ANNUAL TECHNICAL REPORT

2063/064 (2006/2007)

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

Central Veterinary Laboratory

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Preface

On behalf of editorial board, I am pleased to present the publication of our Annual Technical Report, 2063/064 in front of you. This issue includes various activities and remarkable works conducted at Central Veterinary Laboratory (CVL), five Regional Veterinary Laboratories (RVLs) and National Avian Laboratory (NAL), Bharatpur during the fiscal year 2063/064 (2006/2007).

We did several remarkable works during last year such as surveillance on Avian Influenza,conducted by FAO,OSRO USA Project. Development of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test, setting tissue culture laboratory unit at CVL, and collaboration with international reference laboratories. Moreover CVL is producing Salmonella antigen which will help to all RVL,NAL & districts to conduct screening of Salmonella thus help in reducing the Salmonella infection in the farm.

Our effort will continue to develop these diagnostic laboratories as centre of excellence. We are in the process of upgrading CVL, NAL and RVLs into Biosafety Level - II laboratories.Also that CVL is trying to receive accreditation for international certification under ISO.

I would like to express my cordial thanks to all the RVLs as well as NAL for providing their annual progress report and technical articles in due time. I would also like to thank Dr.Banshi Sharma, Dr. Karuna Sharma Bhattarai, Dr. Salina Manandhar ,Dr.Vinaya Kumar Karna,and Dr. Pragya Koirala Sharma for their support in publishing this report. My special thanks goes 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
Officiating Chief
Central Veterinary Laboratory

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CENTRAL VETERINARY LABORATORY
TRIPURESHWOR, KATHMANDU

1. Introduction

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, 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 shortcoming days.

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, 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; 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, Kabhre Palanchok, Chitwan, Rupandehi, Dang, Banke, Jumla, Dadeldhura and Kanchanpur, and 60 primary laboratories available one in rest of the DLSOs work in their domain of activities. 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 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 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 with ample of opportunity. Moreover, CVL, RVLs and NAL work with the following objectives in the country.

Support the national disease control and surveillance programme.

Act as national reference laboratory.

Conduct disease investigation and research.

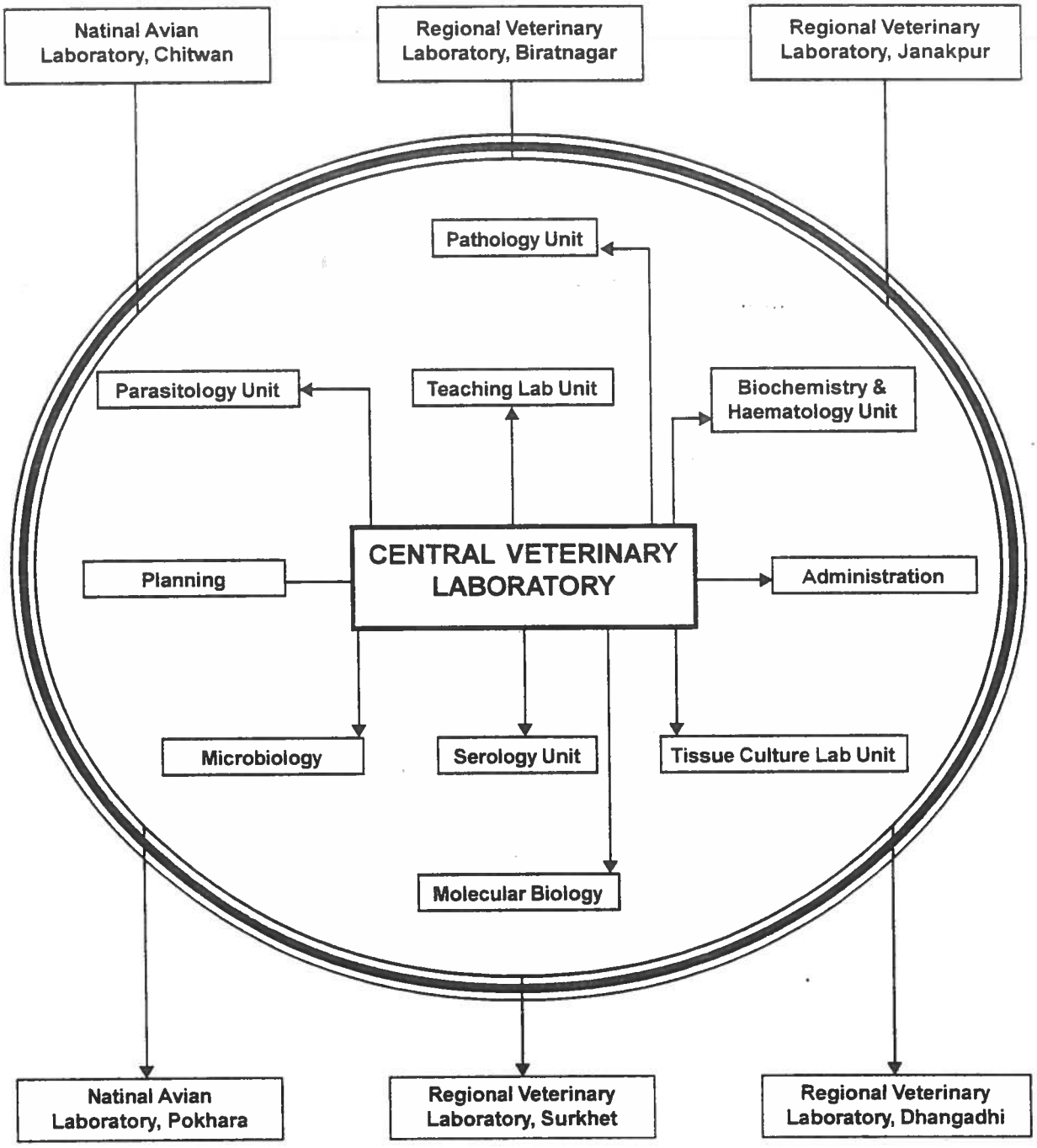
Acquire, adopt, upgrade and disseminate new as well as different diagnostic test methodologies for animal and poultry diseases.

Assist in formulating epidemic control strategies.

Capacity building of veterinarians and veterinary technicians by organizing training on laboratory technology.

Strengthen and coordinate regional and district laboratories.

3. Organization Chart



4. Annual Work Programme & Annual Progress Of CVL (2063/064)

S.N.	Activities	Unit	Target	Budget allocated	Achievement	Progress %
1.	**Diagnostic Services**					
1.1	Parasitology	Number	1600	140	1920	**100**
1.2	Microbiology	Number	2000	320	3502	**100**
1.3	Pathology	Number	1200	250	1207	**100**
1.4	Serology	Number	4500	650	5949	**100**
1.5	Haematology	Number	500	100	713	**100**
1.6	Biochemistry	Number	500	145	578	**100**
1.7	Molecular Diagnosis	Number	30	210	38	**100**
1.8	Rabies Diagnosis	Number	30	130	41	**100**
1.9	Tissue culture Diagnosis	Number	6	308	6	**100**
1.9	Dispatch of samples to other laboratories	Number	300	80	649	**100**
2.	**Investigation Programs**					
2.1	Endemic Outbreak Investigation	Times	12	291	14	**100**
2.2	Serum Bank Management	Times	12	100	12	**100**
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	350	1	**100**
3.3	Training on Laboratory Technology (2 Week Officer level)	Time	1	0	1	

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	50	1	**100**
5.2	Participation in regional workshops (5 regions)	Times	5	80	5	**100**
5.3	Workshop on Prog. & Budget of the next F/Y	Times	1	45	1	**100**
6.	**Publication**					
6.1	Annual Technical Report including Regional & NAL	Times	1	90	1	**100**
7.	**Contract Service**					
7.1	Sweeper & Gardener/Lab.Mechanics	Times	3	100	8	**100**
8.	**Purchase**					
9.1	Technical Books & Journals	Times	3	100	4	**100**
Total						
Administrative Expense						
Grand Total						

5. Human resource situation, CVL (2063/064)

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	2	2	-
3.	Veterinary Officer	III	5	5	-
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 2063/064)

S.N.	Name of Staff	Post	Class	Starting	Rema
1.	Dr. Rebati Man Shrestha	Chief vet. off.	G.I		
2.	Dr. Poornima Manandhar	SVO	G.II	057.12.22	
3.	Dr.Banshi Sharma	SVO	G. II	064-4-8	
4.	Dr. Karuna Sharma	SVO	G.II	062	
5.	Dr. Kedar Bahadur Karki	SVO	G.II	060.10.21	
6.	Dr.Salina Manandhar	VO	G.III		
7.	Dr. Vinaya Kumar Karna	VO	G.III	059.01.22	
8.	Dr. Pragya Koirala	VO	G.III	062.	
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		
15.	Mr. Narayan Pd. Ghimere	VT	NG.I	060.07.24	On Leave
16.	Mr. Laxman Sijapati	S.Man	NG.III		
17.	Mr. Purna Maharjan	S.Man	NG.III	053.12.20	
18.	Mr. Hari Pd. Pyakurel	S.Man	NG.III	054.12.02	
19.	Mr. Prahlad Basnet	S.Man	NG.III	057.12.01	
20.	Mr. Hari Bhakta Karki	S.Man	NG.III	059.01.01	
21.	Mr. Bhimsen Adhikari	S.Man	NG.III	057.08.01	

Administration/Account					
22.	Mrs. Kamala Shrestha	Typist	NG.I	055.07.11	
23.	Mr.Nirmal Poudel	Account.	NG.I		
24.	Mr. Buku Prasad Acharya	Kharidar	NG.II		
25.	Mr. Macha Kaji Maharjan	Driver	L.V.	055.07.01	
26.	Mrs. Chiri Maya Maharjan	Peon	Lo.lev.	055.10.01	
27.	Mr. Santa Raj Budathoki	Peon	Lo.lev.	059.11.01	
28.	Mrs. Bhima Acharya	Peon	Lo.lev.	055.04.01	
29.	Mr. Hari Gobinda Shrestha	Peon	Lo.lev.	059.11.06	
30.	Mr. Chandra Bdr. Rana	Peon	Lo.lev.	056.08.23	
31.	Mr. Anoj Bajracharya	Peon	Lo.lev.	058.11.01	

7. Details of budget sanction & expenditure, CVL (2063/064)

Budget line	Budget head	Budget (Rs.)	
		Sanctioned	Expenditure
1.01	Salary	33,20,650.90	33,20,650.90
1.02	Allowance	0.00	0.00
1.03	Transfer allowance expense		
1.04	Clothes	44,950.00	44,950.00
1.05	Food	25,293.00	25,293.00
2.01	Water and electricity charge	5,05,460.10	5,05,460.10
2.02	Telecommunication charge	1,35,953.26	1,35,953.26
2.03	Official	19,44,902.62	19,44,902.62
2.05	Repair & maintenance	4,69,913.72	4,69,913.72
2.06	Fuel and other provision	3,16,415.95	3,16,415.95
2.07	Consultancy and other service charge	99,931.47	99,931.47
2.08	Miscellaneous	57,000.00	57,000.00
4.02	Medicine	40,911.21	40,911.21
4.04	Programme	5,66,923.79	5,66,923.79
4.05	Travel & daily allowance (programme)	2,71,957.00	2,71,957.00
	Total	78.00,263.02	78.00,263.02
	Salary		
	Medicine & treatment		
	Grand total	78.00,263.02	78.00,263.02

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
86 Japan(K R 1	4,33,923.79	4,33,923.79	4,33,923.79	0.00
Total	80,51,000.00	78,00,263.02	78,00,263.02	0.00

PATHOLOGY UNIT

1. Introduction

Pathological test procedure in a biomedical diagnostic laboratory acts as the opening door wherefrom process of disease diagnosis begins. Pathology laboratory unit of central veterinary laboratory includes post mortem unit (necropsy examination) and histopathology unit (histological examination). As a referral veterinary laboratory, CVL receives a large number of specimens from all over the country either directly or through the respective regional veterinary laboratories (RVLs). Besides, district livestock service offices (DLSOs), veterinary practitioners and hatcheries as well as farmers deliver specimens for the purpose of disease diagnosis.

Disease diagnosis starts with necropsy examination in case of dead animals. Besides, history, clinical findings, epidemiological information, and sometimes line of treatment also provide various clues to formulate a tentative diagnosis. Similarly histological examination lays provisional diagnosis of a disease/condition. Therefore, it becomes essential to confirm the case with the application of specific test methods. For this purpose, necropsy technique plays very important role in the procurement of different specimens suitable for various diagnostic techniques. In this way, post mortem unit provides sizeable number of samples to central veterinary laboratory.

Histology unit processes the tissue samples and provides the result over 7-10 days period. Nowadays, this technique is regarded as an obsolete test procedure; nevertheless, its importance has been enormous in a laboratory with limited diagnostic facilities. Its value as a diagnostic procedure is still high in the diagnosis of diseases of neoplastic origin, chronic courses, some of the viral diseases and disease of prion origin where blotting technique is not available. Also its diagnostic value can be looked positively in the use of research works on various diseases.

2. Post-mortem examination

The total cases received for necropsy examination during F/Y 2063-064 were four hundred and thirty-one. Of them, cases of commercial poultry were 396 (91.87%) and the cases of animals and birds other than commercial poultry were 35 (8.12%). Commercial poultry includes cases of broilers-388 (90.02%), layers-41 (9.51%) and parents-12 (2.78%). Rest of the 35 cases comprised of 15 cases of pigs followed by seven cases of ducks, four cases of local poultry, three cases each of dogs and fish, two cases of goats, and one case of turkey. The monthly distribution of cases for necropsy of commercial poultry has been given in table 1. Similarly, the monthly distribution of cases for necropsy of animals and birds other than poultry is given in table 2.

3. Histological examination

Histopathology laboratory unit received a total of 44 sets of specimen from different species of animals and birds during 2063/064. These specimens comprised 23 cases of

commercial poultry, 13 cases of pigs, three cases each of goats and pigs and one each cases of buffalo and mule.

Among the total cases of commercial poultry, 20 cases were received from broilers and three cases from layers. Among the three cases of layers, two cases were interpreted as for the occurrence of inclusion body hepatitis (IBH) and one case for Marek's disease. Similarly, the three cases of broilers were diagnosed as Gumboro disease, two cases for IBH, one case for acute inflammation. The remaining 14 cases of broilers were studied for haemorrhagic proliferative proventriculitis-gizzard erosion (HPPGE), among which the sections of gizzard in eight cases could not be studied due to faulty histological technique and the rest six cases could not be confirmed with the histological lesions of HPPGE.

Similarly, among the 13 cases of swine, seven cases were received to evaluate the lesions of swine fever, three cases for haemorrhagic septicaemia, two cases of abortions and one case of unknown death. Among seven cases of pigs for swine fever, five cases were interpreted as the cases of acute inflammation and the rest two cases could not be appreciated for any significant pathological condition. Similarly, three cases of HS were interpreted as pneumonia without exploring the occurrence of *Pasteurella,* the two cases of abortions could be correlated with Streptococcal meningitis and pyelonephritis and one case was diagnosed as Eperythrozoan infestation.

Among the three cases of dogs, two cases were interpreted as pneumonia and one case as chronic wound. Similarly, the three cases of goats were diagnosed as inflammatory condition and could not be correlated with any specific disease as the specimens were insufficient for study.

The case of buffalo could similarly be not correlated with any particular disease/condition and it was interpreted as inflammation. The case of mule was however, interpreted for the occurrence of a course of virological episode or chronic inflammatory response which was, later on, confirmed as the case of moldy corn poisoning.

	4			5			6			7			8			9			10			11			12			1			2			3				
	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	B	L	P	
1. Bacterial Infection				1	1																															2	2
2. Manifestations of *Escherichia Coli*																																					
Colibacillosis	15	1		20			7			8			7			13			15			3			7			8			1			6	112		
Colibacillosis+ Salmonellosis					1																														1	
Colibacillosis+ Mycosis				5						1	2					2			1			1													12	
Colibacillosis+ Visceral Gout	1																																		1	160
Coliseptic aemia	4			5			2			3			1									1			1						1			17		
Omphalitis	3								1	4																		5			4			17		
3. Coccidiosis																																					
Caecal Coccidiosis (CC)					1	1																						1						3	4	
CC +Colibacillosis																																			1		
4. Chronic Respiratory Disease										2			3																						5	26	
Complicated CRD				2			1						3						1			3						8						21		
5. Enteritis								1																											1	1	
6. Fatty Liver Syndrome										1												1						3							5	5	
7. Gumboro Disease (IBD)	4			2			1			3												1													11	14	
IBD+ Coccidiosis	1																																		1		
IBD+ Colibacillosis													1																						1		
IBD+ Ranikhet Disease							1																												1		
8. Haemorrhagic Proliferative Proventriculitis & Gizzard Erosion (HPPGE)				4			12	1		4	1		11	1	1	2	1		5			6			7			3			1	2	1		63	63	
HPPGE+ Caecal Coccidiosis																						1													1	1	
HPPGE+ Colibacillosis								1																	1			1							3	3	
HPPGE+ CRD																															1				1	1	
HPPGE+ Visceral gout																			1																1	1	
HPPGE+RD													1																						1	1	70
9. Lichi Heart Disease (LHD)	4	1		1	1											1																		8	8	
LHD+ Coccidiosis																																	1		1	1	

IHD+IBD	1																																				1	
IHD+Mycotoxicosis	1																																				1	
IHD+Salmonellosis					1																																1	
IHD+IBD+Colibacillosis	1																																				1	
IHD+IBD+Salmonellosis	2																																				2	15
10. Lymphoid Leukosis (LL)																																				1	1	1
11. Mineral deficiency																																			1	1	2	2
12. Mycosis	5	2		3						2	1		1			1			1							1		1	1		1	2		4			26	26
13. Mycotoxicosis																			1																		1	1
Mycotoxicosis+Salmonellosis																			1																		1	1
14. NAD/Putrefied specimen/Undiagnosed	1	2		2	2		1									1			1						1	1		4				1					17	17

Table 2: Monthly distribution of cases of necropsy examination of animals and birds other than poultry, CVL

Animals/birds	Diseases/Conditions	4	5	6	7	8	9	10	11	12	1	2	3	Total	Remarks
Dog	CVGE	1												3	Vetro-legal case, referred to forensic lab.
	Strychnine Poisoning								1						
	Hepatitis												1		
Duck	Colibacillosis	1		2										7	
	Colisepticaemia		1												
	Mycosis												1		
	Mycotoxicosis	1													
	Salmonellosis					1									
Fish	Cases of malicious poisoning	1												3	Vetro-legal case, referred to forensic lab.
	Undiagnosed											1	1		One case referred to Fish lab, Balaju.
Goat	PPR							1						2	
	Undiagnosed								1						
Local Poultry	Colibacillosis										1			4	
	Mycosis								1						
	Pasteurellosis +Mycosis												1		
	Parasitic enteritis											1			
Peacock	Avian encephalomyelitis												1		
Pheasant	Undiagnosed										1				
Swine	FMD							2						15	
	FMD+Swine fever								1						
	Haemorrhagic Septicaemia									1	2				
	Swine Fever	1						3		1					
	Swine Fever+Mycosis												1		
	Undiagnosed		1								1				
Turkey	Turkey Disease	1												1	
Total														35	

SEROLOGY UNIT

1. Introduction

Serology unit is responsible to conduct various serological tests to detect antigen and antibody for the purpose of diagnosis, screening, monitoring and surveillance of bacterial and viral diseases of animal and poultry. Similarly this unit is performing competitive enzyme-linked immunosorbent assay (C-ELISA) test to measure the antibody titer in response to Goat plague vaccine in sheep and goat under National Peste des petits ruminant (PPR) control Program. Other serological test used in this laboratory are agar gel immunodiffusion (AGID), plate agglutination test (PAT), Serum samples are received from RVLs, NAL, DLSOs, commercial poultry farms, private veterinary practitioners, farmers and postmortem unit of our own laboratory. Samples thus received are tested in serology laboratory unit and more often, the test results are reconfirmed from OIE reference laboratories as per need.

