes. Out of 26 faecal samples examined so far 21 were positive for different nematodes. However, Fluke infestation was not recorded in any of those samples. Major round worms identified were belonged to *Strongylus*, *Strongyloids* and *Trichuris*.

Haematological Examination:

Haematological examination of the clinically ill kids was carried out to assess change in major blood parameters such as PCV, Haemoglobin, and blood protozoan parasites. Finding is presented in the table below:

Table 5: Result of Haematological Examination

S.No.	Test	Avg. Value
1	Haemoglobin	9.1g/dl
2	PCV	20.4%

Microbiological Examination :

Nasal swabs were collected from clinically sick kids showing signs of pneumonia for microbiological examination. Bacteria identification was done on the basis of cultural characteristics of the colony, and gram's staining. Major bacteria identified were Gram positive cocci, Gram negative rods, *Staphylococcus*, *Streptococcus*, *Pasturella* etc.

Diseases involved in Kid Mortality:

The most common cause of death of a kid was identified as Pneumonia, followed by diarrhoea, Paralysis, Anuria. It is presented in the table below:

Table 6 : Diseases involved in Kid Mortality

S.No.	Type of Disease	No. of Death
1	Infectious Pneumonia	18
2	Vermiceous pneumonia	13
3	Paralysis	7
4	Anuria	6
5	Diarrhoea	12
6	Others	4

Pneumonic conditions in the kid were identified as infectious and parasitic depending on the clinical symptoms and presence or absence of fever.

Conclusions:

It is observed that kid mortality is positively related with the type of birth, evidencing that those born as triplets or twins had lower rate of survival compared to kids born single. Higher mortality was observed during the rainy and winter season and the common diseases identified to cause death were pneumonia, diarrhoea, Anuria, Paralysis etc. Since, highest mortality appeared to be caused due to pneumonia of infectious nature i.e. either parasitic or bacterial; there is ample room for decreasing the rate of kid mortality by better management and adoption of proper health management and parasitic control program.

6. DETAILS OF BUDGET EXPENDITURE (2062/063)Table No. *Budget expenditure of Regional Veterinary Laboratory , Dhangadhi during 2062/063*

Budget Line	Budget Head	Approved Budget(Rs)	Received Budget(Rs)	Expenditure (Rs.)
1.01	salary	1145000.00	1145000.00	946896.80
1.02	Allowance			
1.03	TADA & Transfer Expense	21000.00	21000.00	3210.00
1.04	Dress	12000.00	11975.00	11975.00
1.05	Food and Nutrition	8000	8000	8000
2.01	Water and Electricity	105000.00	105000	105000
2.02	Telephone	66000.00	66000.00	66000.00
2.03	Office concerned	417,000.00	417000.00	405000.00
2.05	Repairs	94,000.00	94,000.00	91000.00
2.06	Fuel for vehicle & other use	91,000.00	91,000.00	87,000.00
2.07	Consultancy & other services	10,000.00	10000.00	9000.00
2.08	Miscellaneous	25,000.00	25,000.00	21965.00

4.02	Medicine purchase	60,000.00	60,000.00	57850.00
4.04	Programme Expense	110,000.00	110000.00	110000.00
4.05	Programme TA/DA	160,000.0	160000.00	158000.00
6.01	Furniture	20,000	20,000	20,000
	Total	23,44,000	2289503.50	2289503.50

8. Manpower of Regional Veterinary Laboratory ,Dhangadhi

S. No	Name of the staff	Post	Class	Sanctioned	Availa-ble	vacant
1.	Dr. Diker Dev Bhatt	Senior VO	GII	1	1	-
2.	Dr.	VO	GIII	1		-
3.	Mr. Shyam Prasad Pathak	JT	NGI	1	1	-
4.	Mr.Narandr Raj Joshi	JT	NGI	1	1	-
5.	Mrs.Mina Sharma	JTA	NGII	1	1	-
6.	Ms.Menaka Shrestha	Typist	NGI	1	1	-
7.	Mr.	Store keeper	NGII	1		-
8.	Mr. Ganesh Boahra	Accountant	NGI	1	1	-
9.	Mr. Keshav Raj Pandey	JTA	NGII	1	1	
10.	Mr.	Driver	None	1		-
11.	Mr.Prem B.Chaudhary	Peon	None	1	1	-
12.	Mr.Lal B.Shaond	Peon	None	1	1	-
13.	Total			13	9	

MOLECULAR BIOTECHNOLOGY DEVELOPMENT AND ITS IMPLICATIONS IN NATIONAL VETERINARY SERVICES OF NEPAL

Dr. Banshi Sharma
Chief, Molecular Biology Unit
Central Veterinary Laboratory, Nepal

Introduction:

Livestock is an integral part of agricultural production systems, focusing principally on manure for cropland and for providing almost all of the draft power used in cultivation. The contribution together with production of food (meat, milk, and eggs), fiber, hides/skins and transportation amounts to about 15% of GDP which is 28% of agricultural GDP (Livestock Master Plan, 1993). Nepal is a mountainous country with great biological diversity. Our country is divided into mountains, high hills, mid hills, Shivalik range and Terai plain. Livestock rearing is more in mountain and high hills (Sharma, B., 2002). Poultry farming is popular in rural areas adjacent to big cities. There is problem of bird flu in South Asia, Nepal is infected with bird flu since January, 2009. There has been sixteen billion Nepalese Rupees investment in commercial poultry sector by private enterpreniurs. Both of our neighbours have been suffering from bird flu from Nepal is a new and 146 th member of WTO. Nepal is also member of OIE. Therefore, there is Importance of trade of livestock and its products to neighboring countries.

The diagnostic services for early detection of diseases is quiet important in this context. In the current situation, the epidemiology of bird flu is not known clearly. In bird flu, there is role of migratory birds. Migratory birds will transmit disease to water fowl such as ducks. The molecular epidemiology of bird flu is hazy . Therefore, poultry industry is in continuous threat from China and India from last four years. The production cost is high in Nepal than our neighbouring countries. Nepalese farmers are vulnerable to free market in the context of WTO as there is no agricultural subsidy in poultry production sector. The new challenge in the world comes from emerging microbes due to reassortment of virus and environmental pollution will proliferate such activities.

The National Veterinary Services has been working with following visions.

- National veterinary services should be able to handle the outbreak of emerging diseases with prompt diagnosis, treatment and containment of the disease on the site of emergence.
- The rules and regulation of veterinary services should be harmonized with the guidelines of OIE.
- Laboratory services should be strengthened.
- Veterinary and zoonotic important diseases should be sorted out and program disease should be prepared.
- Production of Vaccines for economic and zoonotic important diseases in country to safeguard stakeholders' need.

Objectives of Animal Health Services

- Early detection and control of TADs and publication of their containment guidelines based on internationally accepted scientific data.

Central Veterinary Laboratory (CVL) works with the objective of securing healthy national herds/flocks of animals and birds throughout the nation based on scientific evidence of the occurrence of diseases of livestock and poultry. Besides, CVL also works on epidemic investigation as well as surveillance and investigation on various diseases/conditions as its approved annual programme. The direct benefit of the performance of various laboratories

has been experienced in the field of veterinary medical care based on valid laboratory test results. To achieve these multidimensional activities, CVL works with the application of a series of laboratory test procedures through its various laboratory units; Pathology, Parasitology, Microbiology, Serology, Hematology and Biochemistry units, and Molecular Diagnosis with a considerable progress in the later. At present the molecular based diagnosis of Avian Influenza is in the course of advancement. Similarly, setting up of tissue culture laboratory unit is in progress and expected to conduct virus isolation, identification and serotyping in shortcoming days.

CVL is always aware in adopting modern disease diagnostic technologies. Endeavour is continuously made in improving its performance in the form of research-oriented activities rather than routine diagnostic works. We are in the process of development of Standard Operating Procedure, test protocols, measurement traceability and biosafety system so that good laboratory practice is followed in our all the diagnostic laboratories. We are already adopting test verification system through international reference laboratories which will help, at least, in the accreditation of CVL for international certification under ISO.

- To provide diagnostic facilities throughout the country, CVL works through its five Regional Veterinary Laboratories (RVLs) located one in each of the development regions of the nation as well as through National Avian Laboratory. To provide the diagnostic services smoothly throughout the nation, fifteen basic laboratories established in 15 district livestock service offices (DLSOs). The basic laboratories are capable to perform microbial culture and antibiotic sensitivity test. Specimens that could not be processed in the aforementioned laboratories due to insufficient facilities are referred to central veterinary laboratory. In this way, CVL works as reference veterinary laboratory in Nepal. The role of veterinary profession has been modified tremendously in the course of civilization, economic liberalization and trade globalization worldwide. Nepal is also not unfamiliar with their impact and benefit. To grab the opportunity of global trade, Nepal is compelled to follow the guidelines provided by Office International des Epizootics (OIE) for the provision of Sanitary and Phyto-sanitary (SPS) agreement under WTO that seeks scientific procedures and evidences in the course of disease diagnosis as well as production chain. The role veterinary diagnostic laboratories are now therefore expanded and challenging with ample of opportunity.

Vaccine production for immunization of livestock and poultry birds for sustainable economic growth.

- **1961:** Initially Veterinary Investigation Laboratory established at Tripureshwar.
 - *At the same time this laboratory was producing Goat Tissue Vaccine (GTV) to conduct the Rinderpest Eradication Program.*
- **1968:** poultry vaccines production initiated - Newcastle disease and Fowl Pox.
- **1971:** Biological Product Division established with the realization of the necessity of a separate institution for the veterinary vaccines production
 - Now known as CBPL
- **Till 1985:** vaccines produced in small volume for limited disease only.
- **After 1985:** Capacity enhanced
 - addition of new machines and equipment
 - Significant improvement in the quality and quantity of vaccines produced.

SN	Name of vaccine	Production Year
1	ND (Mukteswar and F strain)	1968
2	Fowl Pox	1968
3	ND (La Sota)	1998
4	IBD (Gumboro)	1998
5	ND I-2	under production

- **Till 1997:** all the poultry vaccines were produced from the fertile eggs from local hatcheries.
- **After 1997:** Introduction of SPF eggs for vaccine production with the financial support from SVSLDC project of EU.
- Tissue culture rabies vaccine for animal use.
- Presently this lab produces 20,000 doses / year.
- The lab is strengthened by the cooperation of JICA/ WHO.
- Currently research is going on for producing tissue culture rabies vaccine for human use.
- Japanese expert has been working in the lab.
- Virus typing of FMD.
- TAD's diseases focus on such as blue tongue.
- Responsible for preparing FMD Control Strategy.

Veterinary Standards and Drug Administration Office

- Veterinary drug act is in cabinet. This is policy document.
- Regulatory program and supervision on veterinary services and veterinary inspection.
- Preparing of different standards for strengthening veterinary services.
-

Zoonotic disease awareness campaign had been conducting by Veterinary Public Health. Key role for awareness in meat inspection activities has been carried out. Central Epidemiological Centre (VEC) has been reporting major animal disease to OIE. There has been trimester reporting of disease situation of Nepal to OIE. VEC publishes its reporting 2 times in a year. FMD, PPR, Rabies, HS and internal parasites such as liver flukes are some of important disease in animal. There are two major projects in veterinary services i.e. Avian Influenza FAO /USAID / STOP-AI and Avian Influenza Control Project (WB).

Present Issues on livestock and poultry diseases

- Continual threat of Avian Influenza.
- Bio-security and disease prevention.
- Poor animal and poultry production system.
- Service delivery and its linkage with government system.
- Prompt diagnostic facilities and biotechnology tools in RVLs.
- Availability of inputs -its quality
- Farm registration and statistics
- Livestock and poultry market, slaughter house and meat inspection.