2. Programmes and progress

The result of different test for specific disease has been presented from table 1-10 with the overall progress in table 11. A total number of 9775 sera were tested during 2063/064 and more than 50% of the samples were found test positive in outbreak cases. Of the total samples, 5057 sera intended for seromonitoring were tested to detect antibodies in response to PPR live vaccine or PPR infection of which 80% were found positive.These sera were collected from 56 districts.

Screening for Brucellosis was conducted in six districts by testing 67 samples collected from cattle, buffaloes, pigs and dogs. All samples were found to be negative (Table 6).

Antibodies against salmonella infestation were detected in Chitwan, Bhaktapur, and Nawalparasi and Saptari districts when 907 poultry serum samples were tested by conducting plate agglutination test. Among them, 335 samples were found positive threatening the poultry production of country (Table 2). Prevalence of Mycoplasmosis was detected in 71 samples out of 952 samples tested while 881 samples turn to be negative PAT (Table 3) and antibodies against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* of 226 serum samples were found negative for both (Table 5). Antibodies against infectious bursal disease, Newcastle disease and infectious bronchitis were detected in 210, 226 and 198 samples out of 226 samples tested in vaccinated flock that suggests good vaccination program in commercial poultry (Table 4).

A total 182 sera were collected from pigs for sero-survey of Japanese encephalitis (JE) in 2062/063 and subjected to C-ELISA test. The prevalence of JE was 40 %. (Table 9). A total 79 sera were collected from poultry for sero-survey of Avian Influenza (AI) in 2062/063 and subjected to C-ELISA test. All samples were negative for AI (Table 10).

Table No. 1: C-ELISA test for diagnosis of PPR in Sheep and Goats

(Cases of Disease outbreak) F.Y. 063-064

S.No.	District	Animal Species	No.of Tested samples	PPR Results		Remarks %Positive
				Positive	Negative	
1	Jhapa	Goat	129	21	108	16 %
2	Morang	,,	79	44	35	56 %
3	Dhankhuta	,,	18	15	3	83%
4	Sankhuwashabha	,,	16	3	13	21 %
5	Bhojpur	,,	20	0	20	0 %
6	Solukhumbu	,,	19	4	15	19
7	Udayapur	,,	98	71	27	73
8	Saptari	,,	138	52	86	38
9	Siraha	,,	260	184	76	71
10	Dhanusha	,,	50	16	34	32
11	Mahottari	,,	45	15	30	33
12	Sarlahi	,,	116	58	58	50
13	Sindhupalchok	,,	83	42	41	51
14	Khathmandu	,,	243	192	51	79
15	Nuwakot	,,	24	7	17	29
16	Rasuwa	,,	123	33	90	28
17	Dhading	,,	23	9	14	39
18	Makwanpur	,,	61	26	35	43
19	Rautahat	,,	8	4	4	50
20	Bara	,,	65	30	35	46
21	Parsa	,,	86	61	25	71
22	Chitwan	,,	23	21	2	91
23	Tanhu	,,	55	44	11	80
24	Kaski	,,	69	40	29	58
25	Myagdi	,,	69	57	12	83
26	Gulmi	,,	35	19	16	54
27	Rupandehi	,,	60	26	34	43
28	Kapilbastu	,,	56	24	32	43
29	Salyan	,,	35	3	32	9
30	Banke	,,	200	28	172	14
31	Kailali	,,	21	9	11	45
32	Kanchanpur	,,	41	29	12	61

33	Dadeldhura	,,	32	28	4	71
34	Pyuthan	,,	15	5	10	33
35	Lamjung	,,	3	0	3	0
36	Ramechhap	,,	32	5	27	16
37	Taplejung	,,	22	5	17	23
38	Sunsari	,,	65	27	38	42
39	Bajura	,,	11	4	7	36
40	Humla	,,	15	3	12	20
41	Jajarkot	,,	15	3	12	20
42	Dailekh	,,	5	2	3	40
43	Bardiya	,,	10	6	4	60
44	Surkhet	,,	12	12	0	100
45	Terhathum	,,	10	0	10	0
46	Bhaktapur	,,	14	3	11	22
47	Nawalparashi	,,	26	10	16	39
48	Gulmi	,,	35	19	16	54
49	Dang	,,	14	3	11	22
50	Kavre	,,	64	28	36	44
51	Gorkha	,,	10	2	8	20
52	Rukum	,,	27	18	9	67
53	Baglung	,,	40	32	8	80
54	Parbat	,,	40	36	4	90
55	Shyanja	,,	34	28	6	82
56	Darchula	,,	16	13	3	81
57	Dolkha	,,	49	15	34	31
58	Sindhuli	,,	22	5	17	23
Total			**2983**	**1499**	**1484**	**50%**

Table No. 2: Plate Agglutination test for *Salmonella pullorum* & *Mycoplasma* in poultry (F.Y. 2063-064)
PAT Test result for Salmonellosis (F.Y. 2063-064)

S.N	District	Species	Total Samples	Positive	Negative
1	Chitwan	Poultry	745	325	420
2	Mkwanpur	Poultry	60	0	60
3	Saptari	Poultry	40	0	40
4	Rupandehi	Poultry	18	8	10
5	Kathmandu	Poultry	44	2	42
Total			**907**	**335**	**572**

Table :3 PAT Test result for Mycoplasmosis (F.Y. 2063-064)

S.N	District	Species	Total Samples	Positive	Negative
1	Chitwan	Poultry	790	44	746
2	Makwanpur	Poultry	60	5	55
3	Saptari	Poultry	40	7	33
4	Rupandehi	Poultry	18	4	14
5	Kathmandu	Poultry	44	11	33
Total			**952**	**71**	**881**

Table 4: Sero- monitoring of Poultry Disease by Immuno-comb(F.Y. 2063-064

S. No.	Districts	IBD		ND		IB	
		+ve	-ve	+ve	-ve	+ve	-ve
1	Chitwan	172	14	186	0	158	28
2	Saptari	38	2	40	0	40	0
	Total	**210**	**16**	**226**	**0**	**198**	**28**

Table 5: Screening of Poultry Disease by Immuno-comb test (F.Y. 2063-064

S. N.	Districts	MG		MS	
		Positive	Negative	Positive	Negative
1	Chitwan	0	186	0	186
2	Saptari	0	40	0	40
	Total	0	226	0	226

Table No. 6 Sample tested for Brucellosis (F.Y. 2063-064)

S.No.	District	Animal Species	Total no. of Tested	Number of	
				Positive	Negative
1	Kathmandu	Dog	3	0	3
2	Rupandehi	C,B,G.Pig	30	0	30
3	Lalitpur	Cow	4	0	4
4	Sankhuwasabha	Cow. Buff.	23	0	23
5	Rupandehi	Man	1	0	1
6	Surkhet	Goat	6	0	6
	Total		67	0	67

Table No. 7 Serum Collection & Storage Record (F.Y. 2

S.No.	District	Animal Species	No.of Serum Collected
	CVL.Tripureshwor		
1	Siraha	Goat	210
2	Dhading	,,	124
3	Bara	,,	200
4	Nuwakot	,,	132
5	Dolakha	,,	64
6	Ramechap	,,	131
7	Rasuwa	,,	85
8	Prsa	,,	120
9	Sindhupalchowk	,,	235
10	Chitwan	,,	160
11	Kavre	,,	190
12	Makwanpur	,,	157
13	Rupandehi	,,	120
14	Nabalparashi	,,	
15	Kapilbastu	,,	120
16	Jhapa	,,	212
17	Dhankuta	,,	135
18	Udayapur	,,	195
19	Saptari	,,	210
20	Ilam	,,	195
21	Bhaktapur	,,	70
22	Lalitpur	,,	70
23	Kathmandu	,,	70
24	Argakhanchi	,,	80
	RVL.Janakpur		
16	Dhanusha	,,	201
17	Saralahi	,,	201
18	Sindhuli	,,	208
19	Mahottari	,,	201
20	Rautahat	,,	201
	RVL.Pokhara		
19	Kaski	,,	120
20	Tanahu	,,	120
21	Shyanja	,,	120
22	Parbat	,,	120
23	Baglung	,,	120

24	Myagdi	,,	120
	RVL.Biratnagar		
33	Morang	,,	75
34	Jhapa	,,	77
35	Dhankutta	,,	76
36	Saptari	,,	76
37	Sunsari	,,	75
38	Udaypur	,,	55
39	Siraha	,,	80
40	Ilam	,,	75
	RVL.Surkhet		
41	Dang	,,	100
42	Surkhet	,,	50
43	Banke	,,	75
44	Bardiya	,,	50
45	Rolpa	,,	50
46	Pyuthan	,,	50
47	Salyan	,,	50
48	Jumala	,,	29
Total			4223

Table No. 8 JE C-Elisa Test Report **F.Y. (2063/064)**

S.No.	District	Animal Species	No.of tested samples	Number of		Av.Pos.Sero-conversion
				Positive	Negative	
	Rautahat	Goat	150	135	15	**90%**
2	Parsa	,,	2oo	160	40	**80%**
3	Bara	,,	200	170	30	**85%**
4	Kavre	,,	150	135	15	**90%**
5	Dolkha	,,	100	83	17	**63%**
6	Ramechhap	,,	100	82	18	**62%**
7	Rasuwa	,,	25	19	6	**76%**
8	Nuwakot	,,	75	59	16	**79%**
9	Dhading	,,	125	106	19	**85%**
10	Kathmandu	,,	87	74	13	**85%**
11	Lalitpur	,,	60	36	24	**60%**
12	Bhaktapur	,,	33	28	5	**85%**
13	Chitwon	,,	150	128	22	**85%**
14	Sindhupalchok	,,	100	85	15	**85%**
15	Dhanusha	,,	150	127	23	**85%**
16	Sarlahi	,,	150	114	36	**76%**
17	Sindhuli	,,	150	125	25	**83%**
18	Makwanpur	,,	150	114	36	**76%**
19	Kaski	,,	25	21	4	**84%**
21	Lamjung	,,	50	38	12	**76%**
22	Gorkha	,,	75	58	17	**77%**
23	Gulmi	,,	75	45	30	**66%**
24	Palpa	,,	50	35	15	**70%**
25	Rupundehi	,,	100	78	22	**78%**
26	Tanhu	,,	50	40	10	**80%**
27	Shynaja	,,	75	59	16	**79%**
28	Myagdi	,,	75	58	17	**77%**
29	Baglung	,,	75	60	15	**80%**
30	Arghakhanchi	,,	50	38	12	**76%**
31	Nabalparashi	,,	100	95	5	**95%**
32	Kapilbastu	,,	150	117	33	**78%**

33	Morang	,,	75	56	19	**75%**
34	Jhapa	,,	77	60	17	**78%**
35	Dhankuta	,,	76	58	18	**76%**
36	Saptari	,,	76	60	16	**79%**
37	Sunsari	,,	75	56	19	**75%**
38	Udayapur	,,	55	43	12	**78%**
39	Siraha	,,	80	64	16	**80%**
40	Ilam	,,	75	59	16	**79%**
41	Dang	,,	100	78	22	**78%**
42	Surkhet	,,	50	39	11	**78%**
43	Banke	,,	75	59	16	**79%**
44	Bardiya	,,	50	38	12	**76%**
45	Rolpa	,,	50	37	13	**74%**
46	Pyuthan	,,	50	43	7	**86%**
47	Salyan	,,	50	38	12	**76%**
48	Jumla	,,	29	25	4	**86%**
49	Baitadi	,,	76	57	19	**75%**
50	Doti	,,	148	110	38	**74%**
51	Dadeldhura	,,	100	76	24	**76%**
52	Kailali	,,	154	123	31	**80%**
53	Kanchanpur	,,	75	60	15	**80%**
54	Bajura	,,	200	174	26	**87%**
55	Bajhang	,,	24	18	6	**75%**
56	Achham	,,	75	57	18	**76%**
	Total	**5057**	**4049**	**1026**	**80%**	

Table No. 9 JE C-Elisa Test Report F.Y. (2063/064

S. N.	Disrtict		Sample tested	Test results		Positive (%)
				Positive	Negative	
1	Dadeldhura		24	4	20	16.67
2	Udaypur		20	5	15	25.0
3	Saralahi		18	0	18	0
4	Mohottari		18	3	15	16.67
5	Kathmandu		40	20	20	50.0
6	Lalitpur		30	17	13	56.67
7	Bhaktapur		32	27	5	84.38
	Total		**182**	**72**	**110**	**40%**

Table No. 10 AI C-Elisa Test Report F.Y. (2063/064

S. N.	Disrtict	Test method	Sample tested	Test results		Positive (%)
				Positive	Negative	
1	Dhanusha	C-ELISA	20	0	20	0
2	Kaski	C-ELISA	19	0	19	0
3	Morang	C-ELISA	20	0	20	0
4	Surkhet	C-ELISA	10	0	10	0
5	Kailali	C-ELISA	10	0	10	0
	Total		**79**	**0**	**79**	**0**

Table 11: Summary of serological test results (2063/064

S. N.	Disease	Test method	Sample tested	Test results		Positive (%)
				Positive	Negative	
1	PPR (Routine test)	C-ELISA	2983	1499	1484	50.25
2	PPR (Sero-monitoring,Vaccinated samples test)	C-ELISA	5075	4049	1026	79.78
3	Buucellosis	PAT	67	0	67	0
4	Salmonellosis	PAT	407	170	237	41.76
5	Mycoplasmosis	PAT	417	22	395	5.27
6	Infectious Bursal Disease	Immunocomb	113	105	8	92.92
7	Newcastle Disease	Immunocomb	113	113	0	100
8	Infectious Bronchitis	Immunocomb	113	99	14	87.61
9	Mycoplasmosis (MG)/MS)	Immunocomb	226	0	226	0
10	Japanese Encephalitis	C-ELISA	182	72	110	40%
11	Avian Infuenza	C-ELISA	79	0	70	0
	Total		9775	6129	3646	63.00%

MICROBIOLOGY UNIT

1. Introduction

This unit is responsible for bacterial,fungal and viral disease diagnosis and investigation of epidemics. In addition, it involves in research and on development activities such as antigen production and development of test procedures; penside test for the diagnosis of Peste des Petits Ruminants. Salmonella antigen thus produced is supplied to different RVLs NALs and private practitioners on demand. Similalry, we are also involved in the thesis guidence of graduate and post-graduate students from 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.

Microbiology unit receives a wide variety of samples from the field, veterinary hospitals, farmers, DLSOs, animal quarantine check-posts and post mortem unit of our own laboratory. Besides, we receive primary isolates from RVLs as well as NAL for the test verification and result verification . Microbiology laboratory unit comprises four sub-units; bacteriology and mycology, virology, rabies diagnosis unit, and washing and sterilization units through which various activities are performed.

2. Programmes 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 drug sensitivity test to the isolated organism that facilitate the proper line of treatment. The major samples include milk, various tissues, eggs (commercial poultry), blood and urine followed by swabs, and pus from different species of animal. Similarly water samples are also received from different hatcheries for the appreciation of microbes present therein. This unit receives samples from commercial poultry farms the post mortem unit of CVL and occasionally from RVLs for reconfirmation and verification of the test. This unit special plays role a vital in mastitis control by identifying the organisiums & drug in time. Thus, this provides targeted antibiotics to the animals.

Progress

During the F/Y 2063/064, a total of 781 samples were received from different species of animals. Among them 615 samples were found positive for bacterial culture. Organisms were isolated using different types of culture methods. Similarly , out of 126 samples different species of fungus were isolated from 102 samples.

A total sample received from different species of animals and birds from post mortem unit of CVL was 781 which included 728 samples from poultry, 20 from swine, 14 from bovine, 9samples from caprine and 5 from mule equine, one from canine, 2 from rhino and 2 from cat. Various organisms like *E. coli*, *Salmonella* spp, *Staphylococcus* spp, *Streptococcus* spp and *Pasteurella* spp were isolated from 615 positive samples as shown in table 1.The higest organism isolated was E-coli 237followed by Staphylo 139, Salmonella sps 132,Streptococcus 126 Kleibsella 2, and Brahmhenella 1. This table also reveals that more than one bacterium has been isolated from a single sample.

Table 1: Result of bacteriological culture of post mortem samples (CVL)063/064

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Poultry	728	589	139	*E.coli-228* *Salmonella spp131* *Staph.spp130* *Strepto.spp119* *Klebsiella.spp4* *Proteus.spp 1*	
2.	Swine	20	7	13	*E.coli 6* *Strepto.spp 1* *Staphylococcus spp 1* *Salmonella 1*	
3	Bovine	14	5	9	*Pasturella spp 5*	
4	Rhino	2	2	0	*Staphylococcus spp 1* *Bramhenella spp 1*	
5	Ovine	9	7	2	*E.coli 3* *Staphaylococcus spp 4* *Streptococcus spp 4*	
6	Horse	5	5	0	*Staphylococcus spp 3* *Streptococcus spp 2*	
7	Canine	1	-	1	-	
8	Cat	2	0	2	-	
	Total	**781**	**615**	**166**		

A total of 222 milk samples received from field condition. Out of them, 167samples were screened as positive through California Mastitis Test (CMT). The major isolates were ***Staphylococcus, E.coli Even penicillium sps(fungus) was isolated from mastitic milk sample. Streptococcus*** and ***Klebsiella. Bacillus*** was also isolated from few milk samples. The various isolates of milk samples are presented in table 2.

Table 2: Result of bacteriological culture of milk samples

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	222	167	55	*Staphylococcus spp* *E.coli* *Streptococcus spp* *Klebseilla spp* *Pseudomonas spp* *Bacillus spp* *Micrococcus spp* *Penicillium Fungal sps*	**99** **85** **29** **10** **4** **2** **2** **1** **2**
	Total	**222**	**167**	**55**		

Similarly, a total of 43 vaginal swabs of bovine were received from field condition and the various isolates from34 positive samples were *Staphylococcus, Klebseilla. E.coli, Bacillus and Pseudomonas*, the details of the result has been shown in table 3.

Table 3: Result of bacteriological culture of vaginal swabs

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	43	34	9	Staphylococcus spp Straptococcus spp Bacillus spp Klebseilla spp E.coli	**17** **15** **16** **04** **04**
	Total	**43**	**34**	**9**		

The total blood samples received from field condition were 31constituting2samples of canine and 23 samples of bovine (21), and three each of swine and 2 from equine. Most of these samples were found negative with only 3 samples positive. The detail of the result has been given in table 4.

Table 4: Result of bacteriological culture of blood samples

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	23	2	21	*Pasturella spp*	2
2.	Poultry	1	-	1		
3.	Canine	2	-	2		
4	Swine	3	1	2	*E.coli/Staphylococcus spp*	1
5	Horse	2	-	2		
	Total	**31**	**3**	**29**		

Only two ear swabs from canine were received with only one found to be positive for the presence *E.coli* as shown in table 5.

Table 5: Result of Bacterial Culture of Ear Swab Samples

S.No.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Canine	2	1	1	**E.coli**	
	Total	**2**	**1**	**1**		

The total number of urine samples of different species received from field condition was 12 Among them, 8 samples were found positive with the presence of various isolates like *Staphylococcus* spp, *E.coli* and *Streptococcus* spp. The result of bacteriological culture of urine samples has been shown in table 6.

Table 6: Result of bacteriological culture of urine

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	6	5	1	*E.coli* *Staph spp* *Streptococcus spp*	2 2 1
2.	Canine	6	3	3	*E.coli* *Staph spp*	1 2
	Total	**12**	**8**	**4**		

Similalry, the total pus samples and nasal swab received from field conditions were 2 each .. Among the pus, only two samples of canine were positive revealing the presence of *Staphylococcus* spp and are given in table 7. The total nasal swabs found to be negative the result is given in table 8.

Table 7: Result of Pus Culture

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Canine	1	1	-	*Staph.*	1
2.	Bovine	1	1	-	*Streptococcus spp*	1
	Total	**2**	**2**	**-**		

Table 8: Cultural results of nasal swab

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Horse	2	-	2		
	Total	**2**	**-**	**2**		

The Lab also received water samples from different hatcheries. During 2063/064, a total of 30 water samples were received with 20 samples found positive and 10 negative. The various isolates are given in table 9. The total egg samples found to be negative the result is given in table 9.

Table 9: Result of bacterial culture of water/egg

S.NO.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Egg	7	-	7		
2.	Water	30	20	10	*E.coli, Staphylococcus spp,*	15/10
	Total	**37**	**20**	**17**		

The total sample intended for fungal culture were126 . Among them, 84 samples were received from poultry and rest from canine, bovine, swine, pigeon and caprine. The result of isolated fungi is shown in given table 11.

Table 10: Result of Fungal Culture of different species of animals

S.NO.	Species	Total	Positive	Negative	Isolate Fungus	No.
1.	Canine (skin scraping)	32	28	4	*Aspergillus spp* *Candida spp* *Penicillium spp*	12 14
2.	Poultry (PM sample)	84	67	17	*Aspergillus spp* *Candida spp* *Penicillium spp*	23 10 34
3.	Pig	3	-	3	-	
4.	Maize samples mule feed	7	7	-	*Penicillium spp*	
	Total	**126**	**102**			

2.2 Virology unit

This unit is responsible for diagnosis of viral diseases applying patholgenicity test method and strain identification of different viruses. The sources of samples of this unit are mainly the post-mortem unit of CVL and a few from field condition.

Diagnosis of PPR is done by pen side test, which can be done in field condition. Occular 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. Those samples were send to OIE reference lab for reconfirmed and verified by the OIE reference laboratories of Australia and Italy.

Progress

During 2063/64, a total of 1383 samples from chicks, ducks and goats were tested for different viral diseases among which only 25 samples found positive. A total of 65 sample tentatively diagnosed as New Castle disease were received from Kathmandu, Jhapa, Dhangadi, Pokhara etc district of which 19 samples were found positive as shown in table 12.

Similarly, a total of 44 serum samples received from different Jhapa, Sunsari and Morang etc. district were tested for New Castle disease applying HI method. None of them were found positive as shown in table 11.

Similarly, a total of 6 samples suspected for swine fever Chitwan & Bhaktapur received from district were examined through FATmethod, out of them 3 were found positive as show in table 11

(Table.11) Examination of Swine fever through FAT test method

S. No.	District	No. of sample tested	No. of sample positive	No. of sample negative
1	Chitwon	2	2	-
2	Bhaktapur	4	1	3
	Total	6	3	3

A total of 1263 samples were tested for avian influenza through rapid test method which revealed none of the samples positive for its occurrence as shown in table 13.

Table 12: Test Results of Avian Influenza by Egg Inoculation method

S.N.	District	Total samples	Positive samples	Negative samples	Remarks
1	Kathmandu	13	10	3	ND
2	Chitwon	23	-	23	-
3	Jhapa	9	5	4	ND
4	Dhangadi	11	2	9	ND
5	Pokhara	4	2	2	ND
6	Morang	3	-	3	-
7	Sindhuli	1	-	1	-
8	Dhanusha	1	-	1	-
Total		65	19	46	-

(Table.13) Examination of Avian Influenza disease by AI virus antigen test. (Rapid Test)

S. No.	District	Sample size		Results
		Tracheal swab	Cloacal swab	
1.	Jhapa	51	-	All swabs were negative in **Rapid Test**
2	Saptari	26	-	
3	Sunsari	36	-	
4	Morang	109	94	
5	Dhankuta	-	-	
6	Kavre	50	50	
7	Kathmandu	-	50	
8	Nuwakot	58	-	
9	Nawalparasi	111	-	
10	Chitwan	342	289	
Total		**783**	**483**	

Table 14: Result of AI disease by (HI) Method

S.No.	District	Species	No.of sample tested	No. of sample positive	No.of sample negative
1	Jhapa	Chick	18	-	18
2	Morang	Chick	5	-	5
3	Makwanpur	Chick	9	-	9
4	Sunsari	Duck	3	-	3
5	Sunsari	chick	9	-	-
	Total		**44**	-	**44**

Table 15: Examination of PPR by penside test method

This section also received 5 PPR suspected samples from Ramechhap,Lalitpur district.The samples were analyzed by Penside test and found 3 samples positive

S.No.	District	No.of sample tested	No. of sample positive	No.of sample negative
1	Ramechhap	2	1	1
2	Lalitpur	3	2	1
Total		5	3	2

2.3 Rabies diagnosis unit

One of the most important works done during 2063/064 in this unit is diagnosis of rabies. Although it is the part of virology, various test related to diagnosis of this disease are performed separately than others because of public health importance. Various diagnostic tests conducted for rabies diagnosis include Negri body test, fluorescence antibody test and biological test.

A total of 40 samples were received from field condition comprising129 cases from dog and three samples from cow, and one from goat, and six samples from buffalo.. Among them, 30cases were diagnosed as positive through all the aforementioned tests. Detail of the test results is given in table 16.

Table 16: Result of different tests for diagnosis of rabies

District	Species	Total samples	positive	negative
Kathmandu	Canine	26	17	9
Lalitpur	Cattle	2	2	-
Chitwon	Buffalo	1	1	-
Bhaktapur	Buffalo	1	1	-
Gorkha	Canine	1	1	-
Dhangadi	Goat	1	1	-
Dhangadi	Buffalo	1	1	-
Kaski	Cattle	1	-	1
Kathmandu	Buffalo	1	1	-
Rolpa	Canine	1	1	-
Pyuthan	Buffalo	1	1	-
Rupandehi	Canine	1	1	-
Nuwakot	Buffalo	1	1	-
Total		40	30	10

BIOCHEMISTRY/ HAEMATOLOGY UNIT

1. Introduction

The Biochemistry unit of CVL mainly deals with the analyses of urine and serum samples. The samples are directly brought by the farmers, received from DLSOs and RVLs, and also collected from field during disease investigation and epidemics. These samples are processed in this laboratory unit based on the standard operating protocol of the unit and other protocols as per need. Following tests are done for biochemical analysis.

1. Calcium estimation
2. Phosphorous estimation
3. Zinc estimation
4. SGOT, SGPT
5. Protein and albumin estimation

2. Biochemistry unit

Urine samples are studied and analysed with the use of following techniques.

1. Commercial kits (Multistix/Uristix)
2. Microscopic examination

Urine sample is examined for specific gravity, sugar, ketone bodies, albumin, bilirubin, triple phosphate, calcium oxalate, RBC, pus cells. One hundred and twenty urine samples comprising of 55 samples from canine and rest from cattle were tested during F/Y 2063/ 064. The urine samples of cattle received were mostly requested to rule out haematuria while the same from canine were tested against diabetes, urinary caliculi, kidney function impairment, jaundice, ascites, and haemoglobinuria. The result of microscopic examination of urine showed in most of the cases, the presence of pus cells and calcium oxalate. Similarly the serum samples were analysed for phosphorous, calcium, zinc, total protein and glucose estimation of 606 samples are presented in Table No 1. Table 1 shows vatious types of biochemical estimations of sera of dogs and cattle.

3. Haematology Unit

The responsibility of this unit is to analyze the different parameters of whole blood samples collected in ethylene diamine tetraacetic acid and blood smear received from DLSOs, Avian lab RVLs. Central Veterinary Hospital and those collected during disease out breaks.

Specimen of whole blood is studied for haemoglobin content, packed cell volume, total erythrocyte count, total leukocyte count, differential leukocytes count, total platelets count and erythrocyte sedimentation rate. Table 2

Seven hundred and thirteen blood samples were analysed for the above mentioned parameters during F/Y 2063/64. Cattle-219, Buffalo-169, Dog-229, Horse-99 were also examined for the occurrence of blood protozoan parasites. Thirty one samples were positive for *Babesia* spp, 6 for *Anaplasma* spp and only 1 sample for *Trypanosoma* spp.

Table 1: Vatious types of biochemical estimations of sera of dogs and cattle

S. N.	Sample tested	No. of samples	Dog	Cattle
2	Calcium estimation	154	-	124
3	Phosphorous estimation	162	-	132
4	Glucose estimation	49	49	-
5	Zinc	135	-	135
7	Total Protein	78	46	
	Total	578	95	

Table 2: Parameter of tested samples of various species

S. N.	Species	Total	Hb	PCV	ESR	TLC	DLC	Blood protozoa
1	Cattle	219	58	48	35	65	46	19
2	Buffalo	169	57	34	16	35	38	-
3	Dog	229	35	35	14	25	32	95
4	Horse	99	27					
	Total	713	177	117	75	125	106	114

PARASITOLOGY UNIT

The Parasitology unit is involved in routine examination as well as investigation of different digestive tract parasites and non-digestive tract endoparasites 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/ovas of different nematodes, cestodes, trematodes and other common parasites found in gastrointestinal system of livestocks.

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 asses 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 Trivuwan University and Purwanchal University in field of Parasitology. So far unit has prepared the profile of different parasite spp present in goat ,buffaloes ,Monkey, Captive Elephant .As this servs 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 a total of 3450 samples of cattle, buffaloes, goats, dogs, poultry and monkeys were tested out of which 1885 were found positive for parasites.Of which 1306 samples were positive for Fasciola spp in different spp of animal likewise 56 samples were found to be positive for Paramphistome 498 samples were positives for strongyles,11 skin scraping were found positive for sarcoptic spp mite,3 blood samples from cow were found positive for Babesia spp protozoa.The various types of parasitological examination conducted during the F/Y 2063/ 064 have been presented in tabular from as shown below.

Table: Results of various types of parasitological examination (2063/064)

(***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	-	-	-	-	-	-	-	-	-	-	-	
		Goat	7	6	25	20	45	76	43	10	54	12	10	12	320
		Sheep	-	-	-	1	-	-	-	-	-	-	-	-	
		Buffalo	40	34	32	64	30	25	40	57	40	50	86	65	563
		Elephant	10	10											20
		Dog	-	-	-	-	-	-	-	-	-	-	-	1	
2	Paramphistomum spp	Cow	17	7	2	1	-	9	2	12	-	-	-	-	50
		Goat	1	-	-	1	-	-	-	-	-	-	-	-	2
		Buffalo	2	-	-	-	-	-	-	-	-	-	-	-	2
		Sheep	-	-	-	1	-	-	-	-	-	-	-	-	
		Monkey	-	-	-	-	-	-	-	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	-	-	-	-	
6	coccidia	Poultry	-	-	-	-	-	-	-	-	-	-	-	-	
7	Ascasids	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			194	179	216	279	258	298	277	382	261	302	330	294	3450

MOLECULAR BIOLOGY UNIT

1. Introduction

Molecular techniques are very sensitive, fast and reliable tools for 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 2063/064, total 34 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.

NATIONAL AVIAN LABORATORY BHARATPUR (2063-2064)

Pathological Examination of carcasses

Clinical sign and symptoms are similar in many diseases & for differential diagnosis post mortem is the basic & first attempt. Post mortem examination of dead body is the general and popular process but not the final. Nowadays, well established and equipped Laboratories have different section for proper diagnosis like Bacteriology, Virology, Histology, Serology, Bio-chemistry etc. For Postmortem freshly died animals or birds are should be recievedbecause decaying, decomposed due to delay will give false positive or falselesions and will not be suitable for collection of samples.

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

Followings are the Suspected diseases only from PM findings and referred based are follows :-

S.N.	Disease names	Month												Total
		Shrawan	Bhadra	Aswin	Kartic	Mansir	Pous	Magh	Phalgun	Chaitra	Baishak	Jestha	Ashard	
1.	IBD	0	21	0	5	4	0	1	20	9	4	3	10	77
2.	Ascaris	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.	Cocciodiosis	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.	Tep 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	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 syndrom	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.	Pegion 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 Diagnosis ,E .Coli

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 are another vital function of this unit. In fiscal year 2063/064 525 cases were referred to bacteriology unit. Among them majority isolated bacteria were salmonella, E. coli, streptococcus and staphylococcus etc. Simultaneously we had performed drug sensitivity test to Isolated bacteria.

1. Bacteriological unit

S.No.	Disease name	Positive test	Percentage	Remarks
1.	E.Coli	24	55.81%	
2.	Fungal	6	13.95%	
3.	Pasteurellosis	5	11.62%	
4.	Salmonellosis	4	9.30%	
5.	Streptococcus	4	9.30%	
6.	Total positive test	43	100%	

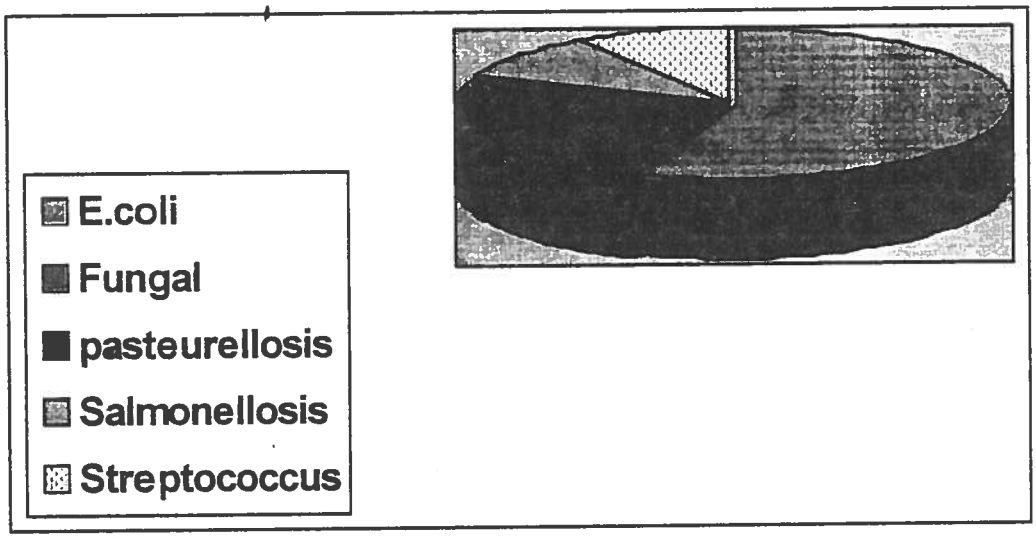


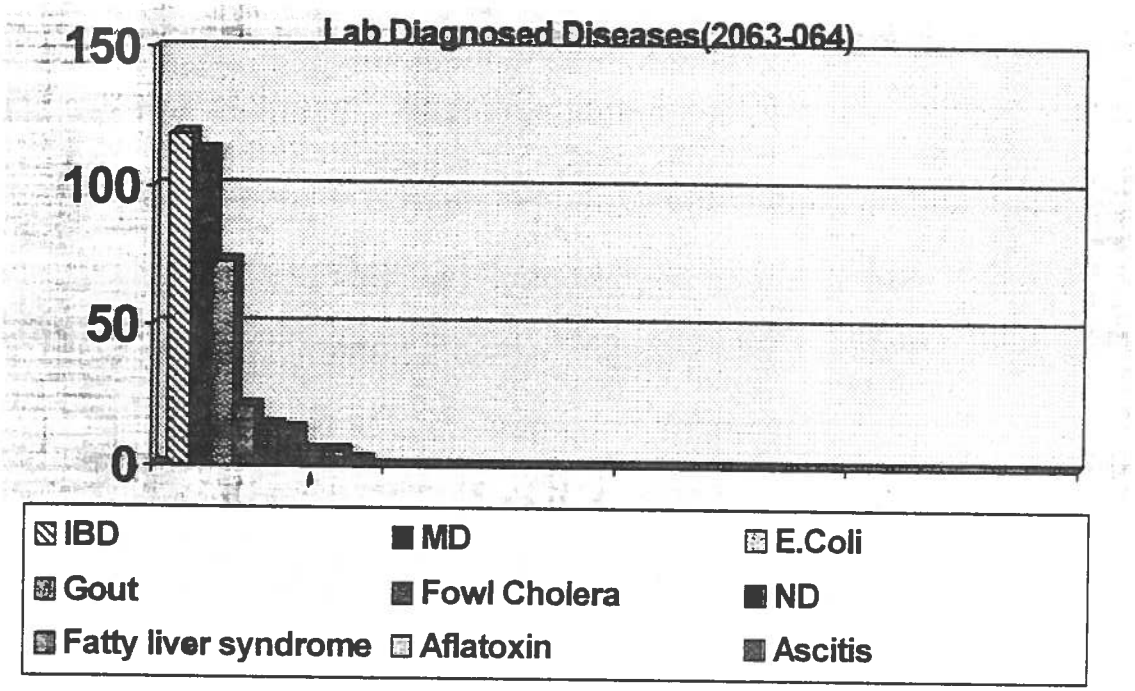
Diagram of Identi fied Bacterial disease at NAL

2. Histopathological

This unit is well equipped than that 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 no then Automatic Machines are in use.