Recommendations

- Disease monitoring system with molecular diagnostic technology shall be enhanced.
- There must be research tie up with different institute regarding epidemiology of disease.
- Sharing of information on biotechnology between institutes.
- Coordinated approach on disease eradication effort with SAARC regions such as HPAI.
- Veterinary services should be strengthened in accordance with OIE guidelines.
- Biological Products Laboratory shall produce under GLP after completion of new laboratory building.
- BSL-3 lab shall be established to diagnosis emerging diseases such as HPAI.
- Collaboration on research work.
- Networking on similar work group.
- Public private partnership in disease diagnosis.
- Human resource development and provision of getting skilled manpower in right place.
- Establishment of genome research centre

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Acute Sudden Death Syndrome in Mule Herd of Udayapur district: Clinical Laboratory Investigation

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Abstract

A clinical epidemiological investigation of acute sudden death syndrome due to which 31 mules from a total herd of 900 died within the period of 5 months from 30 May 2006 to 25 September 2006 was conducted in Udayapur District of eastern Nepal. On Postmortem examination of dead mule revealed acute severe congestion and hemorrhages in liver, lung, spleen heart, and intestinal mucosa were found. Histopathological examination of tissue from these organs revealed infiltration of mononuclear cells that was indicative of chronic nature of the condition. Apparently 15-20% moldy grain (maize, gram) was continuously used as feed and on laboratory analysis of same grain was having 60 -110 CFU/gm of *Penicillium* spp of fungus. Remaining animals of the herd were when treated with toxin binder (Toxicurb, Varishta), and other supportive therapy with liver tonic like bioliv, digevet, mineral mixture (promin) and immunocare for 10 days which were using same types of grain with absence of mortality, may be indicative of the disease syndrome similar to condition Equine Encephalomalacia being caused by moldy corn poisoning. Laboratory findings and response of treatment indicates that during and immediately after rainy season feeding of stored grains are going to be detrimental to equine species for which appropriate preventive measures need to be practiced.

Keyword:

Mule, Moldy corn, *Penicillium*, Hepatocare, Varishta, Toxicurb, bioliv, Immunocare, Equine Encephalomalacia

History of Outbreak:

The District Livestock Service Office Udayapur reported the periodic death of total 31 adult mules during 30 May 2006 to 25 September 2006 with showing symptoms like Sweating, Trembling, Tremor, Sudden death. Initially death was suspected to be due to some acute infectious disease like anthrax was suspected and the mule herds were vaccinated against this disease. When there were reports of death of mules in same herd detail toxicological investigation for insecticide, pesticide was carried out from the intestinal tissue which revealed the absence of any residue of any chemicals. A detail clinical laboratory field investigation was carried out to ascertain the likely cause of periodic but sudden death of mule in herd.

Material and Methods:

- 1: Preliminary Field Investigation.
3. Samples collection from herds and dead animal for laboratory investigation.
4. Intervention for controlling the mortality.

Preliminary Field Investigation:

Mules were found generally fed on dry whole maize,grame grains, which are purchased from market only small part of it is utilized from domestic production. These grains were having moldiness upto15-20%and are not processed before feeding. Mules in the herd were in normal feeding but average poor health . Dr. Steve Hooser, Dr. Duane Murphy in 2003 reported Equine leukoencephalomalacia (ELEM), commonly called "Moldy Corn Poisoning",

is a disease of the central nervous system that affects horses, mules, and donkeys. It is commonly associated with feeding of moldy corn over several days to weeks. The clinical signs include apathy, drowsiness, pharyngeal paralysis, blindness, circling, difficulty backing, staggering, hyperexcitability, seizures and eventual recumbency. However, in some cases, sudden death may be the only clinical sign observed. Once animals show the neurological signs, death usually occurs within 48-72 hours. Post-mortem examination of dead Mule revealed severe congestion of liver, lung, peen, heart, Serosangqinus fluid in thoracic cavity. Hemorrhage in stomach mucosa. Histopathological changes were observed in Liver, Fatty degeneration of hepatocytes. Mononuclear cells infiltration in the form of few nodules. Lungs Perivascular cuffing. Infiltration of mononuclear cells in the form of nodules. Kidney: Deep medullary region reveals infiltration of mononuclear cells. Spleen: The number of white cells seems to be increased in the area of white pulp. Dr. Steve Hooser, Dr. Duane Murphy in 2003 reported histologically in cases of Moldy Corn Poisoning diffuse vacuolization of hepatocytes, fatty degeneration, centrilobular necrosis with inflammatory cell infiltrate, bile duct proliferation, bile stasis, increased mitotic figures within the hepatocytes, or periportal fibrosis. Bacteriological culture of tissue specimen, swab blood: revealed no growth of any bacteria. Blood parasite examination turn out to be Negative. Chemical toxin in tissue did not revealed the presence of any chemical insecticides or pesticides. Carpological examination for Intestinal parasites revealed the presence of mixed Strongyls spp. G Ayele, G Feseha, E Bojia and A Joe* in 2005 reported Prevalence of gastro-intestinal parasites of donkeys in Dugda Bora District, Ethiopia and in their finding the parasites encountered were Strongyle (100%), *Parascaris equorum* (50%), *Anoplocephala* Spp. (7.4%), *Gastrodiscus aegypticus* (6%), *Oxuris equi* (3%) and *Fasciola* (1.5%). Gross faecal examinations revealed *Gasterophilus intestinalis* and *Gasterophilus nasalis* (20.9%). 81.7% of donkeys sampled were severely infected, 8.3% heavily, 3.8% moderately and 6.2% mildly infected. Mixed infections were detected in 54.8% of the donkeys. Cultural identification of larvae (n=28) demonstrated *Strongylus vulgaris* (100%), *Cyathostomes* (100%), *Strongylus edentatus* (66.6%), *Trichostrongylus axei* (40%), *Strongloides westeri* (33.3%), *Triodontophorus* (50%) and *Dictyocaulus arnfieldi* (20%). Samples of feeds maize, grame revealed the growth of *Penicillium*, *Aspergillus*, *Candida* spp on mycological media. 6×10^6 - 11×10^6 CFU/gm *Penicillium* spp colonies recorded in feed samples. K. M. Keller¹, B. D. Queiroz¹, L. A. M. Keller¹, J. M. M. Ribeiro¹, L. R. Cavaglieri², M. L. González Pereyra², A. M. Dalcero² and C. A. R. Rosa¹ February 2007 conducted The Mycobiota and Toxicity of Equine Feeds and reported that Feed contamination can lead to nutrient losses and detrimental effects on animal health and production. Feed mould counts ranged from $<1 \times 10^2$ to 1×10^5 cfu/g. The most frequent genus isolated was *Aspergillus* (40.54%), followed by *Penicillium* (18.38%) and *Fusarium* (16.22%). Advised for proper drying of grain before feeding. 2% Copper sulfate to be mixed in grain before feeding. Commercial toxin binders, Varishta, Toxicurb @ 1kg/tonne of grain for 15 days along with Liver tonic, Hepatocare, Immunomodulators, Promin, Vitamin B complex.

Result and Discussion:

Approximately 15-20% moldy corn infested with *Penicillium* spp was found being fed regularly to these herd. Intermittent but acute sudden death during post rainy season suggests due to moldy grain poisoning. Histopathological changes in the tissue of lung, liver, kidney are similar to the findings of other workers. Histologically, a center of necrosis with no recognizable structure will be observed. The transition between normal and necrotic tissue will often show hemorrhage, edema, congested blood vessels and neuronophagia. In animals with the hepatotoxic syndrome, livers will be swollen and a diffuse yellow-brown color. Irregular nodules and pale foci can be seen in hepatic parenchyma. (Dr. Steve Hooser, Dr. Duane Murphy 2003). Pathogenicity of *Fusarium* revealed that it causes hepatic congestion with mild triaditis, pulmonary congestion, and splenic lymphoid hyperplasia (Karki 2003). The Mycobiota of feed grain samples from the affected herd which revealed 6×10^6 - 11×10^6 cfu of

Penicillium spp fungus had a detrimental effect on health of these mules as similar situation has been reported by Stacey Oke, Susan Piscopo and K. M. Keller. Susan Piscopo reported the gross pathological change in tissue and presence of serosanguinous fluid in thoracic cavity of dead mule and acute sudden death was found to be similar to study carried out from the University of Illinois attempted to determine if horses purposely fed and injected with fumonisin develop increased serum sphingolipids and decreased cardiac function preceding neurological signs. The horses did develop cardiovascular problems, including decreased heart rate, heart contractility, and cardiac output. Increased concentrations of sphingolipids were detected in both serum and heart muscle, and all horses developed neurological signs consistent with leukoencephalomalacia. The authors speculate that decreased cardiac function might have caused uncontrolled dilation of blood vessels in the brain, causing swelling, increased intracranial pressure, and brain damage. The finding of 60-110cfu of penicillium fungus in grain which were staple ration for these mules was above the limit safe for equine species. K. M. Keller¹, B. D. Queiroz¹, L. A. M. Keller¹, J. M. M. Ribeiro¹, L. R. Cavaglieri², M. L. González Pereyra², A. M. Dalcero² and C. A. R. Rosa¹ reported that Feed contamination can lead to nutrient losses and detrimental effects on animal health and production. Feed mould counts ranged from $<1 \times 10^2$ to 1×10^5 cfu/g. The most frequent genus isolated was Aspergillus (40.54%), followed by Penicillium (18.38%) and Fusarium (16.22%) is suggestive to the cause of death of mule in these herd.

Dr. Steve Hooser, Dr. Duane Murphy in 2003 reported clinical signs of fumonisin poisoning in horses are usually related to liquefactive necrosis of the white matter of the brain and include progressive ataxia, depression, anorexia, delirium, aimless wandering, recumbency, coma and death. Death can occur from 12 hours – 1 week after onset of clinical signs. At necropsy, lesions in the cerebral cortex can range from none to multifocal areas of hemorrhage and necrosis, to the presence of large cavitations of liquefactive necrosis. Histologically, there may be diffuse vacuolization of hepatocytes, fatty degeneration, centrilobular necrosis with inflammatory cell infiltrate, bile duct proliferation, bile stasis, increased mitotic figures within the hepatocytes, or periportal fibrosis. Equine leukoencephalomalacia Histopathologically, there are multifocal areas of liquefactive necrosis within the cerebral cortex with infiltration of macrophages. Differential diagnoses should include rabies, equine encephalomyelitis, equine herpes virus, botulism, head trauma, hepato-encephalopathy, and bacterial meningoencephalitis. Clinical symptoms exhibited by mules and time period from onset of symptoms and death and periodic mortality pattern in herd and gross and histopathological changes of this findings broadly in agreement with above researchers. Further more a positive response to treatment with toxin binder, adaptogen, Immunomodulators further confirm the sudden death was due to moldy corn poisoning. As before this investigational intervention no attempt for treatment and control for moldy grain for equine species were made findings of this intervention need to followed up.

Conclusion:

As during monsoon season and post monsoon season sudden death of mules plying in the mountain and high mountain region for transporting the domestic and commercial good often reported which often goes unreported and recorded Findings of this investigation indicates that moldy feed grains and ingredients are infested with toxic fungus is emerging as a new health hazard for equine species. Simultaneous use of toxin binder, adaptogen like liver tonic, mineral mixture and Immunomodulators drugs promises to help in minimizing health risk in livestock and poultry production should be looked into. Further monitoring of finding of this investigation in other species is suggested.

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Aknwoledgement:

We would like to aknwoledg our sincier thanks to Dr.Rebati Man Shreshtha Chief Central Veterinary Laboratory,Tripureshwor ,Dr Sadanand Deo SVO and Chief Regional Veterinary Laboratory Biratnagar ,Dr Ganesh Raj Panta, SVO, Dr. Banshi Sharma, SVO, Dr. Salina Manandhar Dr.Vinaya Kumar Karna Dr. Keshab Prasad Sah,Dr. Shanker Yadav VO, Mr. Shankar Yadav Livestock Development Officer RLSD Biratnagar ,Mr.Gyan Bahadur Bogti,Mr.Bai Bahadur Kunwar,Mr.Ashok Shreshtha,Mr.Prakash Devkota Vet. Technicians of CVL and Officer and technical Staffs of DLSO Udayapur,Mr. Samir Gurung sales officer Polchem group in Nepal, Mr. Nishant Bhattarai sales representative of Varshagroup in Nepal and members and office bearer of Mule Transport entrepreneur of Udayapur for their respective help in conducting this investigation

PREVALENCE, ISOLATION AND ANTIBIOTIC RESISTANCE PATTERN OF SALMONELLA IN CHICKEN MEAT OF CHITWAN

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Abstract

A study of raw poultry meat samples from the local meat market of Chitwan was carried out during March 2008 to June 2008 with special emphasis on isolation and identification of *Salmonella* and their drug resistant pattern. A total of 40 raw meat samples were collected and analyzed. *Salmonella* spp. was found in 22.5% out of 40 meat samples.

Among those isolated salmonella, Gentamycin and Ciprofloxacin was highly sensitive drug and tetracycline was found to be resistant drug. Out of 9 salmonella isolate 55.55% were resistant to nitrofurantoin and cephalosixin

.About 55.5% was highly sensitive to sulphaphenazole. 22.2% were highly sensitive to tetracycline and 77.8% were resistant to it. From those isolated salmonella, 66.6 % were highly sensitive, 22.2% were resistant and 11.11% were moderately sensitive to cotrimaxazole.

INTRODUCTION

1.1 Background

Meat is a delicious and energetic food for most of the people and is a good source of protein, vitamins, minerals especially iron. Poultry meat is a leading source of animal protein for human consumption in many countries. The local poultry industry provides more than 80% of the total poultry used by consumers. Along with this growth, poultry meat has been increasingly implicated in food-borne illness. Meat is also a good source for the growth of micro-organism. In the advent of public health hazard salmonella has been one of the top names in Nepalese context. Therefore, isolation of *Salmonella* is done in poultry products. Estimates of the incidence of *Salmonella* in poultry meat and poultry products vary considerably.

In Nepal almost 50% of 23.02 million poultry, birds are found in central region which are predominantly commercial. The annual growth in commercial chicken eggs and meat production were estimated 10.6% and 18.3% respectively up to year 2001 (Shrestha *et al.*, 2005). In Nepal, commercial poultry contributes 8% of AGDP and 4% of national GDP (Karki, 2005). Emergence of high yielding breeds' gradual development in management and disease control measures has all made poultry a large segment of agribusiness in Nepal. The common broiler breed available in Chitwan is Vencobb, Arboracre, Hubchics, Avian 34, Marshal etc. Similarly the layer breeds in Chitwan are Hyline Brown, Lohman Brown, Babcock, Isa Brown, Keystone Brown etc (Thapa, 2005).

Salmonellosis has caused a burning problem for poultry raiser as well as consumers. Since it is a zoonotic and egg borne disease, has a great importance in transmission of disease in the country. Poultry are a common source and, in recent years, much attention has been focused in determining the prevalence of *Salmonella* during the different stages in the poultry production chain. Poultry are commonly infected with *S. pullorum* and *S. gallinarum* and the organisms are host-specific.

Salmonella spp. is among the most common causes of human bacterial gastroenteritis worldwide, and food animals are important reservoirs of the bacteria. In recent years, an increase in the occurrence of antimicrobial drug-resistant *Salmonella* spp. has been

observed in several countries. Fatality rates are higher for patients with infections caused by drug-resistant *Salmonella* resistance increased to a number of commonly used antibiotics, possibly related to overuse of antibiotic.

The antibiotic does not technically cause the resistance, but allows it to happen by creating a situation where an already existing variant can flourish. "Whenever antibiotics are used, there is selective pressure for resistance to occur. It builds upon itself. More and more organisms develop resistance to more and more drugs," says Joe Cranston, Ph.D., director of the department of drug policy and standards at the American Medical Association in Chicago.

The extensive use of antimicrobials in human and animals has led to an increase in bacterial multi-drug resistant among several bacterial strains. This phenomenon of multiple resistances represents a worldwide problem both for veterinary and public health sectors. Bacterial resistance is observed especially when the antibiotics are abundantly used and that the bacteria can be transmitted easily between the individuals. Various antimicrobials in intensively managed food animals including chickens are often administered through feed or drinking water either for therapy, prophylaxis or growth promotion. *Salmonella* sp is one of the most frequently isolated bacteria in avian production units. The increasing single and multiple antimicrobial-resistant *Salmonella* strains isolated from human cases of salmonellosis have been associated with widespread use of antimicrobial agents in food animal production. This may represent a public health risk by transfer of resistant *salmonella* strains to humans through the consumption of contaminated food and food products

1.2 Rationale of study

A food-borne disease caused by salmonella is an important public health problem worldwide. Most salmonella infections in humans result from the ingestion of contaminated poultry meat. Salmonellosis is still prevalent around the world and is a global issue due to its impact on public health. And the use of antibiotic in any environment creates selection pressures that favor the survival of antibiotic-resistant pathogens which will fulfill the incidence antibiotic resistance profile in local condition. So, a study revealing microbiological aspect of such samples would help control health hazards via public awareness.

1.3 Objective

General objective

To determine the prevalence, isolation, identification of *Salmonella* in chicken meat of Chitwan and antibiotic drug resistant pattern of isolated salmonella species.

Specific objective

- To determine the prevalence of *Salmonella* in chicken meat of Chitwan
- Isolation, identification and biochemical test for *Salmonella*.
- To determine the antibiotic drug resistant pattern of isolated salmonella species.

1.4 Scope of study

This research is mainly focus to determine prevalence of salmonella in market meat and hygienic status of meat. This study shows the contamination of salmonella in meat and its impact on human consumption.

1.5 Limitation of study

This study is carried out within limited time period within Bharatpur municipality.

2. Literatures Review

The genus salmonella of the family Enterobacteriaceae, named for USDA veterinary bacteriologist Daniel E. Salmon (1850-1914), consist of more than 2400 serologically distinguishable variants.

Salmonella spp are one of the major bacterial causes of food-borne gastroenteritis. Bacteria of the species *salmonella* are normal inhabitant of the digestive tract of birds and other animals and most strain do not produce disease to those animals. The majority of infected animals become sub-clinical excretors. However, salmonella can survive for 9 month or more in environment in sites such as moist soil, water, fecal particles, animal feeds and animal product. The isolation of salmonella is reported more often from poultry and poultry product than from any other source, this result is due to high prevalence of salmonella infection in poultry.

P. Manandhar (2006) reported 11.4% (14/123) meat samples were positive for *Salmonella*. Eight samples of chicken, that is, 14.5%, five samples of buffalo (13.5%), and one sample of goat (3.3%) were found to be positive for *Salmonella*.

Out of 652 PM sample *Salmonella* spp was isolated from 204 sample (annual report 1987/88 AHRD, NARC).

30% out of 190 bird showed positive for salmonellosis (S. Shrestha, 2005). and 8% salmonella out of 61 meat samples was reported by Rima Devi Shrestha (2002).

9.3% salmonellosis was reported out of 525 PM cases on fiscal year 2063/64 (NAL annual report).

Acharya (2007) had conducted isolation of *Salmonella* from 116 poultry meat samples. He found that 38 samples (32.76%) were *Salmonella* +ve. The positive percent was 26.09% for Chitwan Valley.

Dhakal and Manandhar (2005) had reported out of 751 samples tested 12% were positive for salmonella, among them 59% were public health importance salmonella.

Salmonella was isolated from 67 (6.8%) of the total samples examined over the period under consideration. The contamination rates were 3.3%, 6.1%, 17.5%, and 51.6% in day-old chicks, raw chicken breast meat, crops and livers respectively.

The overall resistance magnitude regressed from 9.2% in 1994 to 8.2% in 1997. Resistance to Streptomycin (30.6% of 3897 strains), tetracycline (27.3%), and sulfisoxazole (23.6%) was highest.

A high frequency of resistance to antimicrobial agents including tetracycline (85%), ampicillin (47%), co-amoxiclav (23%) and chloramphenicol (21%) was detected.

The antibiotics to which isolated *Salmonella* strains were most often fully resistant included nitrofurantoin (48.7%), furazolidone (48.7%) and streptomycin (46.2%) (Molla B, 1999).

S. gallinarum was highly sensitive to Chloramphenicol and Cephalexin. Erythromycin and nitrofurazone were moderately sensitive and penicillin, Amicillin, tetracycline and gentamycin were drug resistant. (Annual report, 1996/97, AHRD, NARC).

A total of 435 meat samples (236 from the slaughterhouse, 199 from retailers) were tested. Among them, 275 (63%) were positive for *Salmonella*, 43% (101/236) from the slaughterhouse and 87% (174/199) from the retailers. Resistance rates were lower to streptomycin (22%), sulfamethoxazole (15%), spectinomycin (1%), chloramphenicol (1%), and tetracycline (0.4%) while low-level resistance to quinolones was detected.

Highest resistance was to tetracycline (45%), streptomycin (41%), sulfisoxazole (19%), gentamicin (10%), and trimethoprim/sulfamethoxazole (8%) out of 104 isolated salmonella.

2.1 Morphology

Salmonella measure 2-4µm in length and 0.5µm in width. Most species except one serotype comprising *salmonella gallinarum* and *S. pullorum* are motile due to peritrichus flagella. Many of them are fimbriate and some are capsulated and mucoid colonies. The amount of capsular material varies enormously from strain. Spore formation doesnot take place. The organism is Gram negative.

2.2 Cultural characteristics

Salmonellae are aerobic and facultative anaerobe and grow well on ordinary media without the addition of blood and serum at 37 °C. On agar surface, colonies of the organism are grayish, smooth round measuring 2-3 µm in diameter. In broth the organism produce uniform turbidity after overnight incubation.

The salmonellae commonly exist in the intestines where other related organisms are present and sometimes the number of bacteria may be low. For reliable isolation the use of enrichment and selective media have been devised which enhance the growth of salmonellae and inhibit the growth of other enteric organisms.

Selenite broth and tetratheonate broth with the addition of **0.215 mg per ml of sulphathiazole** are enrichment media which inhibit the growth of other enteric organisms. The solid selective media are SS agar, bismuth sulfite agar, deoxycholate citrate, Mac Conkey brilliant green agar and brilliant green-phenol red agar. Preliminary cultivation in enrichment media followed by selective media has proved more reliable than direct plating on selective media. The material from the intestine is inoculated in selenite broth and incubated at 43 °C for 24hours, subculturing is done on brilliant green agar and incubating at 37 °C. The Selenite broth is further incubated at 43° C for 24 hours and subcultures are made on brilliant green agar plates.

Salmonellae do not ferment lactose and sucrose. They usually produce H₂S. The organism is easily killed at 60°C in 20 minutes and destroyed by chemical disinfectants but are resistant to certain chemical like brilliant green, tetratheonate and sodium deoxycholate.

2.3 Biochemical properties

Table 9: Biochemical properties of *Salmonella*.

Tests	<i>Salmonella</i>	Test	<i>salmonella</i>
Indole (I)	-	Methyl red (M)	+
Voges-proskauer (Vi)	-	Citrate (C)	+
Urease	-	Ornithodecarboxylase	+
Lysine decarboxylase	+	H ₂ S	+
Catalase	+	Oxidase	-
Motility	+	Mucoid colony	-
Acid from lactose	-	Maltose	+
Mannose	+	Mannitol	+
Rhamnose	+	Sorbitol	+
Sucrose	-	Xylose	+
Dulcitol	+	Inisitol	d

+ = 90-100% strains positive, d = 26-75% positive, - = 0-10% positive. test read after 48hrs at 37°C

3. RESEARCH METHODOLOGY

This study was carried out from March 21 to June 13, 2008 at bacteriology lab, NAL.

3.1 Site of sample collection

Sample were collected from different retail poultry meat shop of Narayangard and Bharatpur municipality, Chitwan for this study

3.2 Sample size:

40 sample were collected for the isolation of bacteria.

3.3 Laboratory works:

Bacterial examination of meat samples and antibiotics resistance pattern of salmonella isolates was conducted in bacteriology lab at NAL Bharatpur.

3.4 Sample Examination

Collection of sample:

Meat sample was collected in a sterile polybags from different shops and transported to the bacteriology lab within 2 hours, stored in refrigerator and examined within two hours.

Pre enrichment of sample:

The 1gm of sample was inoculated in the tube containing double concentrated lactose broth and the tubes were incubated at 37°C for 24 hrs.

Enrichment of sample:

1 ml of pre enriched culture was transferred to the 9ml of Selenite F broth and reincubated for 24 hrs at 37°C.

Primary culture in Selective media:

From selenite broth, a loopful of enriched inoculate was streaked in Brilliant Green Agar(BGA) and SS Agar. The culture plates were then incubated for 24 hrs at 37°C.

Pure Culture:

Salmonella suspected colonies in BGA and SS agar were picked up with sterile loop and inoculated in Nutrient agar to obtain pure culture. These culture plates were then incubated for 24 hrs at 37°C.

Biochemical test for identification of *Salmonella*

Table 10 :The following test were done for the confirmation of *salmonella* .