Among them Following diseases were found, after the interpretation of prepared slides in different time.

S.No.	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%	



3. Virology & Serology Unit

Diagnosis activities of this unit also increasing day by day .Due to lack of resources we were unable to conduct various test but we will start in future(recently) .

The important and valuable testactivities:-

* Egg Inoculation for ND, MD & IBD
  Route: Allantoic Cavity
  Yolk Sac
  Chorioallantoic Membrane

The following tests are being conducted in this unit:

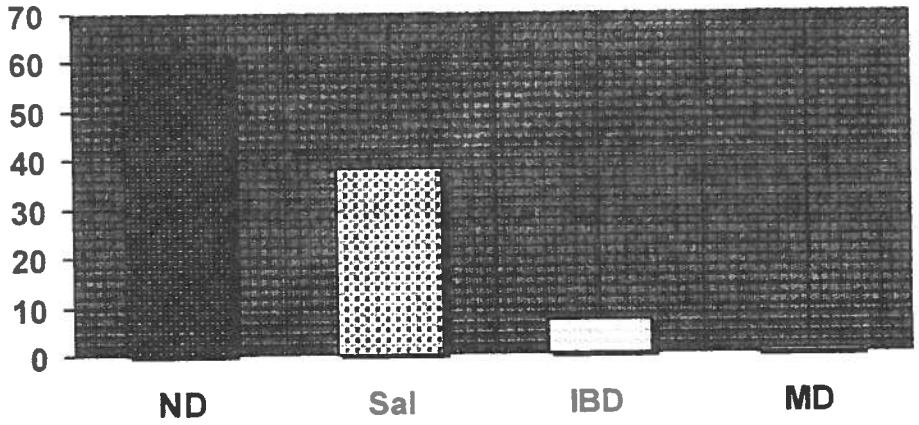
* Haemagglutination (HA) & Haemagglutination Inhibition (HI)

* Immunocomb (IB, IBD, & ND)
* Plate/Slide agglutination
* AGPT
* ELISAdue to lack of Elisa Kit it is currently not in function)
* Biochemical analysis
  - -Calcium
  - -Phosphorus
  - -Total protein
* Rapid test for AIV
* Serum / Swabs collection, preservation and dispatch to further investigation.

Table :List of viral poultry Disease being tested:

S.N.	Disease name	positive test	Percentage	Remarks
1.	ND	62	57.40%	
2.	Salmonella pullorum	38	35.18%	
3.	IBD	7	6.48%	
4.	MD	1	0.92%	
5.	Total positive test	108	100%	

Lab Diagnosed posative Cases.



Annual Program & Progress of NAL

B.T.No 40-3-500(General) (2063/064)

S.N.	Activity	unit	target	Budget allocated	progress %
1.					
1.1	Pathology	Number	3000	69000.00	100
1.2	Histopathology	,,	500	81000.00	100
1.3	Microbiology	,,	900	84000.00	100
1.4	Biochemical	,,	500	93000.00	100
1.5	Virology	,,	900	183000.00.	100
1.6	Serology	,,	1000	121000.00	100
2	Laboretory maintainance				1
2.1	Lab.water system	Times	1	56000.00	100
2.2	Lab.Animal	,,	1	37000.00	100
3	Investigation & surveillance of Avian Disease				
3.1	Epidemic Investigation	,,	3	70000.00	100
4	Reporting & Publication				
4.1	Annual Technical Report	,,	1	23000.00	100
4.2	Epidemiological Reporting	,,	24	12000.00	100

Annual Program & Progress of NAL

B.T.No 40-3-220/40-4-220(WTO) (2063/064)

S.N.	Activity	unit	target	Budget allocated	progress %
1	Lab Top computer	Number	1	75000.00	100
2	A/C	,,	1	75000.00	100
3	Digital Camera & Fax Machine	,,	1	50000.00	100
4	Laboretory bulding Construction & maintenance	,,	1	200000.00	100
5	Officer label TADS Analysis Training	,,	1	10000.00.	100
6	TADS Investigation	,,	3	235000.00	100

Man Power Situation of National Avian Laboratory , Bharatpur.
(F/Y 063-064)

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.	Vet. 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.	Peon	-	2	2	-	
TotalAdministration			4	4	-	
Grand Total			11	11	-	

Staff of National Avian Laboratory,Bharatpur .
(At the end of F/Y 2063-064)

S.N.	Designation	Post	Class	Remai
1	Dr. Tika Ram Neaupane	SVO	G.II	
2	Dr. Daya Ram chapagain	VO	G III	
3	Dr.Peetambar S.Kushwaha	VO	G III	On Le
4	Mr. Endu Raya Yadav	VT	NG I	
5	Mr. Shailendra Bhandari	VT	NG.I	
6.	Mr.Ram Pd. chaudhari	JVT	NG II	
7	Mr. Deo Kumar Yadav	JVT	NG II	
Administration/Account				
8	Mr. Bishwo Nath Adhikari	Asst.Accountant	N GII	
9	Mr.Rishee Ram Acharya	Kharidar	NG II	
10	Mr.Bhanu Bhakta Sapkota	Peon	Lo.lev.	
11	Mr.Purna Prasad Sapkota	Peon	Lo.lev	

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 until1990/1991, 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 1991/19992, the RVL has its separate identity. There was provision of manpower and other logistics. The program 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, Terahthum, 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 underÜêhis 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.

To provide proper laboratory diagnosis and improve in the quality of veterinary services, the government has established five regional laboratories, one in each development region of the country. Primary laboratories in 55 and basic laboratories in 15 districts have been established to improve and upgrade existing disease diagnosis system.

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 program.
- 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.
- To support animal health and infertility camps in the region.

Annual Progress Report (2063/2064)

S.N.	Program	Unit	Annual target	Annual Progress	Annual weightage	Progress %
1	**Laboratory services**					
1.1	Parasitological examination	No.	2000	2548	7.13	100%
1.2	Microbiological examination	"	600	1162	7.89	100%
1.3	Pathological examination	"	300	418	3.79	100%
1.4	Serological examination	"	500	718	5.61	100%
1.5	Haematological examination	"	300	406	5.46	100%
1.6	Biochemical examination	"	900	1933	7.28	100%
1.7	Sample sent to other lab.	"	400	1977	6.37	100%
2	**Investigation and Surveillance Program**					
2.1	Study on infertility	Times	6	6	13.66	100%
2.2	Epidemic investigation	"	6	6	22.31	100%
3	Supervision and monitoring of DLSOs	"	6	6	10.62	100%
4	**Veterinary Disease Investigation Workshop**	"	1	1	4.86	100%
5	**Publication and epidemic reporting**					
5.1	Publication of Six monthly epidemiological bulletin	"	2	2	2.12	100%
5.2	Publication of annual epidemiological bulletin	"	1	1	1.97	100%
5.2	Publication of annual technical report	"	1	1	0.91	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 brought 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 2063/064, altogether 2301 fecal samples from different species of animal were received and examined. Among 2301 samples, 1721 samples (74.8%) were positive and 580 samples (25.2%) showed negative result. The result of fecal test revealed that fasciolosis (54.9%) is the most prevalent parasitic infestation followed by paramphitomiasis (26.2%) and nematodiaisis, the least.

Figures of fecal samples examined and types of parasites revealed in this test are followed:

Months	Total Sampes	(+) ve	(-) ve	L.F	Param	Cocci	oeso	Haemo	Ascari	Other
July	195	160	35	89	42	-	11	6	1	11
Agst	171	139	32	92	30	-	10	3	1	8
Sept	174	145	29	93	26	-	9	3	3	17
Oct	170	139	31	58	37	-	11	5	3	20
Nov	122	74	48	46	19	2	4	-	-	7
Dec	70	52	18	29	10	-	5	-	1	5
Jan	51	33	18	17	11	-	4	-	-	1
Feb	275	197	78	83	61	25	12	-	4	7
Mar	170	88	82	45	27	-	6	4	4	5
Apr	255	182	73	109	44	4	13	2	6	9
May	489	381	108	201	116	3	12	7	15	11
June	159	131	28	83	29	5	11	2	2	4
Total	2301	1721	580	945	452	39	108	32	39	105

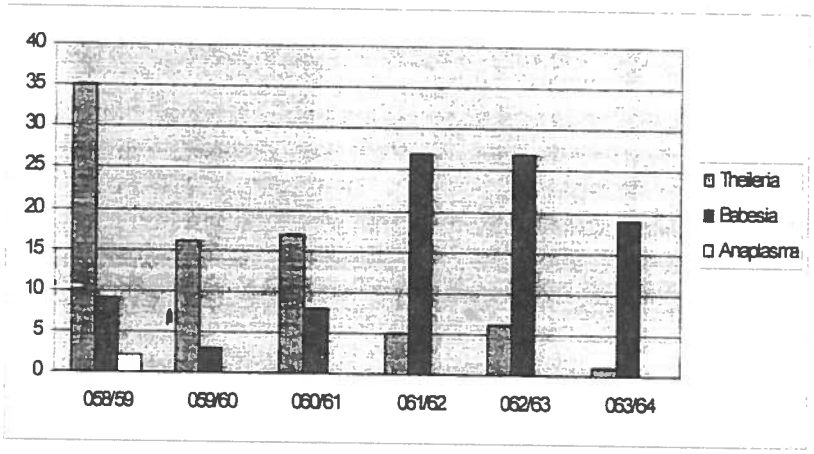
Altogether 17 samples of skin scraping from different species of animals were received and examined. Out of 17, 3 samples were positive (2 sarcoptes and 1 demodex).

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 230 samples were examined for blood parasites. Out of them, 20 samples were positive in which babesioisis was dominant having 19 positive samples. Rest 1 sample was of theileriosis. There is no incidence of anaplasma in the blood samples brought here in fiscal year2063/064.

Comparative chart of blood parasites
confirmed in RVL, Biratnagar
(From F/Y: 058/59 to 063/64)



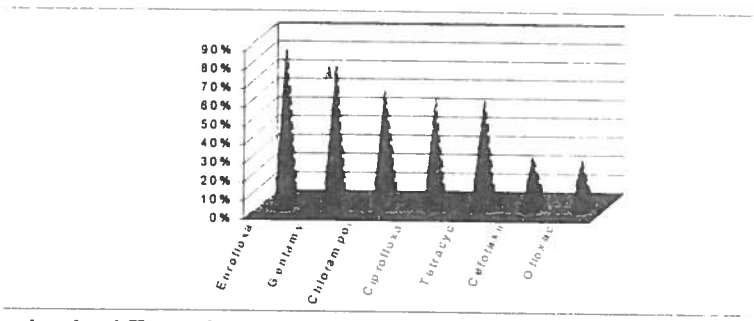
Microbiological Examination:

Altogether 1162 milk samples were registered in this laboratory in the fiscal year 2063/064.Out of them 412 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 (Month wise) 2063/064

Months	Total sample	CMT/MWT Positive	CMT/MWT Negative	% of positive
July	96	57	39	59.4
August	135	89	46	65.9
Sept.	70	41	29	58.5
Oct.	164	97	67	47.4
Nov.	152	38	114	25
Dec.	40	16	24	40
Jan.	24	4	20	17
Feb.	62	12	50	19
March	144	20	124	14
April	117	20	97	17
May	98	47	51	48
June	60	44	16	73
Total	1162	485	677	41.7%

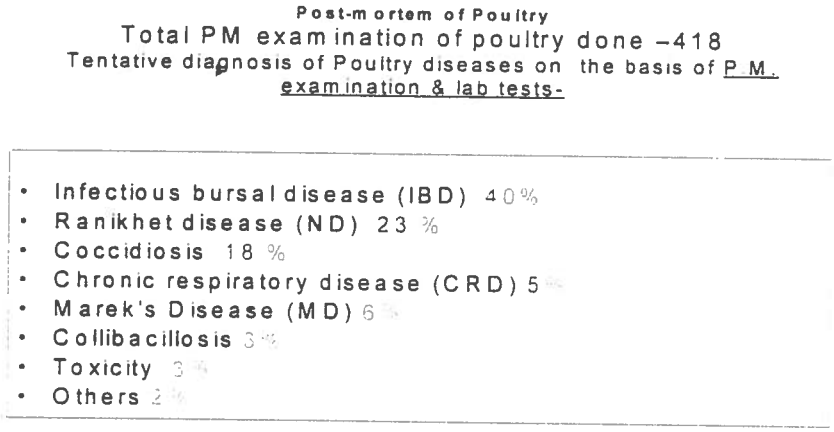
Antibiotic sensitivity Test:



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. Tissues that are collected for histopathological examination are being sent to CVL, Kathmandu.

Altogether 418 dead birds were brought to the lab. On the basis of PM examination and lab tests, diagnosis is done. Out of 418 samples, IBD had higher incidence followed by Ranikhet and Coccidiosis.



Biochemical examination:

Examination of urine and analysis of blood is routinely done to asses the different conditions of urine and blood constituents. Serum samples are collected from farmers, sites of investigation program, etc. Altogether 1933 serum samples were collected and analyzed in the fiscal year 2063/064 for the estimation of total protein, glucose, phosphorus, zinc 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 2063/064 altogether 311 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 400 animals (cattle and goat) and all the samples were negative for this test.

Hypersensitivity test (Tuberculin test) is also done in cattle, buffalo and elephant to screen the animal diseased with tuberculosis. Altogether 7 animals were tested and all the animals examined were found negative for this test.

Sample sent to CVL, Kathmandu in F/Y 2063/064:

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. FMD suspected samples are also sent to FMD laboratory.

S.N.	Type of samples	Number
1	Bird serum	528
2	Bird swab	506
3	Samples of diff.organ (for histopathological exam.)	11
4	Whole blood	11
5.	Sputum (elephant)	2
6.	Serum for PPR	870
7.	FMD	34
8.	Blood smear	11
9.	Mules swab /serum	20
10	Maize and Gram	4
Total		1997

S.N	District	Vaccine dose
1	Jhapa:	3200
2	Sankhuwa:	5000
3	Bhojpur	1500
4	Dhankuta:	4700
5	Saptari:	26600
6	Siraha:	3600
7	Morang	1000
8	Udaypur	10000
9	Panchthar	400
10	sunsari	1000

PPR Vaccine Distribution
At the start of F/Y 2063/64, total dose: **99,300**
Vaccine distributed dose: **57,000**
Remaining dose: **42,300**

Regional Veterinary Laboratory, Biratnagar
National PPR Programme
Sero-monitoring
F/Y-2063/064

LSOs	Udaypur	Sunsari	Morang	Siraha	Saptari	Dhankutta	Illam	Jhapa	Total
accination to e done (No.)	14000	18000	18000	18000	18000	14000	14000	18000	132000
erum to be ollected (No.)	70	90	90	90	90	70	70	90	660
ollected erum (No.)	70	90	90	90	90	70	70	90	660

Bird flu surveillance in EDR
RVL, Biratnagar
(F/Y:2063/64)

S.N	District	Type of bird	Type of farming	Type of sample		Total
				Serum	Swab	
1	Jhapa	Poultry Duck	B & C B	175 5	125 T & 7C 24 T -----	307 29
2	Morang	Poultry Duck	B &C B	107 8	129T &153C — ——	389 8
3	Sunsari	Poultry Duck	B &C B	42 15	—— —— —— ———	42 15
4	Saptari	poultry	C	48	—— ——	48
5	Siraha	Poultry	C	17	—— —	17
6	Dhankuta	Poultry	C	44	—— —	44
Total				521	278 T &160C	899

Abbreviations:

T: Tracheal swab
C: Clocal Swab
B: Backyard poultry
C: Commercial poultry

Sample Positive for PPR confirmed by OVI
(Received from DLSO and QRT), 2063/064

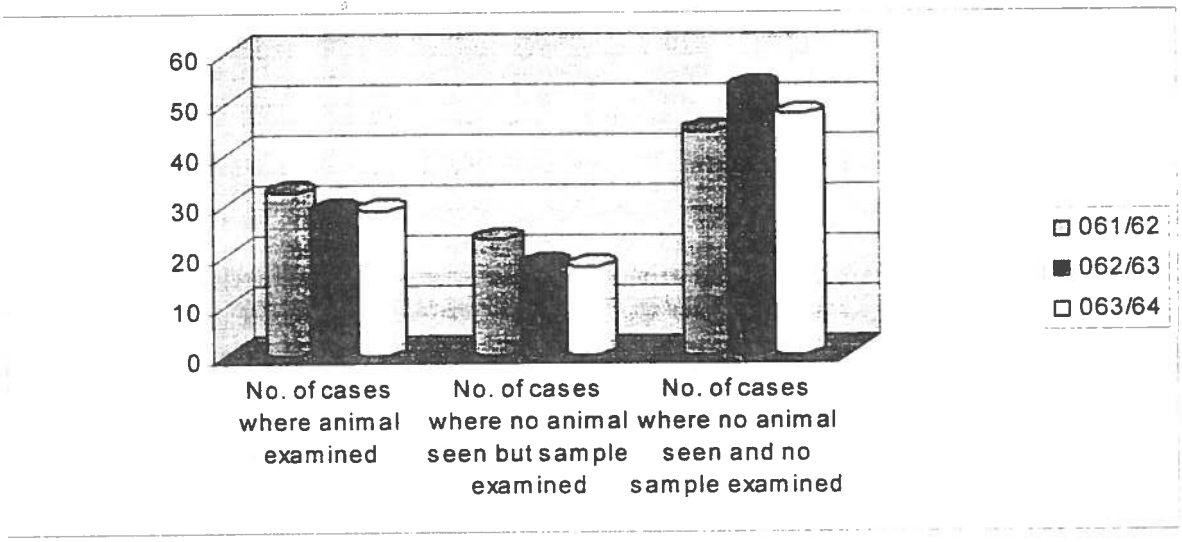
S.N	DLSO	Type of sample	Total sample	Test Report received	Positive sample
1	Jhapa	Serum	65	65	10
2	Saptari	Serum	95	66	24
3	Udaypur	Serum	25	25	20
4	Dhankuta	Serum	18	18	15
5	Solukhumbu	Serum	19	19	4
6	Terathum	Serum	25	1	0
7	Sunsari	Serum	77	51	29
8	Taplejung	Serum	25	25	5
9	Bhojpur	Serum	20	20	0
10	Morang	Serum	19	0	0
11	Okhaldhunga	Serum	29	0	0
12	Siraha	Serum	20	18	18
13	Rani Quarantine	Serum	61	23	0
14	Urlabari Institute	Serum	4	4	1
Total			622	337	126

Epidemiological Reporting Of EDR

Total no. of animals treated, EDR
F/Y 2063/64

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	9262	7515	4546	21323
2	Sunsari	12664	7005	9181	28850
3	Jhapa	4629	3871	3047	11547
4	Siraha	34113	1902	8672	44687
5	Saptari	8373	6584	7489	22446
6	Tehrathum	4920	2777	10177	17847
7	Dhankuta	1873	3055	9595	14523
8	Bhojpur	7371	2807	16813	26991
9	Ilam	6430	5999	9585	22014
10	Panchthar	1806	4128	16844	22778
11	Udayapur	11413	2857	21935	36205
12	Okhaldhunga	4479	3096	7739	15314
13	Khotang	6073	4239	11550	21862
14	Solu	1512	991	2768	5271
15	Taplejung	3565	1141	9964	14670
16	Sankhuwasabha	2565	4091	16994	23650
	Total	121048	62058	166899	350005
	Percentage	36.6	17.7	47.7	

Comparative Chart of Types of Treatment provided in EDR
(F/Y: 061/62 to 063/64)



Vaccination done in EDR

(Based on epidemiological report)

Distraict	B.Q.	H.S.	Anthrax	FMD	Rabies	S. fever
Morang	2181	4731	-	150	162	-
Sunsari	----	8700	-	538	24	1000
Dhankuta	----	179	-	60	-	182
Jhapa	5103	8686	-	628	150	-
Terhathum	1701	1789	-	-	12	-
Sankhuwasabha	11021	10811	-	562	96	82
Bhojpur	11235	11235	-	96	393	-
Ilam	5771	5571	-	???	364	-
Panchthar	3760	3760	-	-	449	-
Taplejung	1353	1353	-	-	205	-
Solu	80	80	-	-	-	-
Okhaldhunga	----	870	-	-	56	-
Khotang	4855	4855	-	-	131	-
Udayapur	12930	12930	550	-	34	-
Siraha	24527	24627	-	-	48	-
Saptari	20701	20701	-	1077	94	-
Total	105218	120878	550	3111	2218	1264

REGIONAL VETERINARY LABORATORY
(CENTRAL REGION) JANAKPUR

1. 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 is achieved through its various laboratory units; pathology, parasitology, and microbioliogy; haematology and biochemistry. Serological and histopathological laboratory test results are obtained by dispatching the relevant specimens to CVL as these diagnostic facilities are not available in RVL, Janakpur.

2. Programme and progress

Programme and progress, RVL, Janakpur (2063/064)

S. N.	Programs and activities	Annual target			Annual Progress	
		Unit	Target	Weightage (%)	Progress	Weightage (%)
1	**Laboratory Services**					
1.1	Parasitological Examination	Number	1000	5/232	178	5/232
1.2	Microbiological Examination	Number	500	6/726	305	6/726
1.3	Pathological Examination	Number	300	3/737	254	3/737
1.4	Serological Examination	Number	300	4/484	315	4/484
1.5	Hematological Examination	Number	200	6/726	454	6/726
1.6	Biochemical Examination	Number	100	5/531	290	5/531
1.7	Sample collection and dispatch	Number	300	2/99	227	2/99
2	**Disease investigation and surveillance**					
2.1	Investigation of infertility in Buffalo	Times	6	27/95	6	27/95
2.2	Investigation of Epidemics	Times	6	19/43	6	19/43
3	Monitoring and supervision					
3.1	Monitoring & supervision of district labs of DLSOs	Times	6	5/979	6	5/979
4	Epidemiological Reporting & publication					
4.1	Quarterly Epidemiological Bulletin publication	Times	4	3/737	4	3/737

4.2	Annual technical report publication	Times	1	0/747	1	0/747
5	Technical workshop					
5.1	Technical workshop on disease investigation	Times	1	5/232	1	5/232
6	**Purchase Program**					
6.1	Purchase of scientific books	Times	1	1/495	1	1/495

3. Laboratory Services

3.1 Parasitology

3.1.1 Coprological examination

In the F/Y 2063/064 altogether 178 Faecal samples from different species of animals such as cattle, buffalo, goats, poultry and dogs were examined. Qualitative Feacal examination of 153 and EPG counts of 25 Feacal samples were counted. Fasciola, paramphistomum, strongyle, Trichuries were major internal parasites identified. Detail description of results of monthly feacal examination during 2063/064 is presented in table 1.

Table 1: Result of monthly feacal examination F/Y 2063/064

Parasites	Months (As per Nepalese Fiscal Year)												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Fasciola	1	1	2	2	2	3	1	-	-	-	3	-	15
Paramphistomum	10	8	6	3	5	6	5	6	9	7	5	10	80
Strongyles	4	2	4	3	-	-	2	-	1	3	2	6	27
Trichuris	2	1	-	-	-	1	-	-	1	2	-	-	7
Mixed infection	3	1	2	-	3	-	2	1	-	-	1	1	14
Total +ve	**20**	**13**	**14**	**8**	**10**	**9**	**10**	**7**	**11**	**12**	**11**	**17**	**143**
Total -ve	**2**	**-**	**1**	**-**	**-**	**2**	**6**	**4**	**5**	**3**	**12**	**-**	**35**

3.1.2 Skin scrapping Examination

Thirty one skin scrapings from buffaloes, buffalo Calf, Ox and Goat were found positive for different external parasites.

Out of that, 9 were from buffaloes, 10 from buffalo calves, 5 from Ox and rest 7 from goats. The detail of the skin scrapping test result is given in table 2.

3.2 Microbiology

A total of 305 samples were received for bacteriological examination among which 181 samples were found positive for different species of bacteria and the rest samples were found negative and details of the tested samples has been present in table 3. Similarly, efficacy of different antibiotics has been shown in table 4.

Table 2: Test result of sking scrappings

Month	Total Sample	Species of Animals				Parasites (Species)
		Buffalo	Buffalo calf	Ox	Goat	
Shrawan	2	1	1	-	-	*Sarcoptes*
Bhadra	3	-	-	1	2	*Sarcoptes* *Psoroptes*
Ashoj	1	1	-	-	-	*Sarcoptes*
Kartik	2	1	1	-	-	*Sarcoptes*
Mansir	3	1	1	-	1	*Sarcoptes*
Poush	2	-	2	-	-	*Sarcoptes* *Psoroptes*
Magh	2	-	1	1	-	*Sarcoptes*
Falgun	3	1	1	-	1	*Sarcoptes*
Chaitra	4	1	1	1	1	*Sarcoptes* *Psoroptes*
Baishakh	5	2	1	2	-	*Sarcoptes*
Jestha	2	1	-	-	1	*Sarcoptes*
Ashadha	2	-	1		1	*Sarcoptes*
Total	**31**	**9**	**10**	**5**	**7**	

Table 3: Monthly receipt of samples at microbiology unit

Duration	No. of Samples	Positive	Negative	Percentage(+ve)
Shrawan	5	3	2	60
Bhadra	20	16	4	80
Ashoj	24	15	9	62.5
Kartic	52	45	7	86.54
Mansir	12	7	5	58.33
Poush	20	12	8	60
Magh	30	19	11	63.33
Falgun	40	30	10	75
Chaitra	26	5	21	19.23
Baishakh	28	10	18	37.71
Jestha	30	4	26	13.33
Ashadha	18	15	3	83.33
Total	**305**	**181**	**124**	**59.34**

Table 4: Antibiotic sensitivity test result

Antibioptics used	Percent efficacy
Enrofloxacin	80%
Ciprofloxacin	73%
Gentamycin	73%
Norfloxacin	68%
Ofloxacin	65%
Oxytetracyclin	50%
Amoxicillin	45%
Penicillin	25%

3. Haematology

A total of 454 blood samples were examined for different blood parameters as well as for blood parasites. Out of 454 samples, 409 blood samples were found Negative for any blood parasites & rest 45 were found positive for different blood parasites. The details are given in the following table No. 5

Table 5: Details of blood sample examination

Month	Total Sample	Anaplasma	Babesia	Theileria	Tryps	- ve
Shrawan	7	0	-	-	-	7
Bhadra	30	2	2	-	-	26
Ashoj	2	1	1	-	-	-
Kartik	112	5	3	-	-	104
Mansir	20	2	2	-	-	16
Poush	25	-	-	-	-	25
Magh	40	-	-	-	-	40
Falgun	67	1	-	-	-	66
Chaitra	25	-	1	-	-	24
Baishakh	35	-	1	-	-	34
Jestha	40	-	4	-	3	33
Ashadha	51	6	6	-	5	34
Total	**454**	**17**	**20**	**-**	**8**	**409**

4. Pathology

The pathological examination includes Post Mortem Examination of the dead animals. A total of 254 cases of Post Mortem Examination were presented during the F/Y 2063/064. All the cases received were birds. No cases of large or small ruminants and other species of animals were received. It is mentioned that all the samples of birds received were brought from the Janakpur Municipality and its adjacent areas. The status of poultry disease in the area is presented in table 6.

Table 6: Trend of disease occurrence in poultry (2063-064)

S. N.	Tentative diagnosis	Total Cases	
		Number	Percent
1.	Coccidiosis	102	40.16
2.	CRD	83	21.68
3.	IBD	33	12.99
4.	Colisepticaemia	14	5.51
5.	Aflatoxicosis	12	4.72
6.	Miscellaneous (parasites, enteritis, mixed in infection)	7	2.76
7.	Salmonellosis	3	1.18
Total		**254**	**100%**

5. Serology

In this unit the serum samples are collected from different districts during disease investigation and surveillance program. Most of the serum samples collected from livestock and poultry were dispatched to CVL and referred laboratories for diagnosis, serum monitoring of vaccinated goat and poultry were also done with the help of CVL. Detail of the serological test results has been shown in table 7.

Table 7: Serological test results

S.N.	Districts	Animal Spp.	No. of Sample	Test requested	Result		Remarks
					+ve	-ve	
1.	Dhanusha	Goat	92	PPR	20	72	sent to CVL
2.	Mahottari	Goat	52	PPR	13	39	sent to CVL
3.	Sarlahi	Goat	31	PPR	13	18	sent to CVL
4.	Bara	Goat	56	PPR	6	50	sent to CVL
5.	Ramechhap	Goat	9	PPR	-	-	sent to CVL
6.	Parsa	Buffalo	35	Brucellosis	-	-	not confirmed
7.	Dhanusha	Buffalo	45	Rinderpest	-	-	serosurveillance
8.	Mahottari	Pig	15	Swin Fever	-	-	not confirmed
9.	Dhanusha	Poultry	17	Bird flu	-	17	sent to CVL
10.	Mahottari	Poultry	11	Bird flu	-	11	sent to CVL
11.	Sarlahi	Poultry	18	Bird flu	-	18	sent to CVL
12.	Bara	Poultry	20	Bird flu	-	20	sent to CVL
13.	Parsa	Poultry	10	Bird flu	-	10	sent to CVL
14.	Makawanpur	Poultry	20	Bird flu	-	20	sent to CVL

REGIONAL VETERINARY LABORATORY
(WESTERN REGION) 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 reas of Nepal. The region shares boundaries with Uttar Pradesh of India in the southand 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 fi bers 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, Hemorrhegic 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 Offi ces, 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 technicins 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 profi le of livestock and poultry diseases and maintain and dessiminate the regional epidemiological infromation 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 form 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 diversifi ed veterinary Laboratory test facilities for the farmers, private veterinary practicinors and district Livestock Service Offi ce 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, fl oatation techniques where necessory. In parasitology unit it not only identify the parasites involved in the infestation but also quantify the prasitic 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 perform microscopic examination of skin scrappings for the identification of mange mite species.

It conducts blood parasite test using blood semar examination and for the blood fi laria examintion using Knot's method.

2. **Microbiological Unit :** Microbiology unit tests diversifi ed samples like milk, tissues, blood, aspirated fluids and tissues etc. Both aerobic and anaerobic culture facilites 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 perform antibiotic susceptibility test and advice for the appropriate antibiotic for the treatments.

   In virology the laboratory is capable for the isloation 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 necopsy of about 900 animals and birds mostly poultry birds.

   It perform 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 intoduce 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, Cretinine, Liver function test with other enzymes etc.

   It is performing the Urine tests by estimating Albumine, Bilirubin, 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 2063/64 & Budget No: 40-4-500/40-3-500)

S. No.	Program and Activities	Annual Target			Annual Progress	Weight-age (%)	Re-marks
		Unit	Target	Weight-age (%)			
1	**Laboratory Services**						
1.1	Parasitological Examinations	Nos.	800	8.07	1048	8.07	
1.2	Microbiological Examinations	Nos.	400	6.78	509	6.78	
1.3	Pathological Examinations	Nos.	700	3.86	840	3.86	
1.4	Serological Examinations	Nos.	400	3.74	1525	3.74	
1.5	Hematological Examinations	Nos.	150	3.34	209	3.34	
1.6	Biochemical Examinations	Nos.	200	3.98	246	3.98	
1.7	Sample collection and dispatch	Nos.	500	8.65	677	8.65	
2	**Disease Investigation and Surveillance Program**						
2.1	Investigation of Nematodiasis in Goats	Times	12	19.18	13	19.18	
2.2	Investigation of Epidemic	Times	15	24.21	16	24.21	
3	**Monitoring and Supervision**						
3.1	Monitoring and Supervision of district based Laboratories	Times	10	7.95	11	7.95	
4	**Computer Training**	Per-sons	3	1.17	3	1.17	
5	**Animal Disease Investigation Interaction Workshop**	Times	1	3.13	1	3.13	
6	**Publication Program**						
6.1	Half Yearly Epidemiological Bulletin publication program	Times	2	2.34	2	2.34	
6.2	Annual Technical Report Publication	Times	1	2.34	1	2.34	
7	**Purchase of Scientific books**	Nos.	4	1.08	4	1.08	

Table 2: Annual work Program and summary of achievements of Regional Veterinary Laboratory, Pokhara (for fiscal year 2063/64 & Budget No: 40-4-220/40-3-220) (WTO Programme)

S. No.	Program and Activities	Annual Target			Annual Progress	Weight-age (%)	Re-marks
		Unit	Target	Weight-age (%)			
A	**Capital Expenditure**						
1	Laptop Purchase	Nos.	1	13.27	1	13.27	
2	Microscope Purchase	Nos.	1	13.27	1	13.27	
3	Refrigerator Purchase	Nos.	1	8.85	1	8.27	
B	**Recurrent Expenditure**						
1	Officer Level TADs, Communication and Risk Analysis Training	Per-son	1	1.77	1	1.77	
2	TADs Investigation	Nos.	3	23.01	209	23.01	
3	PPR Survillence	Nos.	4	39.82	246	39.82	

1. Laboratory Services

1.1 Parasitological Examinations

Faecal samples were examined adopting both qualitative and quantitative methods. In the fiscal year 2063/64 altogether 1048 faecal samples from different species of animals such as cattle, buffalo, sheep, goats, dogs and poultry were examined. Out of 1048 faecal samples examined 356 samples were found to be positive for various internal parasites. Total number of faecal samples of goats examined for EPG count was 57. Upto 6000 EPG of GI nematodes was found in the goats of Syanja and Tanahu districts. Fasciola, Paramphistomum, Coccidia, Strongyloides, Strongylus, Trichuris and Monezia were major internal parasites identifi ed. Two hundred seventy three faecal samples were negative for any parasites. The results of monthly examinations of faecal samples are presented in Table 2.

Twelve skin scrapings from goats; cattle, Horses and dogs were received for the examination and identification of mites. The Four positive samples revealed 3 Sarcoptic and 1 Demodectic species of mites in cattle/goat and dogs respectively.

Table 3: Monthly faecal examination results during 2063/64

Parasites	Months												Total	Total in %
	Shrawan	Bhadra	Aswin	Kartik	Mangsir	Poush	Magh	Falgun	Chaitra	Baisakh	Jesth	Asar		
Fasciola	2	5	4	3	2	0	6	4	0	3	0	2	31	8.8
Paramphistome	4	3	4	2	5	3	2	2	1	3	4	6	39	10.9
Ascaris	0	2	2	0	0	0	0	3	0	0	0	0	7	1.9
Strongyle	5	3	4	13	2	0	0	0	7	0	0	22	56	15.7
Strongyloides	3	0	2	0	3	1	0	0	0	0	3	4	16	4.5
Trichuris	0	0	0	0	0	2	0	0	0	0	0	2	4	1.1
Moneizia	0	0	1	2	0	1	0	0	0	0	0	0	4	1.1
Coccidia	7	9	8	12	22	33	12	18	18	3	18	8	168	47.2
Mixed infections	3	0	1	4	2	0	7	0	1	3	0	1	22	6.3
Others (B.coli)	0	1	2	1	0	3	0	0	0	2	0	0	9	2.5
Total positive	24	23	28	37	36	43	27	27	27	14	25	45	356	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 2063/64 a total of 509 samples were examined in microbiology unit of the laboratory. Out of them 305 sample were milk for the test of mastitis. All these 305 sample were subjected for the Sodium Lauryl Sulphate Test (SLST) screening test for detecting mastitis in the animals. In SLST test of milk sample about 154 samples of animals were found to be positive and then these 154 sample were sujected for the microbiological culture. In the culture 114 sample of milk showed bacterial growth which is summerized in the following table.

Table 4. Bacterial isolation and their percentage in the mastitic milk samples.

Serial No.	Name of the Bacteria isolated	No. of sample Positive	Percentage of Positive sample
1	*E. coli*	63	40.90
2	*Streptococcus spps.*	23	14.93
3	*Proteus spps.*	8	5.20
4	*Staphylococcus spps.*	8	5.20
5	*E. coli + Streptococcus spps.*	6	3.90
6	*E. coli +Bacillus spps.*	2	1.30
7	*E. coli + Staphylococcus spps.*	2	1.30
8	*Bacillus spps. + Staphylococcus spps.*	2	1.30
9	No Growth	40	25.97
Total		154	100.00

For culturing the milk sample the laboratory used Nutrient Agar, Blood Agar and MacConkey Agar. After the growth the bacteria were identifi ed by colony morphology, Gram characteristics, and Certain Biochemical characteristics like, Oxidase test, Catalase test etc. The following is the compilation of Bacterial isolation performed in the RVL, Pokhara from the year 2056/57 to 2063/64.

Table No. 5 : Major Bacteria isolated from the milk sample in RVL, Pokhara from Fiscal Year 2056/57 to 2063/64.