Tests	Reagent	Reaction of Salmonella
Morphology	Grams staining	Gram -ve, rod
Catalase test	3% H ₂ O ₂	+ve
Oxidase test	1-tetramethyl-p-paraphenylenedihydrochloride	-ve
KOH test	3%KOH	+ve
Citrate utilization	Bromothymol blue	+ve
MIO test		
TSI test	TSI agar	+ve

OF test	+ve
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Storage of culture

The culture was kept in the nutrient broth for 2 hrs in incubator for further examination.

Assessment of antibiotic resistant

The nutrient broth containing culture was plated on the Mueller Hilton Agar and the different antibiotic disc were plated on MH agar and then incubated for 24 hrs at 37°C . After 24 hrs the zone of inhibition was examined. The antibiotic disc used were gentamycin(G₁₀),Tetracyclin(T₃₀), Nitrofuratoin(Nf), colistin (Cl), Co-Trimoxazole (Co), Cephalexin(Cp),Sulphaphenazole(Sp), Ciprofloxacin(Cf).

RESULTS

Out of total 40 samples, 9 samples were found positive for *salmonella Spp*. That means about 22.5% were positive for *Salmonella spp*. Among those isolated salmonella, Gentamycin and Ciprofloxacin was highly sensitive drug and 55.55% were resistant to nitrofuratoin and Cephalexin. 55.5% were highly sensitive to sulphaphenazole. 22.2% were highly sensitive to tetracycline and 77.8% were resistant to it. 66.6 % were highly sensitive, 22.2% were resistant and 11.11% were moderately sensitive to co-trimaxazole.

Table 11. Result of drug sensitivity

Drug	Highly sensitive	Moderately sensitive	Resistant
Gentamycin	9/9 (100%)	-	--
Tetracyclin	2/9(22.2%)	-	7/9(77.8%)
Sulphaphenazole	5/9(55.5%)	2/9(22.2%)	1/9(11.1%)
Nitrofuration	1/9(11.11%)	3/9(33.3%)	5/9(55.5%)
Cephalexin	-	4/9(44.4%)	5/9(55.5%)
Cotrimaxazole	6/9(66.6%)	1/9(11.1%)	2/9 (22.2%)
Colistin			
Ciprofloxacin	8/9(88.8%)	1/9(11.1%)	-

DISCUSSION

In this study, it was examined *Salmonella* isolates from retail meats in Chitwan and to determine their antibiotic resistant pattern. In general, findings are similar to those studies showing that *Salmonella* isolates in retail meats are commonly resistant to multiple antimicrobials, including tetracycline, sulfamethoxazole, and streptomycin. *Salmonella* strains isolated from retail meats in Chitwan. Further studies involving larger sample sizes are necessary to more precisely determine if there are differences in antimicrobial resistance between *Salmonella* isolates.

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Investigation of Infertility in cattle of Eastern Development Region of Nepal

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Abstract:

A study was conducted, from March to May 2007, at Ramgunj Belgachhiya VDC of Sunsari district, Nepal to investigate the infertile cows domesticated in rural managemental system. On the basis of gynaecological examination and other necessary information's, 59 anestrus cows were selected to assess Hemoglobin, total protein and phosphorus on 0, 21 and 42 day of study. Values of Hemoglobin, total protein and phosphorus were found to be below the normal on 0 day. Altogether 76.2% animals were found to be badly infested with endoparasites. Medication was done to deworm first followed by mineral supplementation. After mineral mixture supplementation, there were remarkable improvement ovarian conditions. As a result, 67.8% anestrus animals exhibited the symptoms of estrus. There was marked improvement in the blood /serum level of these constituents after mineral supplementation.

Introduction:

In Nepal infertility problem in crossed bred and exotic cattle has been reported to be the most prioritized problem in dairy pockets area in the country (Jha, 2000). A study conducted in Kathmandu valley in improved cattle revealed that among the various reproductive disorders the proportions of anestrus, repeat breeding and abortion was 45%, 27% and 5% respectively (Khanal, 1996). Reproductive disorders such as anestrus and repeat breeding were reported to be 21% and 36% respectively in cows and heifers in Pokhara valley (Sankhi, 1999).

It is generally believed that a crossed bred heifer raised under a very good management condition receiving optimum nutrition and health coverage can conceive at the age between 12 and 18 months depending upon the genetic constitution of indigenous and exotic blood. Delay in establishment of conception in such heifers affects the life time production and economy. Casteneda et. al (1973) reported that if a heifer does not conceive by 720 days of age, she should be culled on economic ground.

There are many causes of infertility, among them it may be broadly classified as infectious and non-infectious. In non-infectious causes, there might be low energy intake, postpartum loss of weight, deficiency of major trace minerals and vitamins. Infertility due to nutritional causes is usually characterized by failure of estrus or cessation of estrus cycle, prolonged postpartum anestrus, silent or weak estrus and repeat breeding are the major problems of animal husbandry (Kumar et. al, 1988). Breeding efficiency of indigenous stock can be improved by better feeding, management and health care.

Delayed puberty and post partum anestrus conditions are common reproductive disorders encountered in rural cows leading to prolonged inter-calving period, reduced milk production and thus affecting the economy of poor farmers. A low plan of nutrition due to lack of sufficient protein and other necessary elements to maintain body weight may cause failure or delay in onset of puberty or onset of estrus cycle following parturition. This condition is mostly seen in heifers maintained on straw or on poor pasture.

The problem of anestrus has been recognized to occur from moderate to high incidence affecting the fertility of the animals and economy of the farmer (Rao; 1972, Kodagali; 1975, Luktuke; 1976; Nayak; 1987).

Anestrus is a multifactorial syndrome, which includes the factors like age, breed, environment, nutritional status, management practices, and general disease problems. Malnutrition influences the reproductive performance. As a result, there may be delay in

puberty in heifers and a prolongation in the post –partum anoestrus condition in cows. In the rural areas of Nepal, dairy cattle are usually maintained on agricultural wastes products with insufficient concentrates and fodder under the backyard system of management.

Concentrates and roughages are needed in a good ratio for proper development, productivity and fecundity of animals. Tree fodder is one of the important sources of animal feed for winter and dry summer. However, good grassland and pasture land are rare and concentrate feeding is very costly in Nepal. Fodder trees are not found sufficiently in most areas, except at some places in the hills. Over exploitation of natural forest and fodder tree has resulted in considerable decline of tree cover and farm productivity in general. Although different kinds of fodder are fed traditionally in many parts of the country, on an average daily deficit fodder is estimated to be about 0.8 kg / animal (Maharjan, 1987). Fodder and feed scarcity becomes the major problems of livestock production (Barakoti, et al, 2000).

The basic ingredient of the most of the ruminant diet in Nepal is straw, which are more seriously deficient in protein and other nutrients. It also contains oxalates, which, in body, bind with the available Ca causing its deficiency leading to Ca and P ratio imbalances.

As a result, marginal deficiency of macro and micro elements are likely to affect fertility adversely without manifesting specific deficiency symptoms.

Lukute and Sharma (1978) examined 47 heifers and 86 cows of rural Bareilly districts of India and found 36.1% heifers and 43% cows had inactive ovaries which could be considered as a case of true anestrus. Naidu and Rao (1981) recorded higher incidence of anestrus in crossbred cows (14%) as compare to heifers (12.37%).

Changes in hormonal and biochemical profile are responsible for the anestrus condition. Biochemical profile can indicate the nutritional status of the animal and thus will further help in diagnosis and management of anestrus. There is no denying that minerals, vitamins and trace elements play an integral role in the action of hormones and enzyme at cellular level. Thus elements under influence of certain enzymes system have been found to definitely affect the reproductive efficiency of the animals.

According to Blood et. al. (1983), the normal values of the Hb, total protein and phosphorus in cattle are 11gm%, 5.7gm/dl and 4-7mg/dl respectively. All the above nutritional elements being recognized to have important roles in regulation of reproduction cycle in animal.

Moddie 1965, Prasad et al 1984 have reported that certain biochemical constituents in blood serum affect fertility status of cow and their reproductive behaviors. Deficiency of various biochemical constituents have been associated with fertility status of the animals (Mc Clure, 1965) The present study was under taken to find out the levels of certain biochemical constituents during fertile and non- fertile period of rural cows.

Objectives:

- To assess the nutritional status of infertile animals.
- To evaluate the response of vitamin – mineral supplementation.

Material and methods:

A study was conducted, during the period of March to May 2007, in Ramgunj Belgachhiya VDC of Sunsari district to investigate the infertile cattle. Altogether 59 animals (heifer with delayed puberty, Post partum anestrus, and repeat breeder), were selected for the investigation for the cause of infertility.

A detail survey was made based on a questionnaire sheet by direct interview the farmers. Breeding history, feeding of fodder and forage, milk yield, body condition score and other necessary information's of the animals were recorded

Each anestrus animals were examined to determine the conditions of ovaries, uterus, cervix and vagina. Fecal examination of all the selected animals was done to know the parasitic infestation in the area.

To serve as control, 10 apparently healthy and normal cyclic cows having free from reproductive disorders were also selected.

Nutritional assessment of all anestrus and control animals were done. For this, blood was collected from jugular vein under aseptic conditions for the estimation of Hb and separation of serum. The serum was then stored at -20 degree centigrade for biochemical studies. Estimation of Hb, Total Protein (TP) and phosphorus was done following the directions of manufacturers provided with the specific kits.

All the infertile cattle were dewormed with anthelmintic named Oxyzan-L (Oxyclozanide-1gm, levamisole-500gm) of Nucare Company, India. The animals were also supplemented with Kalferol boli (Ca, P, Vitamin B12 and D3, Nucare Company, India) @ 2 boli daily for 10 days and Numin forte (mineral mixture) @ 25gm once daily for 40 days.

Farmers were advised to increase the green fodder and include concentrate ration in the feed on regular basis. Common salt and locally available mustard cake (as a protein source) was also included in their diet.

Again on 21st and 42nd days, gynaeco-clinical examination along with blood collection for nutritional assessment was done. Re- evaluation and follow up of selected animals was done. Animals that exhibited the symptoms of estrus were inseminated.

Result and discussion:

Age and type of animal:

Out of selected 59 animals, maximum animals were heifer of age group 2.5-4 years. There were 42 (71.1%) heifers with delayed puberty followed by 10 (16.9%) post partum anestrus and 7 (11.8%) repeat breeding cattle. The age wise distributions of animals are presented in figure 1.

Fig.1:Age-wise distribution of cattle

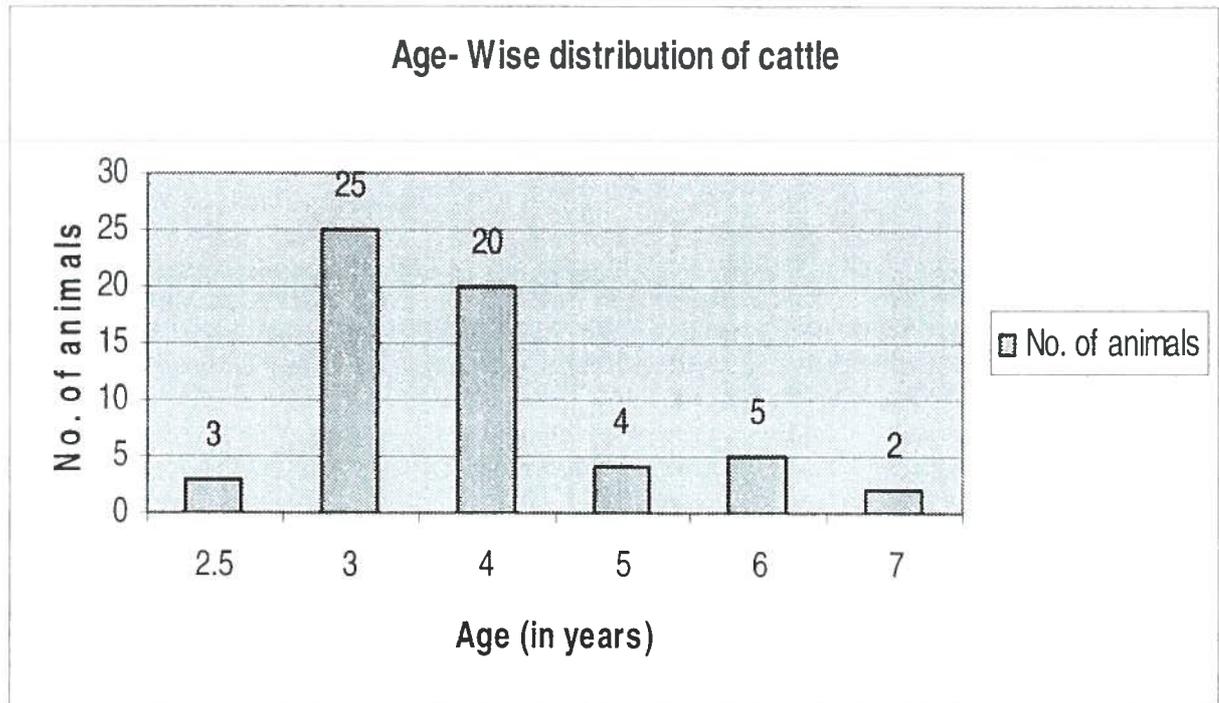


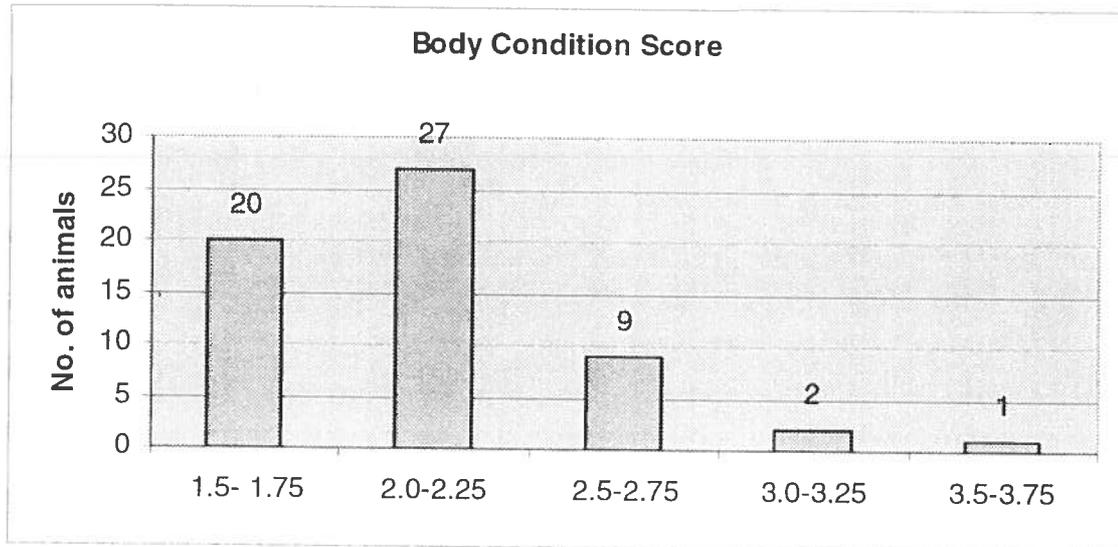
Table1: Distribution of animals based on its type

Type of animals	No. of animals
Heifers with delayed puberty	42
Post partum anestrous	10
Repeat breeding	7

Body Condition Score (BCS):

Figure 2 shows the body score condition of animals on 0 day of study. Altogether 47 (79.66%) of animals were with poor body condition (BCS less than 2.25). The animals were thin and poor weight with rough body coat.

According to Hafez (1993), the onset of puberty is more closely related to the body weight than to age. Dairy cattle reach puberty when the body weight is 30 to 40% of adult weight. Nutritional level modulates age at puberty. If growth is accelerated by over feeding, the animals reach puberty at younger age. When the energy intake in adult is low, follicles fail to develop to maturity and follicular atresia result along with loss of sexual desire anestrous.

Figure 2: Body Condition Score on day 0**Ovary:**

On rectal examination, the ovaries of infertile cattle were found to be small, quiescent and usually flat and smooth, especially in heifers. Altogether, 43 animals were examined to have small and smooth ovary. Growing follicles and palpable CL was found in 7 and 9 animals respectively.

Cervix:

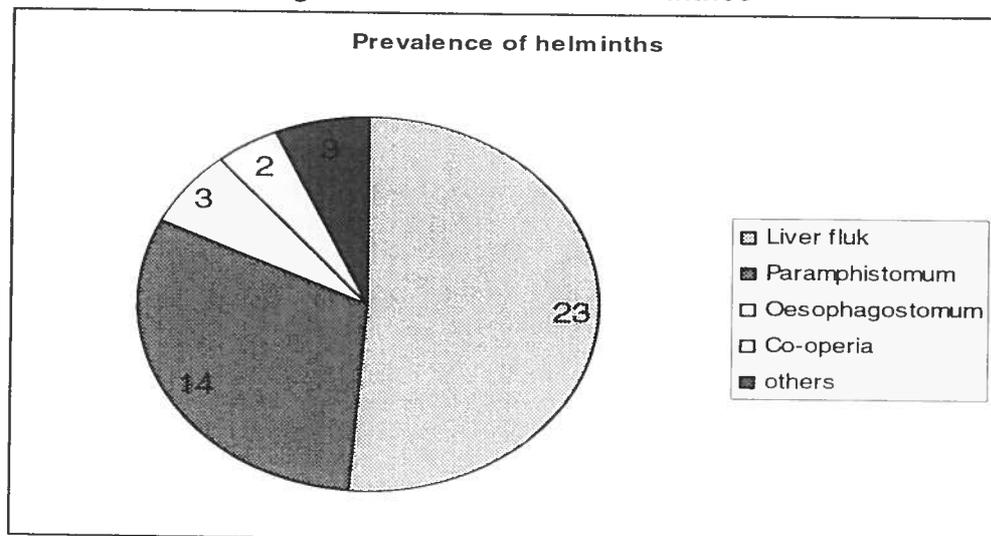
Cervix of most of the animals was found to be normal. In five infertile cattle (8.5%), the cervical condition was examined as cervicitis.

Uterus

No animal was found to have uterine disorder or uterus.

Helminthes infestation:

A detail of fecal examination result has been mentioned in figure 3. Fecal examination result shows that 76.2% animals were found to be badly infested with helminthes. Out of them, 51 % cases had infestation of Fasciola followed by Paramphistomum (31.1%) and Round worms (17.8%).

Figure3: Prevalence of Helminthes

Assessment of biochemical profile on day 0:

In the present study, the average value of haemoglobin (Hb) in anestrus cows on day 0 of study ranged between 6.8 to 9.0 gm % with an average value of 7.8 gm %, which is in accordance with the statement of Hansel, who reported Hb level below 9gm% in all anestrus cows.

However, Awasthi and Kharache (1987) did not find any differences between Hb level in normal and infertile group of cows. Apart from Hb, Total Protein (TP), Phosphorus (P) was also assessed.

The values of Hb, P and TP in the blood / serum of anestrus cows on day 0 along with control animals are presented in the table below:-

Table2: Blood bio chemical profile on day 0

Constituents Level	Hb gm %			Total Protein (TP) Gm%			Phosphorus(P)		
	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max
Normal Values	8.0	11	15	5.7	6.9	8.1	4.0	5.5	7.0
Control animals	8.0	9.5	11	5.5	7.6	9.8	4.3	6.1	7.9
Anestrus cows (0day)	6.8	7.8	9.0	3.8	4.9	6.4	2.0	3.7	6.5

The average value of total serum protein in anoestrus cattle on day 0 ranged between 3.8 to 6.4 gm %. These values of total protein are nearer to the value reported by Samad et.al (1980), sharma et.al (1984), Kavani et.al (1987) and Baruah et.al (1988).

Patil and Deshpande (1979) reported that in anestrus cows, the total serum protein was low as compared to those exhibiting estrus. In control animals, average value of total serum protein was 7.6 gm%. However, high level of protein in the diet of dairy cows has been shown to increase the incidence of anestrus (Gould, 1969).

Low protein level was recorded in the anestrus animals. It was due to the fact that animals had higher infestation of parasites. They were also kept on marginal level of feed containing waste materials and paddy straw.

Role of protein in reproduction is obscure and its deficiency in usual condition is uncommon. Its deficiency is frequently seen in animals having malnourished condition, parasitic condition and lactation. Low intake of protein therefore will put the animals in negative energy balance leading to delayed growth and puberty.

According to Arthur (1989), cows that are in negative energy balance during early lactation are more at risk of becoming anestrus than which are in energy equilibrium.

The average value of serum phosphorus in anestrus cows, on 0 day of study, was from 2.0 to 6.5 with average value of 3.7mg/dl. This value is in consistent with Morris (1976), who suggested serum P level less than 4 mg/dl in affected animals. Like wise, Bansal et. al, (1978) reported that anestrus animals had low level of P in blood serum. The average value in control animals was ranged between 4.3 to 7.9 mg/dl; where as, normal values of inorganic P reported was 4.0 to 7.0 mg/dl (Blood et.al; 1983).

According to Wagner and Morrow, blood level below 3mg /dl usually indicates phosphorus deficiency. Failure of estrus with non- functional or smooth ovaries is observed during the winter months in cows on poor or inadequate amount of feed.

Asdell suggested that P requirement for reproduction is about 10-12 gm daily except during lactation when an additional amount is required.

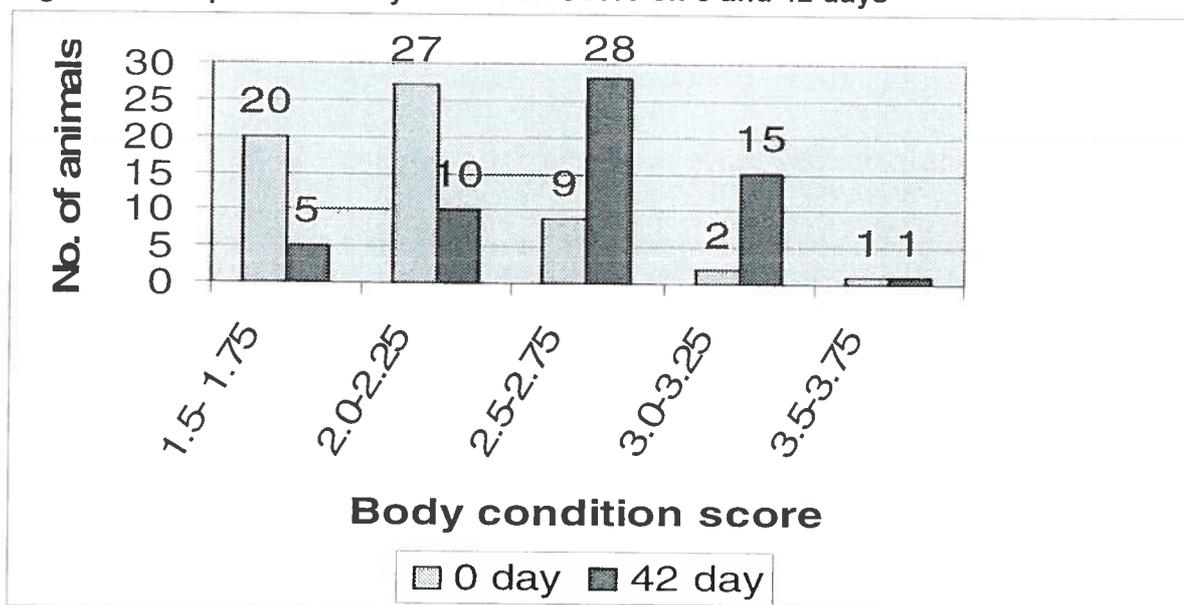
Above finding shows the importance of inorganic phosphorus in reproduction leading to delay in puberty and occurrence of post partum anestrous. Diagnosis of P is only possible by analyzing the blood serum because in most of cases, P deficiency occurred with no clinical sign of aphosphoresis such as lameness, rough coat, hoof abnormalities as described by Blood and Henderson (1968). Deficiency of P usually tends to occur due to poor or low protein intake and P deficient soil.

Mineral Mixture therapy and its effect:

Out of 59 infertile cows supplemented with mineral mixture for 40 days, 40 cows (67.8%) exhibited estrous. Dabas et.al (1987) treated anestrous animals with 60 gm of Nuvimine forte (mineral mixture) for 15 days and observed 75% cows in normal heat. Singh and Vednere (1987) observed 72.22% of induced estrous in cows with sodium phosphate treatment. The difference in findings in present study might be due to feeding, age, climate and managerial conditions.

There was marked improvement in body condition score during the period of 0 day and 42 day. As **figure 4** indicates that on 0 day, 47 (80%) of animals had BCS below 2.25. However, on 42nd day of study it was found that 44 (74.5%) animal showed improvement in their body condition (BCS more than 2.5).