Bacteria Isolated	Number of Isoation (No.in Paranthesis are percentage of Isolated)							
	FY 2056/57	*FY 2057/58*	*FY 2058/59*	*FY 2059/60*	*FY 2060/61*	*FY 2061/62*	*FY 2062/63*	*FY 2063/64*
E. coli	34 (18.88)	34 (19.31)	33 (18.43)	26 (19.26)	34 (18.78)	33 (22.97)	54 (33.13)	73 (47.40)
Staphylococcus sps.	45 (25.00)	42 (23.86)	41 (22.90)	30 (22.22)	47 (25.96)	28 (18.92)	38 (23.31)	12 (7.80)
Streptococcus sps.	35 (19.44)	35 (19.88)	36 (20.11)	16 (11.85)	21 (11.60)	17 (11.48)	17 (10.43)	29 (18.83)
Bacillus sps.	-	-	-	28 (20.74)	35 (19.33)	31 (20.94)	28 (17.17)	4 (2.60)
Proteus sps	21 (11.66)	19 (10.80)	18 (10.05)	13 (9.63)	14 (7.73)	7 (4.73)	9 (5.52)	8 (5.19)
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

All the milk sample showing the growth are then subjected for antibiotic sensitivity testing. The antibiotics used for the sensitivity test are Ampicillin, Chloramphenicol, Cephotaxime, Cotrimoxazole, Enrofl oxacin, Gentamicine and Tetracycline. Following is the table which shows the result of in vitro Antibiotic sensitivity test conducted fot the mastitic milk samples.

Table 6 : In-vitro Antibiotic sensitiviy of mastitic milk samples.

Serial No.	Antibiotic Disc Used	Highly Senitive		Intermediately Sensitive		Resistance	
		No	%	No.	%	No.	%
1	Enrofloxacin	109	94.78	5	4.34	1	0.87
2	Cephotaxime	68	59.13	28	24.34	19	16.52
3	Gentamicine	35	30.43	69	60.00	11	9.56
4	Cotrimoxazole	14	13.86	23	22.77	64	63.36
5	Oxytetracycline/Tetracycline	12	12.00	40	40.00	48	48.00
6	Ampicillin	10	9.80	13	12.74	79	77.45

The above antibiotic sensitivity pattern clearly states that almost all organisms are sensitive to Enrofl oxacin antibiotic with very small number of Intermediate sensitivity and Resistant. Similary, 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 identifi cation. One milk samples was found to be positive for Candida spp. Apart from these other pathological and clinical cases of poulty and other animals the tissue, swabs and tissue fluids were subjected to bacteriological culture. In this the laboratory conducted about 204 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*
Staphylococcus spp.	*15*
Streptococcus spp.	*9*
Bacillus spp.	*19*
Pasturella multocida	*8*
Proteus spp.	*11*
Micrococcus spp.	*3*
Enterobacter spp.	*5*
Salmonella spp	*5*
Escherichia coli	*32*
Pseudomonas spp.	*2*

In the virological works the laboratory mainly conducted virus isolation work in the suspected New Castle Disease in the poulty. Egg innoculation was conducted in the 9 day old embryo and the death of embryo was recorded. Egg innoculation for the NDV isolation was conducted in 8 different samples correlated with the ND outbreaks in commercial poultry and backyard poultry farms. The allantoic fluid was harvested and HA/HI test was conducted for the disase diagnosis.

1.3 Pathological Examinations

Pathological examinations mostly consisted of post mortem examination (PM) of animals and poultry. Eight hundred fi ve poultry, 15 pigs and piglets, 1 buffalo, 1 sheep, 1 dog, 1 cat, 5

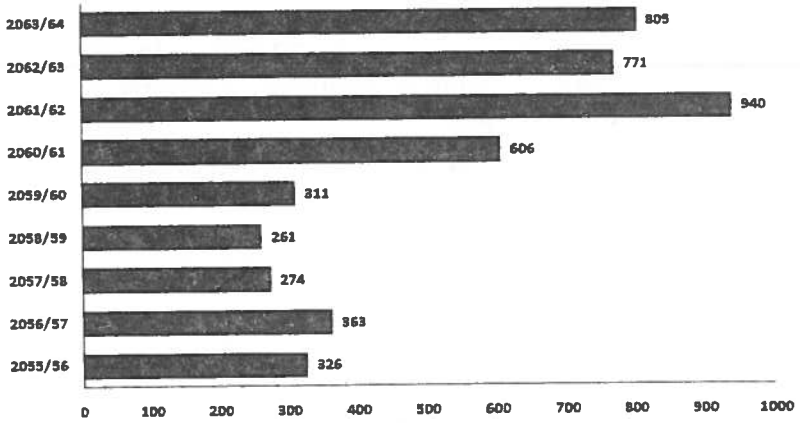
ducks, 3 wild birds and 8 goats were brought for the postmortem examination. In the pathology unit, the cause of death of chickens presented was generally drawn on the basis of both the post mortem lesions observed and laboratory investigation of samples collected during PM examinations. Some times samples collected during PM were sent to Central Veterinary Laboratory, Tripureswor for the confi rmatory diagnosis. Diseases of chickens diagnosed on the FY 26063/64 are summarized in Table 8.

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

S.No.	Disease diagnosed	No. Of Cases	Percentage of Cases
1	Infectious Bursal disease	206	25.60
2	Haemorrhagic enteritis	124	15.40
3	Colibacillosis	68	8.44
4	Coccidiosis	53	6.58
5	Pneumonitis	53	6.58
6	Ascitis	51	6.33
7	Mycotoxicosis	45	5.60
8	Hepatitis	29	3.60
9	Salmonellosis	28	3.48
10	Chronic respiratory disease	26	3.23
11	Omphalitis	17	2.11
12	Vitamin/Mineral deficiency	17	2.11
13	Nephritis	9	1.11
14	Ascariasis	8	0.99
15	Ranikhet disease	7	0.87
16	Hydropericardium	6	0.74
17	Marek's disease	5	0.62
18	Visceral gout	3	0.37
19	Miscellaneous disease	50	6.21
Total		**805**	**100**

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 2063/64 is presented in the following diagram and the most important poultry diseases diagnosed during that period is shown in the following table.

Case load of Poultry Postmortem Examination of RVL, Pokhara from FY 2055/64.



It is clear from the above graphical presentation the Poultry postmortem 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. But in the year 2063/64 it increased by 4.5 % again. 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 poultry postmortem examination.

Table No. 9: Poultry Diseases Diagnosed in Different Fiscal Years from 2055/56 to 2063/64

S. N.	Poultry Diseases Diagnosis	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
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)
2	Haemorrhegic Enteritis/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)
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)
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)
5	Ascites	-	-	-	-	10 (3.21)	9 (1.48)	-	29 (3.66)	51 (6.33)
6	Mycotoxicosis	39 (12.00)	10 (2.75)	10 (3.65)	23 (8.82)	6 (1.92)	-	16 (1.70)	32 (4.04)	45 (5.60)
7	Hepatitis	8 (2.40)	23 (6.34)	-	-	-	16 (2.64)	-	18 (2.27)	29 (3.60)
8	Salmonellosis/ Pullorum Disease/ Bacillary White Diarrhoea	17 (5.20)	23 (6.34)	9 (3.28)	11 (4.22)	42 (13.50)	20 (2.30)	27 (2.90)	33 (4.17)	28 (3.48)
9	Chronic Respiratory Disease (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)
10	Omphalitis	-	-	-	-	6 (1.92)	11 (1.81)	27 (2.90)	28 (3.53)	17 (2.11)
11	Intestinal Helminthiosis	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)
12	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)
13	Marek's Disease	5 (1.50)	20 (5.50)	-	5 (1.92)	2 (0.64)	-	1 (0.10)	4 (0.50)	5 (0.62)
14	Visceral Gout	-	4 (1.10)	-	-	-	-	-	36 (4.55)	3 (0.37)
15	Litchi Heart Disease(IBH-HP)	-	-	-	-	-	14 (2.31)	79 (8.40)	34 (4.29)	-
16	Other Poultry Diseases	41 (12.57)	33 (9.09)	22 (8.03)	54 (20.70)	37 (11.90)	41 (6.76)	159 (16.91)	127 (16.47)	152 (18.88)
Total		326	363	274	261	311	606	940	771	805

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 2063/64, the serum samples tested and their results are presented in table 10.

Table 10: Results of serum samples tested

Species of Animals	*Number of Serum Tested*	*Serum Tested for*	*Test Applied*	*Test Result*
Cow	*26*	*Brucellosis*	*RBPT*	*All Negative*
Buffalo	*13*	*Brucellosis*	*RBPT*	*1 positive*
Goat	*81*	*Brucellosis*	*RBPT*	*All negative*
Poultry	*83*	*Mycoplasmosis*	*PAT*	*11 positive*
Poultry	*1322*	*Salmonellosis*	*PAT*	*121 positive*
Poultry	*7*	*NewCastle Disease*	*HA/HI*	*5 Positive*
Total Serum Sample tested in the Serology Unit 1532				

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 209 blood samples from animals were examined for different hematological parameters. The results of the blood smear examinations revealed 1 Epierythrozone spps and *Dirofi llaria immitis* in a dog and anaplasma spp. in 1 cows.

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 184 samples were examined in biochemistry unit including 62 urine samples. Out of 62 urine samples examined, 27 were diagnosed to be haematuria ,9 proteinuria and 26 miscellaneous cases.

1.7 Sample collection and dispatch:

During 2063/64, a total of 677 serum, blood and tissue samples of different animal species and poultry were collected from the disease outbreak investigation sites. 417 various samples were subjected for the laboratory investigation at RVL, Pokhara. A total of 251 various samples were dispatched to Central Veterinary Laboratory, Kathmandu and 9 samples to National FMD and TADs laboratory, Kathmandu for confi rmatory disease diagnosis. The results of samples referred to CVL, Kathmandu and FMD laboratory, Kathmandu for laboratory investigation is presented in table 11.

Table 11: Results of samples dispatched for laboratory investigation during 2063/64

S. N.	Animal/ Bird species	District	Type of Sample	No. of samples	Investigation requested for	Samples despatched to	Lab. test	Results
1	Goat	Gorkha	Serum	10	PPR	CVL	C-Elisa	3 positive
2	Buffalo	Baglung	Mouth Lesions	2	FMD	FMD& TADs Lab	ELISA	Report Not Received
3	Goat	Kaski	Serum	7	PPR	CVL	C-Elisa	5 Positive
4	Goat	Tanahun	Serum	7	PPR	CVL	C-Elisa	3 Positive
5	Goat	Myagdi	Serum	16	PPR	CVL	C-Elisa	12 Positive
6	Poultry	Kaski	Proventiculus, Liver, Brain, Sciatic Nerve	5	Marek's Disease	CVL	Histo-patholoy	Report not received
7	Goat	Lamjung	Serum	3	PPR	CVL	C-Elisa	Both Negative
8	Goat	Kaski	Serum	7	PPR	CVL	C-Elisa	4 Positive
9	Ducks	Kaski	Cloacal Swab	15	AI	CVL	Rapid Test	All Negative
10	Poultry	Kaski	Cloacal Swab	30	AI	CVL	Rapid Test	All Negative
11	Ducks	Kaski	Serum	15	AI	CVL	ELISA	Report not received
12	Poultry	Kaski	Serum	30	AI	CVL	ELISA	Report not received
13	Cattle	Baglung	Tongue Lesion	3	FMD	FMD & TADs lab	ELISA	Report not received
14	Goat	Tanahun	Serum	8	PPR	CVL	c-ELISA	5 Positive
15	Poultry	Kaski	Tracheal Swab	2	AI	CVL	Rapid test	Both Negative
16	Poultry	Kaski	Cloacal Swab	1	AI	CVL	Rapid Test	Negative
17	Poultry	Kaski	Dead Broiler	2	AI/ND	CVL	HA/HI	ND Positive
18	Poultry	Kaski	Tissue Sample	5	Marek's Disease	CVL	Histopathology	Report Not Received
19	Poultry	Myagdi	Harvested Allantoic fluid	2	ND	CVL	HA/HI	2 Positive
20	Poultry	Kaski	Harvested Allantoic fluid	2	ND	CVL	HA/HI	2 Positive
21	Goats	Mustang	Mouth lesions	3	FMD	FMD& TADs Lab	ELISA	3 Positive
22	Poultry	Kaski	Bacteria in cultrue plates	23	Salmonella	CVL	Bacterial Identification	All Negative for Salmonella species
23	Poultry	Kaski	Bacteria in Selenite Broth	12	Salmonella	CVL	Bacterial Identification	All Negative for Salmonella sps.
24	Goats	Gulmi	Serum	8	PPR	CVL	c-ELISA	2 Positive

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 fi nd 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 confi rmation of disease diagnosis. Samples, not possible to process in this laboratory were referred to CVL, Kathmandu.

A total of 15 epidemics were investigated during 2063/64. Out of 15 outbreaks investigated, some were confi rmed by laboratory while others confi rmations were based on clinical signs and postmortem fi ndings. The outbreaks fi ndings are presented in Table 12.

Table no: 12 Disease Outbreak Investigated during 2063/64.

S. N.	Outbreak Month /Year	District	Species of animal affected	Disease diagnosed
1	Shrawan	Kaski	Buffalo	HS
2	Bhadra	Baglung	Buffalo and OX	FMD
3	Aswin	Myagdi	Goats	PPR
4	Aswin	Baglung	Buffalo	H.S.
5	Aswin	Gorkha	Buffalo	HS
6	Kartik	Mustang	Goat	FMD
7	Mangshir	Lamjung	Goats	PPR
8	Manshir	Tanahun	Goats	Nematodiasis
9	Poush	Kaski	Goat	Cold inflicted Pneumonia
10	Magha	Kaski	Goats	PPR
11	Falgune	Tanahun	Goats	PPR
12	Chaitra	Baglung	Buffalo	Infertility
13	Baisakha	Syangja	Cattle	Infertility
14	Baisakha	Kaski	Poultry	Salmonellosis
15	Jesth	Kaski	Poultry	ND
16	Asad	Kaski	Pigs	Salmonellosis

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, Begnas lake, Rupa Lake, different poultry farms and poultry supplers of kaski district, was visited and some of them were interviewed. Various samples like serum, tracheal swabs and cloacal swabs in antibiotic PBS were collected. The same is conducted in various farms and village poultry of Palpa, Syangja, Tanahun, Parbat and Baglung districts. The collected swab samples were pooled and tested using flu detect kit in the laboratory. All together 500 samples were collected and tested which showed negative for Avian influenza type A virus.

Sero-surveillance of PPR vaccination Programme

During the fiscal year 2063/64 sero-surveillance of PPR vaccination in the sheep and goats programme in the western region was carried out. A total of 6 districts as Tanahu, Shyanja, Myagdi, Baglung, Kaski, Palpa were collected from the vaccinated goats. All the collected serum samples stored in the RVL for sending to Central Veterinary Laboratory, Tripureswor , Kathmandu for the examination of antibody against PPR vaccination.

WTO Program

Under the WTO Program the RVL received some amount of budget to strnthen the laboratory capability. By this program the laboratory purchased one laptop computer, one microscope and one refrigerator. One veterinary offi cer got training on TADs, Communication and Risk Analysis Training, which mainly focused on AI diagnosis. Besides these, the laboratory was ingased in PPR survillence program and TADs Investigation programs. In TADs Investigation it investigated FMD and Ranikhet Disease for 4 times.

Details of Budget Expenditure (2062/63)

The details of budget expenditure of Regional Veterinary Laboratory, Pokhara for the year 2062/63 is shown in Table 19.

Table 19. Details of budget expenditure Budget No: 40-3-500

Budget Line	*Budget Heads*	*Approved Budget (NRs in 000)*	*Expenditures (NRs in 000)*	*Remarks*
1.01	*Salary*	*1154.00*	*930.71*	
1.03	*Transfer TADA*	*8.00*	*2.34*	
1.04	*Dress*	*9.00*	*8.98*	
1.05	*Feed*	*9.00*	*8.97*	
1.08	*Training*	*10.00*	*10.00*	
2.01	*Water and Electricity*	*92.00*	*83.86*	
2.02	*Telephone*	*55.00*	*54.93*	
2.03	*Office materials*	*420.00*	*419.88*	
2.05	*Repairs*	*193.00*	*192.99*	
2.06	*Fuel for other purpose*	*90.00*	*89.99*	
2.07	*Service cost*	*12.00*	*11.97*	
2.08	*Miscellaneous*	*22.00*	*22.00*	
4.02	*Medicine*	*80.00*	*79.92*	
2.04	*Program cost*	*29.00*	*29.00*	
2.05	*Program TADA*	*150.00*	*149.94*	
Total		***2333.00***	***2095.51***	

Details of Budget expenditure Budget No.:40-3-200/40-4-220 (WTO Program)

2.03	*Office Materials*	*190.00*	*189.86*	
2.05	*Repairs*	*50.00*	*49.97*	
2.06	*Fuel for other purpose*	*50.00*	*49.95*	
4.02	*Medicine*	*40.00*	*39.89*	
2.05	*Program TADA*	*35.00*	*35.00*	
Total		*365*	*364.68*	

Manpower of Regional Veterinary Laboratory, Pokhara:

Table 20: Manpower of RVL, Pokhara (by Shrawan, 2063).

S.N.	Name of Staff	Post	Class	Post Sanctioned	Post Available	Vacant
1	Dr. Narayan Ghimire	Senior Veterinary Officer	Gazzetted Second	1	1*	
2	Dr Shiva Prasad Devkota	Veterinary Officer	Gazzetted Third	1	1	
3	Miss Bishnu Kumari Basnet	Veterinary Technician	NonGazzetted First	1	1	
4	Mr Bishnu Prasad Kafle	Assistant Veterinary Technician	Non Gazzetted Second	1	1	
5	Mr Gunaraj Adhikari	Assistant Veterinary Technician	Non Gazzetted Second	1	1	
6	Mrs Til Kumari Sigdel	Typist	Non Gazzetted First	1	1	
7	Mrs Tara Bahadure Rayamajhi	Sub-accountant	Non Gazzetted Second	1	1	
8	Mrs Chitra Kumari Dhakar (Regmi)	Kharidar	Non Gazzetted Second	1	1	
9		Stockman	Non Gazzetted Third	1	0	1
10		Stockman	Non Gazzetted Third	1	0	1
11		Driver	None	1	0	1
12	Mr Bhoj Bahadur Dharti	Peon	None	1	1	
13	Mr Hari Bahadur Gharti	Peon	None	1	1	
Total				13	10	3

REGIONAL VETERINARY LABORATORY
(MID-WESTERN REGION) SURKHET

Introduction

Regional Veterinary Laboratory (RVL), Surketh was established in 1989 and has been located in Ward No. 7, BIRENDRANAGAR Municipality, SURKHET district, Mid Western Region of Nepal. The objective of this RVL is to provide laboratory based diagnostic and investigation services to the farmers of Mid Western Region. Therefore this laboratory has been providing free diagnostic services to 15 districts. Various specimens are received from different District Livestock Service Office (DLSO), farmers and laboratory staff during epidemic investigation and surveillance. In spite of limitation of facility and recourse this laboratory has fulfilled its targeted objectives however there is enough space to improve the quality of work to meet the international standard. This laboratory should be upgraded toward BSL class 2 laboratories.