Figure 4: Comparative Body Condition Score on 0 and 42 days



It may be due to the fact that there was mineral mixture supplementation to the animals for 40 days. Farmers were also advised to increase the daily fodder supply to the animals along with concentrate feed of protein source. There was regular supply of 10 gm common salt per animal per day, which also played the positive response to them.

Bellows et.al (1976) reported that estrum did not return in post partum lactating cow until salt was supplied in diet and mentioned that there was improvement in appetite, nutritive state and body condition after supplementation of salt.

Effect of mineral mixture on blood profile:

During the study period, nutritional assessment was done on days 0, 21st, 42nd. Increasing trend of blood level of Hb, TP, P was observed from 0 to 42nd day. Before treatment, the average value of Hb, TP, and P in anestrous animals was 7.8 gm %, 4.9 gm %, 3.7mg/dl respectively.

On 21st day, the level of Hb, TP and P was 8.3 gm %, 5.7gm% and 4.8 mg/dl respectively. On 42nd day, the values of Hb, TP and P were 8.9gm%, 8.3 gm% and 6.0mg/dl respectively. Values of Hb, TP and P obtained after test of blood / serum have been summarized as below:

Table 3: Comparative blood constituent profile on 0, 21 & 42

Anestrous cows	Hb gm %			Total Protein (TP) gm%			Phosphorus(P)		
	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max
0 day	6.8	7.8	9.0	3.8	4.9	6.4	2.0	3.7	6.5
21st day	7.2	8.3	9.0	5.6	7.5	8.9	2.5	4.8	7.0
42 day	8.2	8.9	9.8	7.2	8.3	9.0	4.4	6.0	7.8

Figure 5: Comparative chart of Hb and TP on 0, 21, 42 day

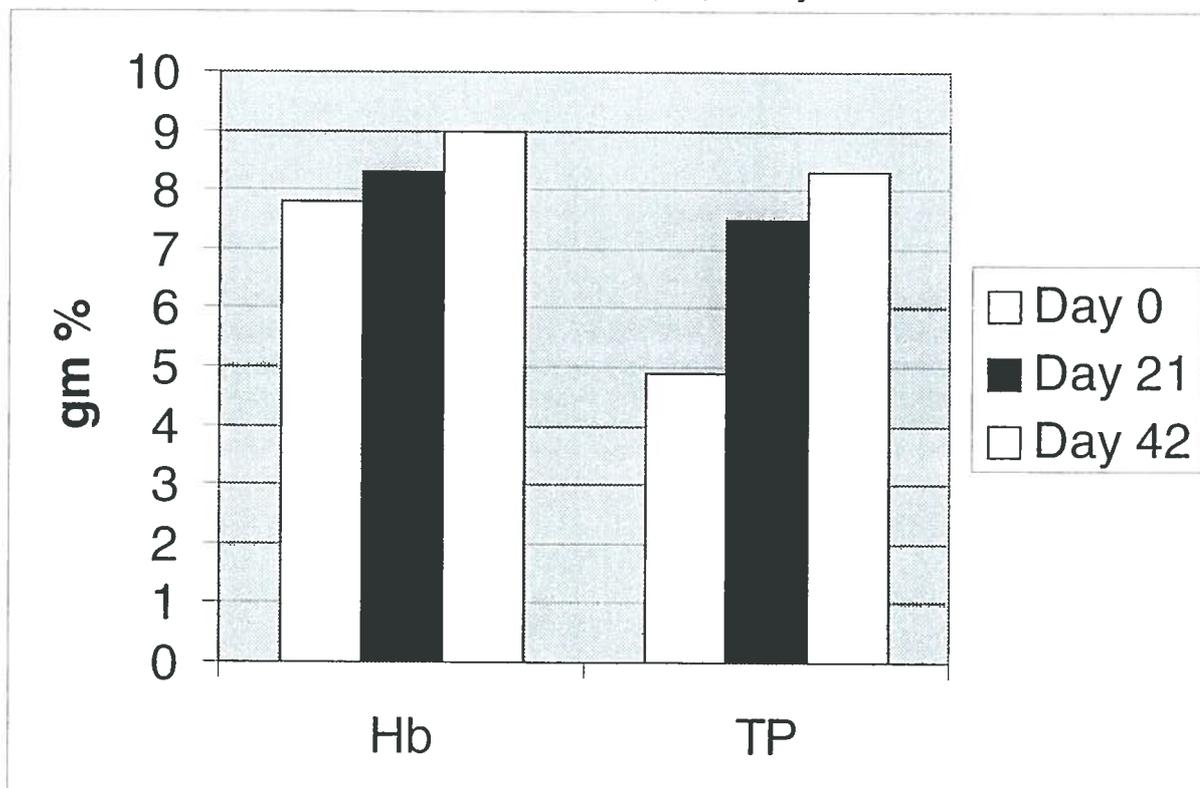
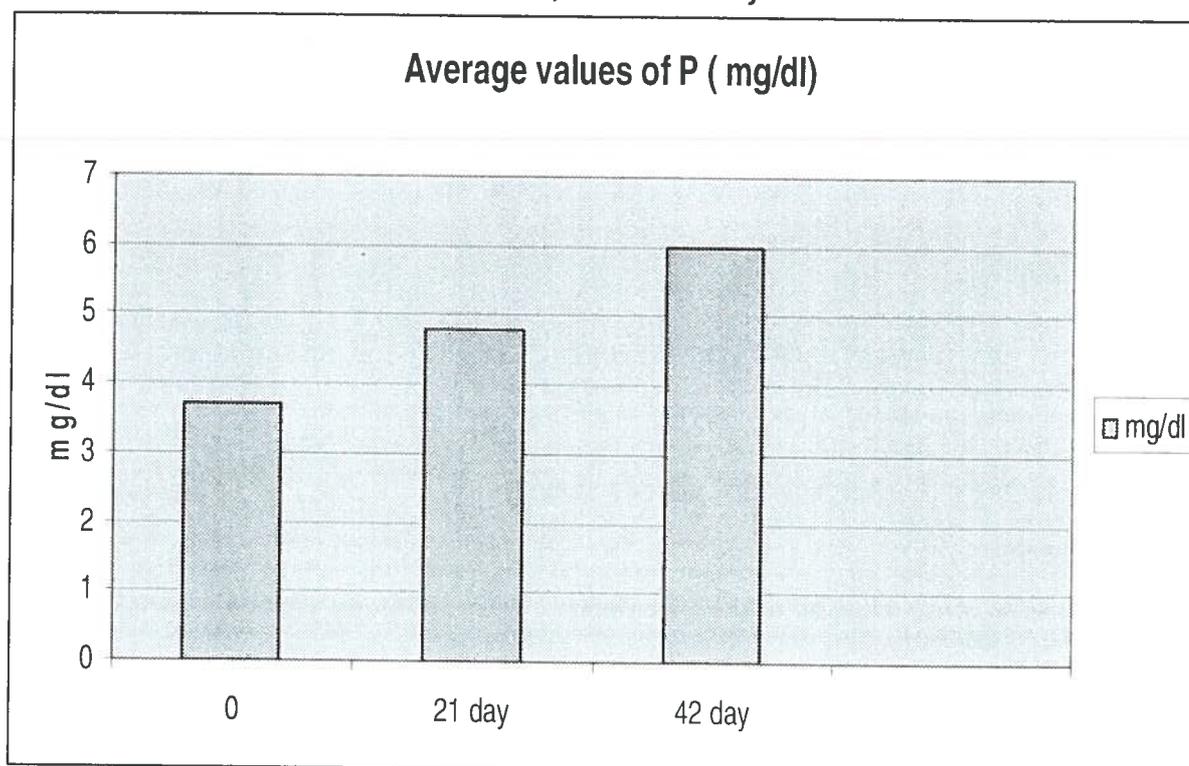


Figure 6: Comparative values of P on 0, 21 and 42 days.

**Effect of mineral mixture therapy on ovary:**

During the study period, all the anestrus animals were examined per-rectally to know the condition of ovaries as well as other genital organs on day 0, 21 and 42. On day 0 of study, small smooth and inactive ovaries were found in most of the heifer, while flat, non-functional ovaries with or without palpable corpus luteum were found in lactating animals.

The results are in accordance to the findings of several workers. Low level of serum protein has been reported to be associated with inactive ovaries (Roberts's et. al). Like wise, low level of serum protein has been reported in anestrus animals having inactive ovaries (Aminuddin et.al, 1984). Occurrence of ovarian dysfunction / ovarian activity at lower phosphorus levels were recorded by Hignett (1952).

On 21 and 42nd day of study, there was gradual improvement in ovarian condition of animal. As a result, during study period, altogether 67.8% animals exhibited signs of estrus. Details about the animals exhibiting estrus has been presented in table 4.

Table 4: Animals that Exhibited Estrous during study.

Duration	No. of animal that exhibited estrous			Total	%
	Heifer with delayed puberty	Post partum anestrus	Repeat breeder		
0-21	2	-	-	2	3.4
22-42	11	4	4	19	32.2
43-90	14	2	3	19	32.2
Total	27	6	7	40	67.8

Only 3.4% of heifer exhibited estrous within 21 days of mineral supplementation. Maximum numbers of animals exhibited estrous between 22 to 90 days of study. Out of 42 heifers, altogether 27 (64.28%) showed the symptoms of estrous. Out of 10 post partum and 7 repeat breeding cattle, 6 (85 %) post partum and 7 (100%) repeat breeding animals exhibited symptoms of heat. Comparatively low percentage estrous was observed in heifer due to the reason that most of heifers were malnourished, poor body condition score and badly infested by helminthes.

Conclusion:

Present study showed that majority of animals was suffering from endoparasitic conditions, anemia and mineral deficiency. After deworming and mineral mixture therapy, there was marked increase in the blood biochemical profile of several constituents. There was significant improvement in body condition of animals resulting in exhibition of estrous by maximum numbers of animals. Thus, it can be concluded that nutritional supplementation is necessary for the inducing estrous in infertile cattle.

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Situation of Khari Disease in Far Western Development Region (FWDR)

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Introduction:

Khari is a chronic debilitating disease principally affecting the buffaloes of the two districts of the far western region viz. Baitadi, and Darchula. While the disease was initially confined to buffalo population in these districts, recently it has been found to affect the cattle population as well. This disease earlier had been described as chronic debilitating disease particularly of stall-fed lactating buffaloes (Singh et.al 1996-97). Same authors have described the disease as seasonal affecting milch buffaloes during winter season reporting partial recovery of the animals during rainy season when the animals have access to plenty of green forages.

The disease is reported to have been in existence there for over 20-25 years, however, its cause could not be attributed to any known etiological factor so far. Locally the disease is also known by many other names such as "Chaukhari", Dhulkhule Khoche, etc. These names have been given since the diseased animal is affected on its hooves from where ash like dust/powder appears and the animal goes lame. The diseased animal progressively becomes weak, debilitated, show signs of lameness with development of furrow or groove on the pedal aspect of the hoof from which grayish ash like powder is shed, milk production in milking animals drop sharply to almost nil and the general body condition become so emaciated the animal becomes hide bound displaying all the bones and its protrusions. Diseased animal usually seen to stand with abducted elbows and the shoulders appear to have been pulled away from the thoracic region. Lesion of skin involves loss of hair, scale formation and extrusion of scaly dust. Psoroptes mites were encountered in 96.4% of skin scraping samples from diseased animals and these mites were incriminated to be one of the causes of the syndrome (Ratala et.al 1992).

Singh et.al (1995-96) observed severe nit (lice) infestation in animals infected with Khari disease. They also found psoroptes mites in skin scrapings. Singh et.al (1996-97) reported that the problem mostly confined in some of the high altitude areas of the districts (5000-6000 feet) where the winter green forage is very limited.

Prevalence of the disease for such a long period of time without any known disease entity has tremendously affected the livestock rearing practice in the region causing incalculable loss to the socio-economic condition of the farmers.

In order to address this serious problem an investigation programme was launched during the fiscal year 2061/62 in combined effort of Regional Veterinary Laboratory, Dhangadhi, and the Central Veterinary Laboratory, Tripureshwor. Khari disease investigation programme was designed as a project to last for three fiscal years from the starting year and continuity is given this year also.