Description of human and financial recourse as well as diagnostic activities has been presented in different table.

Description of human resource, RVL, Surkhet (2063/2064)

S.N.	Name of Staff	Post.	Responsibility.	Contact.
1.	Vacant	Senior Veterinary Officer	Chief	
2.	Dr. Keshab Shah	Veterinary Officer	Act. Chief	
3.	Mr.Indal Sah.	Senior Lab. technician	Planning	9848049920
4.	Mr.Mohan Giri	Laboratory technician	Hematology.	9848047494
5.	Mr.Anand Saru	Laboratory technician	Microbiology	9848029301
6.	Mrs.Rama Bhandari	Laboratory technician	Parasitology	9848041148
7.	Mr.Bhim Bd. G.C.	Clerk	Administration	9848027932
8.	Mr.Lovbikaram Saha	Accountant.	Account	9848025669
9.	Mrs.Bimita Yaral	Clerk	Clerk typist	9848037774
10.	Vaccant	Laboratory assistance	Microbiology	-
11.	Mr.Padam K.C.	Massager	Helper	9848037839
12.	Mr.Karan B.Garti.	Driver	Driving	9848050785
13.	Mrs.Monikala Garti.	Massager	Helper	9848050785

Budget line	Budget head	Budget in ,000 Rs	
		Allocation	Expenditure.
1.01	Salary	1055	1040,623
1.03	Transfer allowance	20	11,614
1.04	Cloth	20	20
1.05	Food	7	-
1.08	Training	10	10
2.01	Electricity and water	100	891,47.47
2.02	Telecommunication	65	65
2.03	Official	372	372
2.05	Repair	170	170
2.06	Fuel	149	149
2.07	Consultancy	20	20
2.08	Miscellaneous	22	22
4.02	Medicine	35	35
4.04	Program	40	40
4.05	Travel & daily allowance	180	180
Total		2265	2217,011.27

S.N.	Activities	Unit	Annual target	Annual progress	Budget ,000
1	Laboratory activities				
1.1	Parasitology	No.	1350	1365	48
1.2	Microbiology	,,	350	384	50
1.3	Pathology	,,	350	709	43
1.4	Serology	,,	510	530	68
1.5	Hematology	,,	350	383	35
1.6	Bio-chemistry	,,	250	344	37
1.7	Sample dispatch	,,	510	717	56
2.1	Micoplasma investigation in goats	Times	12	12	130
2.2	Parasitic burden investigation	Times	15	15	124
2.3	Epidemic disease investigation	Times	6	6	87
3.1	District veterinary lab supervision.	Times	6	6	74
4.1	Animal Health Workshop.	Times	1	1	40
5	Computer training	Times	2	2	10
6	Epidemic reporting and publication	Times	4	4	32
6.1	Annual report publication	Times	1	1	5
7	Technical book purchasing	Times	1	1	15
	Total Program budget				854

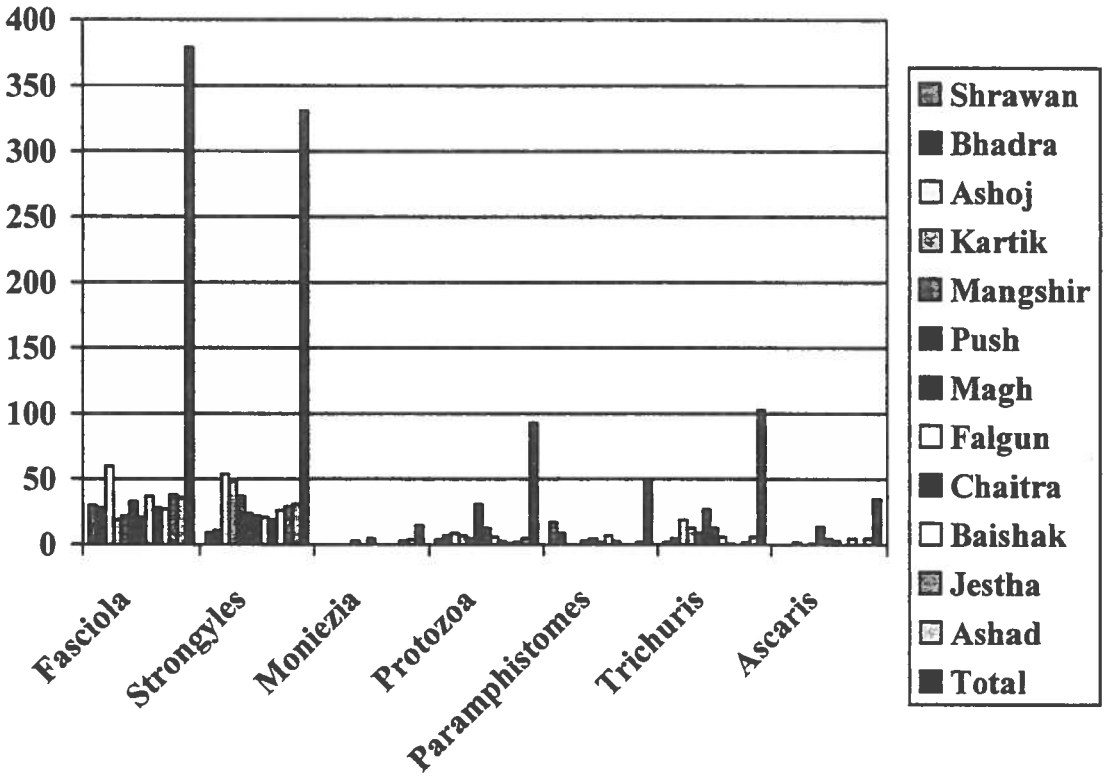
Laboratory activities

Parasitology:

Faecal samples were examined by using differential flotation and sedimentation method to identify the eggs of gastrointestinal parasite. A total number of 1365 fecal sample from different species of animals as cattle, buffalo, goat, pig, dog and sheep were examined and 70% of tested samples were found positive.

The seasonal distribution of parasites has been presented in following.table:

Distribution of parasites across month



Microbiology:

Samples collected for microbiological examination were milk, swabs and tissue. A total number of 384 samples were received out of which 20 were swabs, 174 were milk, and 190 were tissues. Routine bacteriological examine was conducted and different species of bacteria were identified on the basis of colony morphology and biochemical test. The most common bacteria isolate were *Streptococcus, Staphylococcus, Pasteurella, Salmonella* and *E .coli* species.

Pathology:

Only post-mortem examination was conducted at RVL due to the limitation of histopathology. In total, 709 carcasses of poultry, goats and pigs were subjected to post-mortem examination and tentative diagnosis was done on the basis of lesions. Further confirmation of disease was performed with the support of bacteriology, parasitology, serology and

histopathology. The most common diseases of poultry were Infectious Bursal Diseases, Coccidiosis, New castle disease, Collibacillosis, Mycoplasmosis, Broiler ascitis, Yolk sac infection, Salmonellosis and rest were unidentified cases.

Serology

Serological examination was conducted to diagnose two diseases namely salmonellas and brucellosis. Out of 500 tested serum samples only 5% were found positive for Salmonella in Plate Agglutination Test (PAT) and rest were negative. All 30 samples tested for Brucellosis were negative in PAT.

Hematology:

Hematology was subjected to 383 blood samples to examine hemoglobin, total leucocytes, total red blood cell and packed cell volume. Seven percentage of tested sample revealed low level of packed cell volume and total RBC count suggesting the evidence of anemia.

Biochemical Test:

Serum and urine samples were received and tested either in RVL, Surkhet or Central Veterinary Laboratory Kathmandu. A total number of 500 samples were examined in fiscal year 2063/064. Urine sample were analyzed with the use of commercially available multi stick at RVL Surkhet where as serum samples were sent to CVL for the extermination of minerals.

Sample Sent to Other Laboratory:

Seven hundred seven samples were referred to Central Veterinary Laboratory and National Foot and Mouth Disease Laboratory in Katmandu.

Sero- survey of PPR in vaccinated animals:

Serum samples were collected from vaccinated sheep and goats and sheep and sent to CVL for the examination of antibody in response to PPR vaccine.

No of serum sample collected in different districts

S.N.	D.L.S.O.	Serum collection
1	Bankey	100
2	Birdiya	50
3	Dang	100
4	Dailekh	50
5	Surkhet	50
6	Salyan	50
7	Pyuthan	50
8	Rukum	50

Surveillance of Bird flu

Surveillance of Bird flue in poultry was conducted by collecting a total number of 499 samples. Similarly 215 serums, 144 tracheal swabs and 140 Cloacal swabs were collected from commercial and back yard poultry of district Dang, Banke and Bardiya and sent to CVL for laboratory investigation.

REGIONAL VETERINARY LABORATORY
(FAR WESTERN REGION) DHANGADHI

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 Uttranchal 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 livestocks raised by the people in these districts.

The four hill districts of the region viz. Baitadi, Dadeldhura, Doti and Accham keep livestocks 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 loose 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 manage mental, 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.

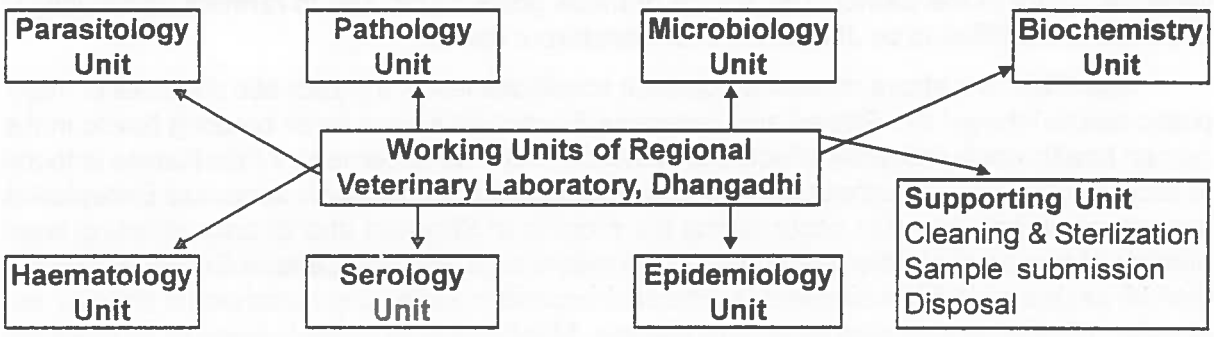
Although, Far western region is still lagging behind in its development efforts of the poultry industry as a business enterpreneur. Broiler farming is carried out in small commercial scale in Kailali and Kanchanpur districts. Recently some farmers of Dadeldhura and Doti are showing interest in poultry 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, Collibacillosis, 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 2063/2064

3.1 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	Coumputer	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%

3.2 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 2063/64
Animal Health Services Programme: 100%
Weight age of progress: 100%

4. Laboratory Services

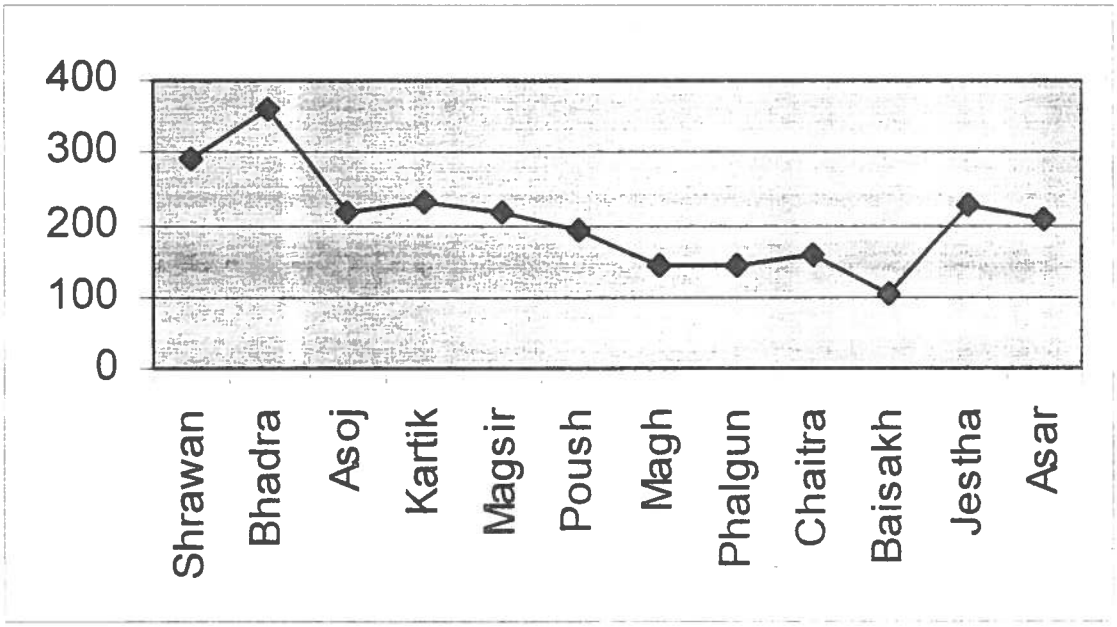
4.1 Parasitological Examination :

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, Stronglyloids, 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 blacks 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.

Parasites (Spp)	4.	5	6	7	8	9	10	11	12	1	2	3
Fasciola	29	60	60	24	32	33	23	47	40	32	61	28
Paramphistomes	56	158	64	38	44	40	28	40	51	57	47	62
Strongylus	26	59	24	16	14	14	21	13	26	16	17	13
Strongyloides	4	11	9	12	1	3	1	5	6	1	0	14
Ascarids	0	7	4	7	1	2	1	3	2	2	2	8
Coccidea	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	2062/5/4	PPR	8	14	
2	Kailali, Godavari	Goat	11	2062/5/27	PPR	1	10	
3	Kanchanpur (Zonal Adm. Office)	Goat	5	2062/7/8	PPR			Not received
4	Dadeldhura	Goat	30	2062/9/21	PPR	26	4	
5	Kanchanpur AQO	Goat	23	062/10/10	PPR			Not received
6	Krishnapur, Kanchanpur	Goat	13	062/11/14	PPR	5	8	
7	Banbedha, Kailali	Goat	10	063/3/8	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	2063/3/9	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	

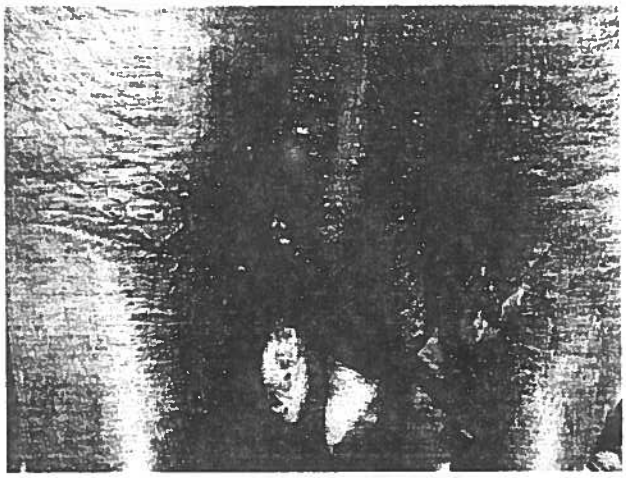
4.3 Haematology:

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 livestocks 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.



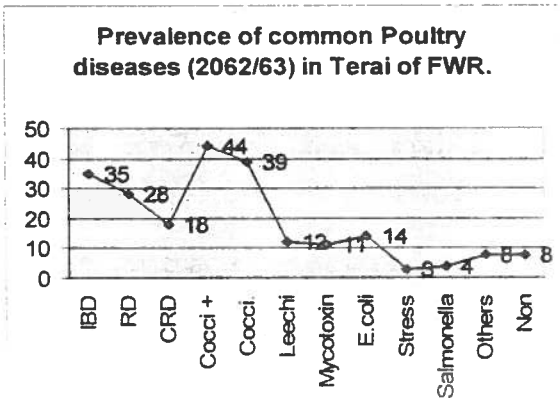
Shown above is the udder and the inner thigh of a milking buffalo which was affected with mastitis and received intra-mammary infusion of pendistrin HS that incited a generalized allergic reactions with formation of rashes all over the body. The udder, teat, inner thigh and lower abdomen were severely 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 2063/64 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.

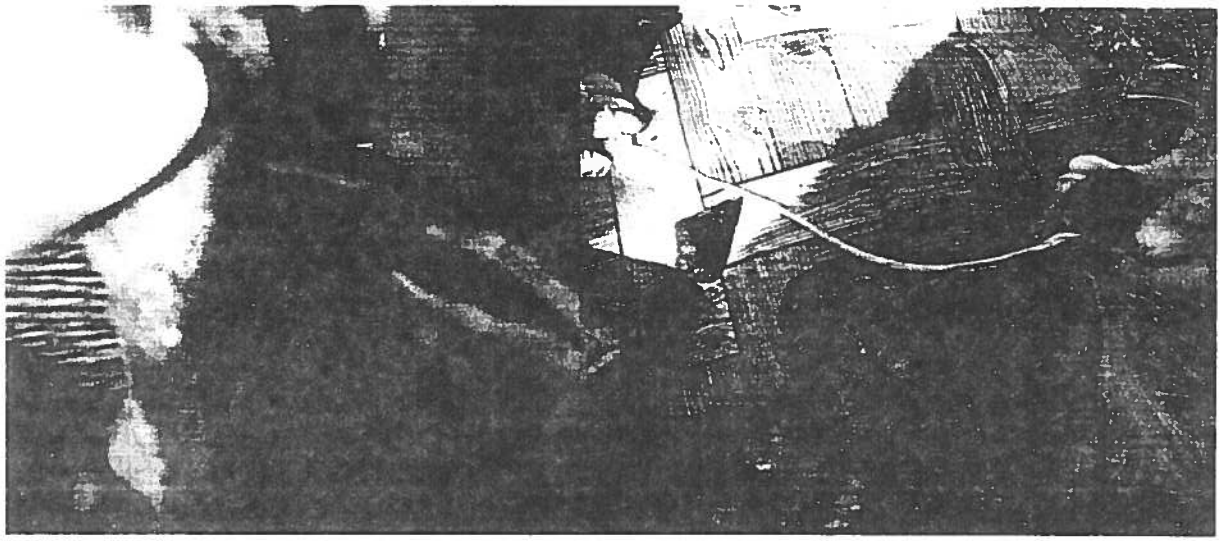
Table: Common Poultry Diseases of Far Western Region



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 below.

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.