At the end of last two years of the investigation and this year investigation we have been able to successfully complete the basic survey, tour and observation of the diseased animals in order to obtain basic epidemiological data concerning the disease and considerable number of samples has been collected from the diseased animals. A few salient features or findings on processing of the epidemiological data, and collected samples will be presented in the following Slides. These slides shows the situation of Khari diseases in FWDR and efforts that have been taken to investigate this diseases so far. Details will be shown in the flowing slide presentation.

Paragraphs while will discuss the results or the findings.

Objectives:

Short term objectives:

- To carryout epidemiological study of the disease that will assist in pointing out the etiology of the disease.
- To conduct drug trials on the diseased animal, which will provide immediate relief to the farmers apart from fulfilling the long term objective of developing cost effective therapeutic regime for the disease?
- To strengthen the diagnostic capability of the Regional Veterinary Laboratory (R.V.L), Dhangadhi.

Long term objectives:

- To find the etiology of the disease.
- To develop effective and reliable therapeutic regime and suggest possible control measures.

Epidemiological survey:

Preliminary survey of the disease situation was conducted using pre-structured questionnaire developed by the RVL, Dhangadhi. Epidemiological information was also collected during our visit for sample collection on separate occasions. It was so found that mostly the adult animals were commonly affected i.e. animals of the age 8.7 years and above were more frequently affected by the disease. But, occurrence of the disease in younger animals was not uncommon. Females were found to be affected more than the male of a species. However, occurrence of the disease in younger group of animals or male is not uncommon. The disease was recorded in a male buffalo calf aged 1 year and a 3 year old male calf during our survey. Though the incidence of the disease was found to be high in buffalo population i.e. 80.35 % of the total animals investigated were buffaloes, but, the disease was quite common in cattle also since it comprised 19.64% of the animals under investigation. Gongol 2004 reported 8% cases in cattle, 12% cattle or buffalos and 80% of cases in buffaloes.

This finding is greatly different from earlier observations and findings, which reported the disease condition only among adult milking buffalo population. The finding is presented in the table below:

Table: Epidemiological status of the Khari disease in Bovine Population

S/No.	Species	Sex		Total	Avg. age	Remarks
		Male	F			
1	Buffalo	1	44	45	8.7 year	
2	Cattle	1	10	11		

In contrary to the finding of Singh et.al (1996-97) which reported the disease as limited to the confined milking adult buffaloes considerable number of dry buffaloes including male and cattle were also found to be affected by the disease as is evident from the table above.

Result & Discussion:

Over hundred samples of different types were collected from among the khari disease affected animals of Darchula and Baitadi. The samples included EDTA Blood, Blood smear, Serum, Hoof dust, Skin scrapings, hay, grass, soil etc. Many of the samples were tested at the RVL and those tests which could not be performed at the RVL were sent to the CVL and other laboratories of the country. We had even approached the Indian Veterinary Research Institute (IVRI) in our attempt to broaden the sphere of laboratory examinations.

The details of various samples collected and their number is given in the table, which follows:

Table: Type and the number of samples collected

S/No.	Type of Sample	Number
1	EDTA Blood	32
2	Blood smear	62
3	Serum	119
4	Skin Scraping	20
5	Hoof dust	14
6	Soil	3
7	Hay	2
8	Fodder	1
9	Grass	4

Bacteriological Examinations:

S.N.	Khariaffected Species	Kind of sample	Media	Result
1.	Buffalo	Hoof scrape	Sabouard	Blastomyces sp. Candida sp.
2.	Buffalo	Hoof Scrape	Selective	Absidia

The above Bacteriological Examination Indicates that the hoof scrap contains the presence of Fungus. There was the evidence of systemic fungal infection too which might generalize the situation of the disease. However, it could be also suggested that the floor of the shed harbour fungus and it might have contaminated the hoof sample during collection. Since the shed where the animal was housed was dark without the provision of any ventilation.

Haematological Examination:

EDTA Blood was collected and blood smears prepared from the fresh blood of diseased animal and examined for various blood parameters. The details of the finding of the blood test is presented in the table below :

Table: Record of Haematological Test

S.No.	Test type	No.	Avg. value	Range	Remarks
1	Hb.	25	6.56 g/dl	5.2-8 gm/dl	
2	PCV	14	35%		
3	ESR	5	43.6 mm/hr.	28-65 mm/hr.	
4	Lymphocyte	36	44%	35%-67%	
5	Neutrophil	36	50%	26%-58%	
6	Monocyte	36	4%	2%-8%	
7	Eosinophil	36	2%		

Haemoglobin content of the animals was found to be below normal in these animals. The test for haemoglobin was carried out by using Shali's method. This invariably suggests that the animal are anaemic, which could have several reasons, i.e nutritional, parasitic, etc. However, the estimation of haemoglobin at the CVL with machine method gave a contrasting result, giving higher values of haemoglobin and well within the normal values, therefore, the values used here corresponds to the findings of the Regional Veterinary Laboratory, Dhangadhi. Since PCV estimation was done at the CVL with the machine it has normal values but it needs to be confirmed by Microhaematocrit method before any conclusion could be drawn. Other haematological pictures reflected rather normal, except, for the Erythrocyte Sedimentation Rate (ESR) which was recorded to be abnormally high. This simply represents that the disease is of chronic nature. But, it can be suggested to look in for presence of urinary problems, and or other neoplastic changes.

Due to lack of expertise, however, we were unable to record the normal/abnormal size of RBC, it's central pallor, the nature of leucocytes, etc. which could provide us with many valuable information regarding the exact etiology of anaemia in animals.

Biochemical Examination:

Biochemical examination of serum was mainly carried out to assess the micronutrient availability to the animals. Therefore, estimation for serum calcium, phosphorus, magnesium, zinc and total protein was done. Estimation of serum calcium level attempted at the RVL gave erroneous results ranging from low of 4.5 to high of 22 mg/dl, therefore, the result is not presented here for analysis unless we get it tested from a reliable laboratory.

Skin Scraping examination for Mange mites:

Skin scraping examination for the presence of mange mites has revealed that approximately 80% of the animals are affected by Sarcoptes mites which cause lesions to develop in their skin surface, resulting in scale formation, hair fall and excoriations. Out of the 20 skin scrapings tested for mites' infestation 16 were found positive. It is detailed in the table below:

Table: Result of skin scraping examination

S/No.	Animal spp.	No. of skin scrapings	Number positive	Identified organism
1	Buffalo	18	15	Sarcoptes mange
2	Cattle	2	1	Sarcoptes mange

Though this finding is consistent with the finding of Ratala and Singh (1993-94) and Singh et.al (1995-96) in so far as infestation of diseased animals with mange mites, however, it is contrary to our finding of sarcoptes mange as compared to their finding of psoroptes mange in their reports.

Thus it can be inferred that the skin lesion noticed in Khari affected animal can be attributed to the infestation of this mite, which may also to some extent contribute to anaemic condition of the animal. However, it cannot be the sole cause of the body condition that occurs in the animal or the cause of the foot lesion that is so common in the animals suffering from this particular disease.

Examination of Hay:

Hay (grass straw), which is locally known as Gajjo is the principal source of feed to the animals of these two districts during the lean period i.e October to May was examined physically, and chemically. It consisted of rough fibrous locally available grasses that were cut, and dried during September-October and stored by staking in the form of a tower as a main supply of feed to the animals during the winter season when there was shortage of feed to animals. It can however, be noted that Dabadghao & Shankarnarayan (1970) in India found the crude protein content of a Heteropogon community was 5 percent untreated and 5.8 percent when treated with nitrogen. The digestibility of the protein is however, low. The grass is reported to be palatable in the early vegetative stage, but unattractive as it matures (L.Beauv. ex Roem. and Schult) . The phosphorus figures as percentage of dry matter is reported to range from 0.09-0.15 which is far below the requirement of the dairy cattle, and the calcium content is barely sufficient, ranging from 0.23 to 0.30 as percentage of the dry matter.

A few grasses which constituted the Hay were also collected individually were sent to the Natural Products Research Laboratory for identification. The result obtained from there is presented in the table below:

Table: Result of identification of Grasses used to make hay

S.No.	Local Name	Scientific Name	Percentage composition in hay
1	Atharne	Heteropogon contortus	60%
2	Ghorade	Chrysopogon	30%
3	Babyo and others	Eulabopsis and others	10%

The same laboratory has also given the test of toxicity of the hay. It has reported that the alcohol extract of the hay used for feeding the livestock subjected to acute toxicity test on mice caused death of 25% of the mice at the rate of 500mg/kg body weight, whereas, no mortality was observed when administered at 300mg/kg body weight.

Hay sample was also forwarded to the Animal Nutrition Division of the Nepal Agriculture Research Council (NARC) for proximate analysis. The result obtained from there is given in the table below:

Table: Result of proximate Analysis of Hay and Grass samples

S/No.	Sample type	DM %	CP %	NDF %	ADF%	Lignin
1	Hay	88.63	6.14	82.69	67.13	9.17
2	Hay	86.15	11.05	56.29	55.19	29.31
3	Grass	65.87	4.06	62.68	55.15	9.76

The availability of crude protein in the straw used for feeding animals is very low as is evident from the result of proximate analysis given in the table above and the finding is consistent with the reports Dabadghao & Shankarnarayan (1970) in India for *Heteropogon contortus*.

Heteropogon contortus is identified as spear grass with stem 30-150 cm long. The root has a diuretic property and the awns injure the mouth and skin. Awn oil is given in asthma. The plant also contains Myo-inositol, galactinol and raffinose. It can be a good hay if cut before flowering containing DM 93%, CP 6%, and CF 37%. When fresh the grass recorded to contain CP 9% and CF 30%. It has been said to cause Birdsville disease (high stepping with forelegs and holding the head high. The hind limbs swayed and dragged the toes of their hind feet. The most severely affected keep their hind legs abducted and if exercised, staggered backwards and fell over) (Aust. Vet-J. 1983, 60:316-7) cited by K.Narayana et. al, 2003 in Poisonous and Medicinal Plants.

Examination of Soil:

Soil samples were also collected and sent to soil laboratory for its test of various parameters. Also the soil test record of the Darchula district was obtained from the Regional Soil Laboratory, Dhangadhi. The result of the soil test of the laboratory as we obtained it from the soil laboratory is given in the table below:

Table: Result of the Phosphorus test of Soil, collected from the Khari affected area of Darchula, (Tested by Soil Lab, Dhangadhi)

S.N.	Name of the farmers	Address	Place of the soil collection	Phosphorus contents		Remarks
				ppm	Kg/h	
1.	Man singh Mahar	Darchula Banjh	Field	0.1120	51.29	Medium
2.	Man singh Mahar	" "	Garden	0.117	54.04	Medium
3.	Man singh Mahat	" "	Fodder soil (upland)	0.049	22.9	Low
4.	Man Singh Mahat	" "	Animal Shed	0.123	56.33	High
5.	Lokmani Thaguna	" "	Garden	0.060	27.48	Low

As we can make out from the table above that the phosphorus content of the soil is also comparatively low, which beautifully co-relates with the phosphorus content of the grass growing in the region.

Examination of the soil of the cultivable land as well as the grazing land or the land from where the grass is obtained for making hay for organic matter content, Nitrogen, Potassium, Phosphorus, Calcium and other micronutrients was also conducted at the Regional Soil laboratory and the findings are illustrated in the table below.

Table: Results of the Soil Examination at the Regional Soil Lab., Dhangadhi.

Name of the farmer	Address	Soil type	P.H.	O.M %	N ₂ %	P ₂ O ₅ kg/ha.	K ₂ O kg/ha.	Ca. (ppm)	Fe (ppm)	Mg (ppm)	Cu (ppm)
Soil from grazing land	Rodidewal, Baitadi	Silt loam	8.8(Salty)	3.71 (Medium)	0.185 (Medium)	43.3 (Medium)	77 (low)	10 (optimum)	1	-	-
Birendra Singh	Rodidewal-8, Baitadi	Silt loam	8.8(Salty)	2.77 (Medium)	0.138 (Medium)	41.9 (M)	85 (Low)	25 (optimum)	1	-	-

Harka Singh Mahara	Khalanga-9, Darchula	Silt loam	7.28	2.45 (Medium)	0.122 (Medium)	50.01 (Medium)	365 (High)	25 (optimum)	-	2.5 (very low)	0 (sufficient)
Lal Singh Karki	Shankarpur-9, Dharchula	Silt loam	5.96 (slight acidic)	2.69 (Medium)	0.134 (Medium)	46.02 (Medium)	270 (Medium)		-	10 (very low)	0 (sufficient)

It can be noted from the above table that while the calcium content of the soil is optimum in all the soil samples, Phosphorus content is relatively low in the soil with very low amount of Magnesium and comparatively low amount of Potassium. The organic matter of the soil along with other important micronutrients like Nitrogen content is also insufficient requiring their supplementation from outside in the form of farmyard manure or chemical fertilizer.

Result of Drug Trials:

A standard protocol for drug trial on diseased animals was developed jointly by the RVL and CVL, which was as follows

Group 1: Ivermectin @ 1ml/50 kg. body weight, two injections at monthly interval.

Group 2: Minamil at the rate of 50 gm. per day for 15 days per month to be used for two months.

Group 3: Ivermectin @ 1ml/50 kg. body weight two injections at monthly interval Plus Minamil at the rate of 50gm per day for 15 days in a month for two months period.

Group 4: Minamil @ 50 gm per day for 15 days per month for two months and Tonophosphan 10 ml per animal, 2 injections at 15 days interval.

Group 5: Ivermectin as described for G1 and Tonophosphan as for G4

Group 6: Ivermectin, Tonophosphan and Minamil at the dose rate described above.

Accordinging the directions were conveyed to the technician looking after the investigation in the field to monitor the administration of the drug together with the response of the animal on respective treatment.

Following the above mentioned treatment it was so observed that there was only slight improvement was observed on those animals under Minamil treatment i.e they were able to move around with better ease than before, however the skin lesion remained unchanged.

Similarly the animals which were treated with Ivermectin improved in the sense that the skin lesion disappeared, and encouraging regrowth of hair in the affected region, but without the improvement of the emaciated body condition.

Much better results were obtained when Ivermectin and Minamil were used in combination for treatment of the animal. This treatment regime supplemented with some amount of concentrate feed to the animal gave encouraging result indicating not only the improvement of the skin lesions but also the body condition and slight improvement in the furrow of the hoof as well.

Very encouraging result was achieved in animals which were given all the three drug regimen revealing recovery of lameness, skin condition, improved body condition and appetite.

These trial regimens are still continuing into the third and final year of the investigation program on khari disease and the final interpretation is expected after one more year of trial.

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Investigation of Nematodiasis to Identify the Treatment Strategies in Goats under Sedentary Management in a Low Hill Village of Western Nepal

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Introduction

Goat husbandry is an important component of mixed subsistence agriculture systems in the hills of Nepal and reared by all communities and classes of Nepalese society for their diverse use as meat, manure, hair and hide but also as a source of cash during emergency. Resource poor farmers in the hills, who cannot invest large sums of money in cattle and buffalo, prefer goat keeping. The goat production of Nepal has been estimated to be 6.97 million and its meat production to be about 39664 mt. in the year 2002/03 (CBS, 2004) that worth about 134 million US\$ at the current market and exchange rates. However, the national production is unable to fulfill the need of the country, hence; a significant number of goats are imported from neighbouring countries.

The goat production system in the country is mostly traditional type and the loss in the production system is high to affect productivity. In general, high mortality, poor kid growth, delayed puberty, higher interkidding interval are the major factors affecting productivity. In the lower hills goats are raised under sedentary management system in smaller flocks of 10-50 animals. In some villages where cropping intensity is high, pasture and labor availability are low and flock size is even smaller, and goats are raised under stall fed management. In the sedentary system, animals graze continuously over limited grazing land during the day time and are housed indoors during night. The grazing areas are generally private crop harvesting in winter, grazing is mainly on the fallow fields.

Among the various factors affecting productivity, gastrointestinal nematode infestation has been regarded as one of the important cause (Shrestha, 1994). Thakuri and Mahato (1990) similarly stated that gastrointestinal nematode infestation in goats in the Eastern hills is recognized to be a serious parasitic problem which causes significant problem to the development of goat farming. Likewise, Karki (1987) reported that GI nematodes are regarded to be the most important disease problem in both the management system, i.e., migratory or sedentary system. Joshi (1991, 1994) recorded high mortality due to GI nematode infestation in sheep and goats under intensive grazing management in mid and low hilly region of the West Nepal and further stated that PGE is one of the major causes of productivity loss in goats of Nepal. Joshi (1996) found that subclinical parasitism was common in goats raised either under sedentary or migratory and further stated that the infection was responsible for the reduction in body weight gain by 93-160 percentage. The community grazing areas were the source of infection for the sedentary animals (Joshi, 1996) and further said that the main period of pasture infection was confined to the wet summer months between Aprils to October with a very low level of infection during the rest of the year. *Hemonchus* spp. was the most prevalent in the sedentary management system.

The most important control program for intestinal nematodiasis in ruminants for PGE is regular drenching with suitable anthelmintics. Ivermectin as injectable is a commonly available anthelmintics. Ivermectin should be used at the dose rate of 50-200 µg/Kg body weight of goats (Soulsby, 1982). Armour et al. (1980) reported that subcutaneous injection with 100-200 µg/kg removed all adults and inhibited larvae of common gastrointestinal nematodes of cattle in the UK.

The following study was conducted to know the drenching interval in the winter time in the sedentary management system in goats.

Materials and Methods

Site Selection : For the field study Khaireni Tar, Tanahun was selected as a study site where all goats were raised as a sedentary management system. All together 56 goats were randomly selected for the study irrespective of feeding, management and age. Among them randomly 10 goats are kept for controlled group and 46 were kept for the treatment group.

Sample collection:

Faecal samples from 56 goats were collected from first visit in Bhadra and all the goats were given ivermectin Injection @ of 1 ml/50kg body wt. basis except the control population of 10 goats. Then, the faecal samples were collected in the months of Asoj, Mangsir, Poush, Magha, Falgun, Chaitra, Baisakha, Jestha and Asar. The first fecal collection was brought in the cool condition to identify the nematode larvae and EPG Counts and then the consecutive faeces were collected for the EPG in formalin swab inserted in the collecting pouch.

Laboratory Examination:

All the faecal samples collected were examined by improved modified McMaster Method (MAFF, 1986) as stated as follows;

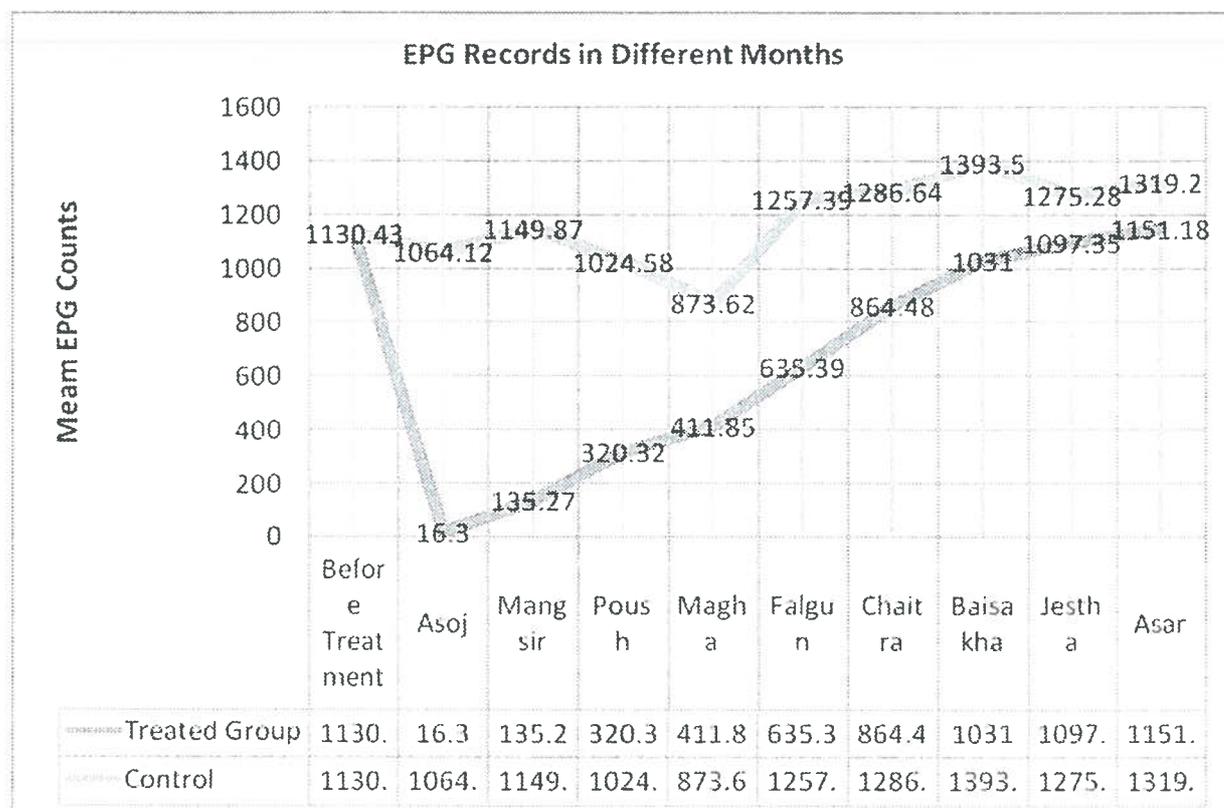
1. Three grams of faecal material was weighed and placed on the mortar and about 42 ml of water was added to it.
2. The mixture was gently grinded using a pistil until the faecal material was uniformly broken down.
3. The mixture was poured through the wire mesh screen and caught the strained fluid in a bowl. The debris left on the screen was discarded.
4. About 15 ml of the solution of the stained fluid after mixing regularly was drawn on the centrifuge tube and subjected to centrifugation for about 2 minutes at 1500 rpm.
5. The supernatant was discarded and the sediment was agitated until loosened and form a homogenous mixture and mixed the saturated salt solution to it and further agitated with a wooden stick.
6. Now the solution was kept for 2-3 minutes and the fluid was drawn in a sufficient amount with a pasture pipette and carefully ran into one counting chamber of McMaster slide for counting the eggs.
7. The number of eggs counted inside the counting chamber was multiplied by 100 to get the EPG count.

The faecal samples collected without a formaline was processed for the larvae identification as follows.

1. About 10 grams of faecal material was weighed and poured on a mortar and same amount of wood dust was added to it and mixed using pistil after sufficient amount of water to make the content moist.
2. The prepared content was poured on the beaker and left at room temperature for about 10-14 days. The moisture content was maintained daily by adding required amount of water to it but not making the content very wet.
3. After 14 days the beaker was fully filled with water and inverted on a petriplate by slightly tilting the petriplate.
4. After 4-5 hours the larvae migrated were come out to the water on the petriplate. Thus the fluid on the plate was drawn using a pasture pipette and collected in a test tube. The centrifuge tube after labelling was kept in a refrigerator for about a night.
5. Next day the supernatant was discarded and the sediment where larvae was deposited was mixed with a 2-3 drops of Lugol's iodine and transferred to the slide and viewed on a microscope using x10 objective lense and counting of the larvae and their identification was carried out on their morphological characteristics.

Results

The result of fecal egg count (EPG) of both control and treatment groups were shown in the chart below.



The fecal sample collected for the larvae identification gave the following result.

- *Trichostrongylus spp*
- *Ostertegia spp*
- *Oesophagostomum spp*
- *Haemonchus spp*
- *Nematodirus spp*

Discussion

The faecal egg count has been regarded as a crude estimate of the worm burden, it has been suggested that in dairy goats, a faecal egg count of >2000 EPG would be indicative of clinical disease and the faecal egg count of 500-2000 EPG could be considered for subclinical parasitism (Lloyd, 1987 cited by Joshi, 1994). The EPG count was found to be 1130.43 in the study in Bhadra states that the flock was infested by subclinical parasitism. The flock was also infected by the mixed type of nematodes.

After treatment with Ivermectin the Mean EPG count reduced drastically to a negligible number as compared to the control group. After treatment the EPG gradually increased in the subsequent months and reached 635 in Falgun month which indicate that the parasitic burden reached the subclinical parasitic disease. So, the goats should be treated in the Month of Falgun again. Therefore it can be said that in mid hills when we treat the goats against the nematodes in late Bhadra the treatment can be repeated after 5-6 months in Falgun.

Limitations and Recommendations

This study does not take the management system, grazing patterns of the goats and also did not include the rainy season in the study which is the most important for the nematode infestations. So, to generalise the results and their varification another study should be conducted by taking all the management systems with wider climatic zones and more population.

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