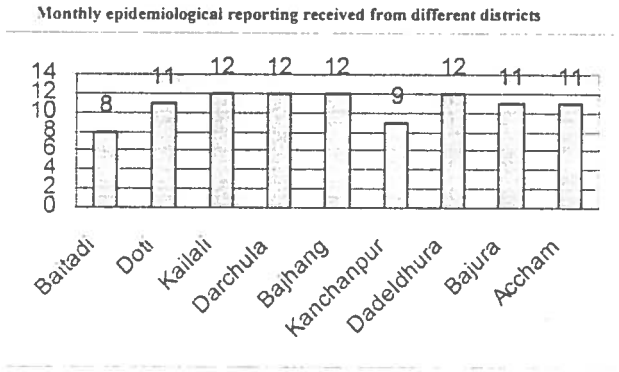
4.7 Epidemic Outbreak investigation :

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	2063	Dadeldhura	Jogbuda	Cattle	Ergot poisoning
Magsir	2063	Kailali	Chaumala	Goat	Kumri
Phalgun	2063	Kailali	Godavari	Goat	PPR
Chaitra	2063	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.



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 DLSO'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 Maharastra 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 20632/064. 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.

Shown below is the picture of RVL technicians taking tracheal swab from a local poultry in Gokuleshwor, Darchula.

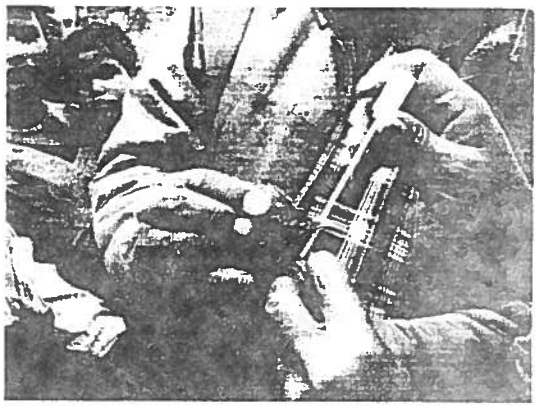


Figure 1: Macrocanthorhincus hirudinaceus attached to the intestinal wall with typical granulomatous lesion (Curtsey: RVL Dhangadhi)

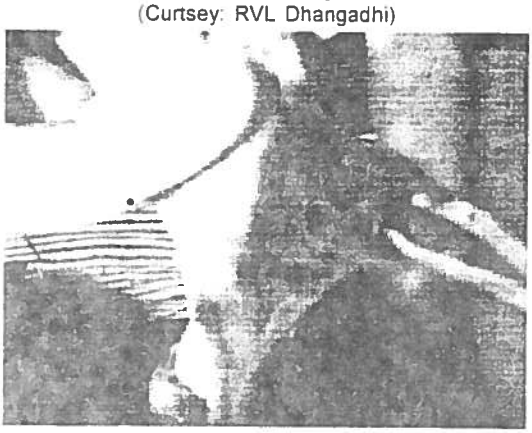


Figure 2: Penetration of the intestinal wall with Macrocanthorhincus hirudinaceus causing peritonitis (Curtsey: RVL, Dhangadhi)

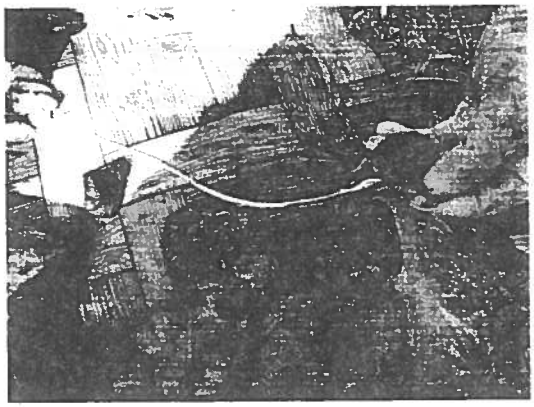
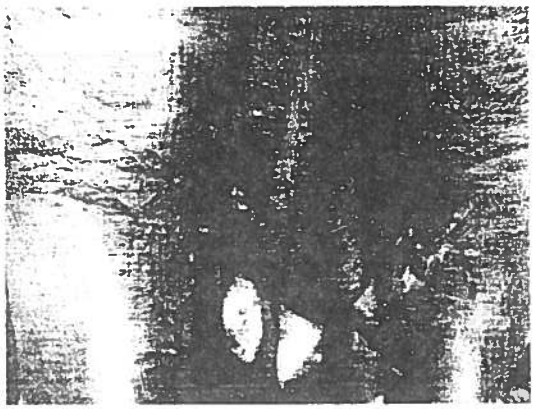


Figure 3: A clinical case of mastitis in buffalo (Curtsey: RVL, Dhangadhi)



An Overview of Sero-monitoring of PPR Vaccination in Nepal

Dr. Poornima Manandhar and Dr. Salina Manandhar

Introduction

Peste des petits ruminants (PPR) is an acute febrile viral disease of sheep and goats caused by single stranded RNA virus belonging to the genus Morbillivirus of family Paramyxoviridae. PPR is one of the most important contagious diseases of small ruminants and is known as sheep and goat plague. It causes death in more than 50% of the affected animals due to high fever, pneumonia, diarrhea and dehydration. The disease was reported in India in 1989. The disease is also enzootic in Pakistan, Nepal and Bangladesh. Nepal reported first outbreak from Dhanusha district in the year 1995. Since then, the disease has been reported from most parts of the country. A regular vaccination against this disease was initiated in enzootic areas since F/Y 2057-2058 under national PPR control programme. So, seromonitoring is one of the parts of the National PPR Control Project. The main objective of this work is to monitor the antibodies in serum collected from the vaccinated sheep and goats from the different districts of the country

Methodology

- Goats were vaccinated against PPR using PPR vaccine produced in our country only
- Serum samples were collected from the animals after one month of the vaccination
- Serum samples were collected from the different districts of the country
- Antibodies were detected and analyzed using Competitive ELISA technique
- Data of three years period were included in this study i.e FY 2061/062 to FY 2063/064

Results

In 2061 to 062 the serum samples were collected from 41 districts and a total of 20879 samples were tested for the antibodies and 13935 were found positive (67%). In 2062 to 063, serum samples were collected from 34 districts and a total of 4655 samples were tested and 2632 were found positive (67%). However, in 2063 to 064, the serum samples were collected from 56 districts and a total of 5075 samples were tested and 4049 samples were found positive (80%) which is the highest comparison to earlier years. Moreover, no. of districts were also covered in 063/64 FY than in 061/62, 062/63 respectively.

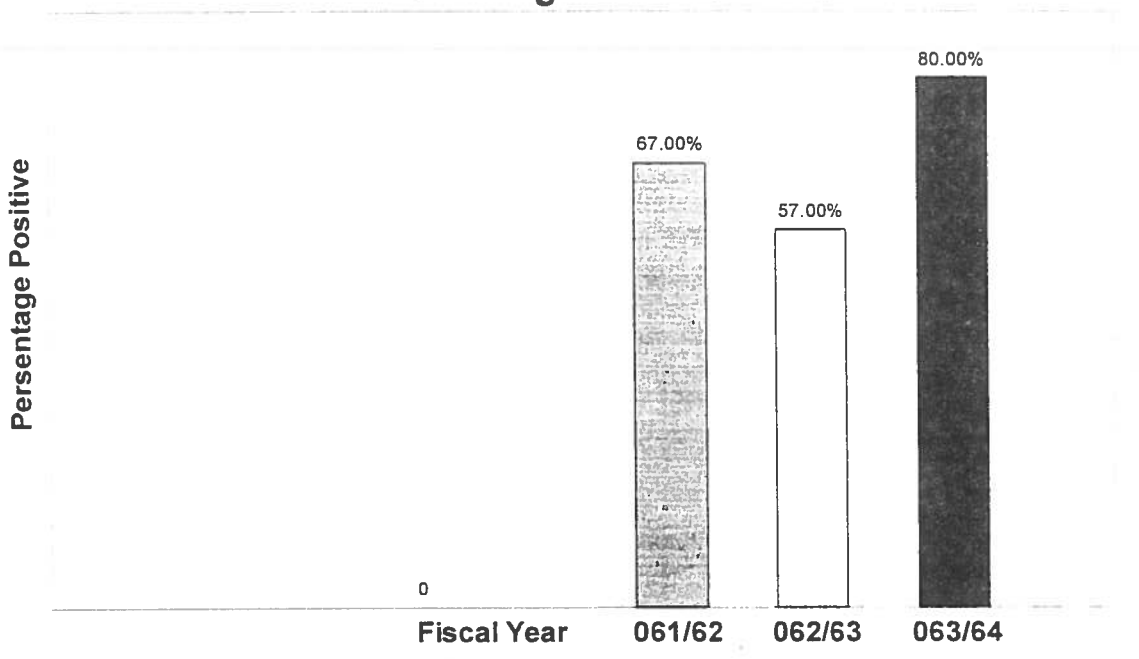


Table 1: PPR Sero-monitoring (C-ELISA) from FY 2061/062 to FY 2063/064

Year	No. of Districts	Sample tested	PPR Results		Percentage (%)	Remarks
			No. of Positive	No. of Negative		
061/62	**41**	20879	13935	**6944**	67%	**Kit was not available and not purchased due to some unavoidable cause. So, all the collected samples could not include in the study.**
062/63	**34**	**4655**	2632	**2023**	57%	
063/64	**56**	5057	**4049**	**1008**	**80%**	

PPR Outbreak From FY 061/062 to FY 063/064

Year	No. of Districts outbreak	Sample tested	PPR Results		Percentage (%)	Remarks
			No. of Positive	No. of Negative		
061/62	25	764	231	533	30.24	Except 12, rest of all the districts experienced outbreak
062/63	47	2407	928	1479	38.55	Except in 21 districts all outbreaks in vaccinated flocks
063/64	58	2983	1499	1484	50.25	Except 11, rest all the districts experienced outbreak

Discussion

The higher sero-conversion is recorded in FY 063/64 and the lowest in 062/63. It might be due to the right time of serum collection after the vaccination. It is in practice that at the last period of the fiscal year the vaccines are supplied and serum are collected in last hour before good antibody is formed in the goats; body. The other reason may be the quality of vaccine was good in the year 2063/064. Sero-conversion is also depending upon the health of the animal. So, it is necessary to monitor quality of vaccine of course of our own vaccine too. It is also necessary to monitor storage of vaccine and cold chain in the field. The lower titer of antibody may also be due to collected serum from unvaccinated population. So, there should be one body to monitor vaccinator and serum collector to avoid such results. However, when look at the outbreak situation, there were outbreaks in vaccinated goats and vaccinated districts too (Table 2.). When look at the whole picture, it rises a question of quality of the vaccine as well as the population in the districts not covered. It may be due to replacement of vaccinated population. It also may be due to introduction of infected goats in the vaccinated flocks. If there is no control and monitoring system the seromonitoring program will not give the good picture of antibody production time duration of the antibody in the body of the animals.

Conclusions

Seroconversion rate is higher in FY 2063/064. The vaccine and vaccination seems better in this year. Awareness and sincerity of farmers and technicians shows improved. However, there are still outbreaks experienced, shows it is necessary to vaccinate the flocks that are newly introduced.

Recommendations

There should be regular monitoring of quality of vaccine.

Regular monitoring of vaccine and vaccinator in the field

Timely vaccination and collection of serum

Monitoring of serum collection

More coverage of districts as per the objective of National PPR control project

Strict quarantine to check unvaccinated and infected animals entering into the country

Acknowledgement

We would like to thanks Dr. Rebati Man Shrestha for his full support. We would like to acknowledge Mr. Ashok Prasad Shrestha for his help to test the samples and rest of the staff who are directly and indirectly involved in this work in CVL. We would also like to acknowledge all officers and other staffs of different DLSOs for their help to collect and send the samples to CVL.

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A Laboratory outbreak investigation of Post-Monsoon Endemic Moist Eczematous Syndrome in cattle . in Jhapa District of Nepal

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Abstract;

An endemic hyperemic moist eczematous syndrome was reported in Cattle and Buffaloes in Jhapa district of Nepal during month of September after prolong spell of drought followed by heavy rainfall causing water logging total 56 cattle and buffalo were affected and out of which 12 animal died. Rest of ill animals were treated with 5%of Antidegnala liquor and Penta-sulphate,liquod toxinbinder. Straw and Skin samples revealed Penicillium sp.Fungus.After long spell of drought period followed by repeated flooding in lowland area in tropical and subtropical there is likely increase risk of fungal infestation in forage.When these forage fed to cattle buffaloes seems to be risky for occurence of Endemic Moist Eczamztous syndrome, either preventive measure for its prevention or early treatment with either with anti Degnala liquor or Use of pentasulphate,loquid toxin binder seems to prevent loss from this condition.

Key word:

Endemic hyperemic moist eczematous syndrome, Cattle and Buffaloes, Jhapa district, Nepal, Antidegnala liquor and Penta-sulphate, Penicillium sp.Fungus, postmonsoon.

Background:

District livestock Service Jhapa reported the incidence of syndrome similar to be as described as Degnala disease. A total 56 cattle were affected out of which 12 of them died during treatment with antibiotic and other supportive medicine. On field observation in outbreak area all sick animals were having hypernic moist eczematous lesion in all over the body ,and on the tail, thigh, legs , udder ,testicle with normal temperature and apitite.All the animals showing the symptoms were diagnosed as suffering from Endemic Moist Eczematous syndrome and were provided treatment with antidegnala liquor and penta sulphate .

Review of Literatures:

Dr Marjorie reported facial eczema is a disease of sheep and cattle which occurs in warmer districts of the North Island during late summer and autumn and is responsible for serious production losses in some years. It is caused by a fungus, Pithomyces chartarum, which proliferates on dead plant material in pasture under warm, humid conditions. The minute spores of this fungus contain a substance, sporidesmin, which produces severe toxic effects in the liver. The appearance of livers of affected animals varies, according to the severity of the damage, from slight mottling with light patches to gross discoloration, distortion, and atrophy of large areas.

Norman Trevor observed that frequently the severely damaged portions are surrounded with new liver tissue. As a result of this damage the functions of the liver are impaired. Blockage of bile ducts may prevent the excretion of waste substances in the bile; for example, accumulation in the fat and skin of bile pigments, derived from the normal breakdown of old red corpuscles, produces the jaundice or yellow staining commonly seen in the carcasses of affected sheep. Of particular importance is the loss of ability to excrete the substance phylloerythrin.

This is formed in the digestive tract of ruminants through the degradation of chlorophyll and is absorbed from the intestine and carried to the liver, where it is normally excreted in the bile. If this excretory mechanism is upset, phylloerythrin passes into the bloodstream which supplies the whole of the body. Phylloerythrin belongs to a class of flourescent pigments which are capable of making the skin sensitive to sunlight, causing reddening, intense itching, swelling, and scab formation. It is these effects, generally showing on the face of affected animals but also on other unpigmented skin exposed to light, such as the teats and udders of cows, which give rise to the popular name "facial eczema". These skin effects are, however, secondary to the much more serious impairment of liver function. The fungus, Pithomyces chartarum, grows only on dead or dying plant tissues, not on the living leaf. Hence the amount of the fungus in a pasture is related to some extent to the amount of this dead material, or litter, present. Growth of the fungus, and its production of spores, is strongly influenced by climate and environmental factors. Temperature, humidity, and the time during which the litter remains wet appear to be particularly important. This explains the typical, although not invariable, association of the disease with a period of warm, wet weather, often following a dry spell during which grass growth has ceased and litter has accumulated in the herbage.The toxic substance, sporidesmin, has been isolated from cultures of the fungus and its chemical structure determined. A single dose of one-thousandth of an ounce is sufficient to kill a lamb of about 60 lb live weight. Sporidesmin itself does not appear to accumulate in the liver, but its effects are cumulative, so that repeated small doses are as effective as a single large dose. Even with a single dose, the full sequence of changes takes some time to develop. Hence photosensitisation usually does not occur until 10 to 14 days after the animal received the toxin, and it may be even further delayed. Both the chemical nature of sporidesmin and its effects on tissues present unusual features which have not yet been fully studied.Facial eczema (FE) is a type of sunburn (sometimes called photosensitisation) affecting exposed areas of pale skin of cattle. It is caused by a poisonous substance called "sporidesmin" that causes liver damage. Sporidesmin is produced on pasture plants, including rye grass, by a fungus called Pithomyces chartartum. This fungus is widely distributed and occurs naturally within dead plant material at the base of standing pasture.FE has been recorded in sheep and cattle on mainland south eastern Australia.

Irfan, 1971; Kalra et al., 1972; Irfan and MaqboolA, 1986 reported in their study that deg Nala disease, which causes necrosis and gangrene of the dependent parts in cattle and buffaloes (Bubalus bubalis) is known to exist in Indo-Pakistan, as a number of cases were recorded stemming from a monsoon rainwater stream in the area of Murdike (Sheikhpura District), near Nala Deg in Pakistan (Shirlaw 1939). A widespread occurrence of the disease has been reported from rice growing areas of Indo-Pakistan which caused considerable economic losses.

Norman Trevor reported as the disease may be seen in stock between several days and several weeks following pick-up of sporidesmin from the pasture. The toxin is absorbed from the intestine and reaches the liver, where it causes severe damage to bile ducts and liver cells. All the outward signs of FE result from the liver damage caused by sporidesmin.

Veterinarian in Austraia reported that the signs of FE range from mild photosensitization (sunburn) to severe jaundice and death, depending on the amount of sporidesmin consumed. Sunburn is the most consistent sign, and usually affects the exposed areas of the skin of the face, ears, teats, and vulva, and areas of skin lacking dark pigmentation, ie. areas covered by white hair. The skin over these areas becomes reddened, and then goes crusty and dark. It eventually peels off leaving large raw areas, which are susceptible to infections. The sunburn is often accompanied by watery swelling of the underlying tissues. Jaundice (yellowing of mucous

membranes) is often seen at this stage.Affected animals lose weight rapidly. Most animals recover from the acute phase, but tend to be unthrifty, often taking many months to regain condition. Some never recover, and either die or are culled. In dairy cattle, the udder and teats are often severely affected, and milk production drops sharply. Loss of weight and general illness are often severe, and death, although uncommon, can occur up to months after the initial liver damage occurs. Clinical Symptoms observed are initial dullness, lethargy and anorexia ,Variable onset of jaundice and photosensitization . some animals may die without either being observed ,photosensitization: sheep - non wool skin including muzzle, ears, face, escutcheon cattle - non black pigmented areas including teats, deer - generalized, some animals develop chronic ill-thrift ,some progress to a hepatic encephalopathy dullness, depression tremor, recumbency.

Researcher in austrailia innumurated that that sheep, cattle, deer susceptible,horses resistant ,evidence for genetic resistance in sheep/ Plant/environmental factors,fungus grows on the dead leaf litter of pasture ,most frequent pasture is perennial rye grass, but can occur on other species ,requires warmth and humidity to promote rapid fungal growth and sporulation ,typical weather conditions involve autumn break rains after dry summer, several days of consistent warmth (T°C>15.5°C) and high humidity (>80%) ,fungus concentrates toxin in spores which may be distributed throughout whole pasture sward most toxic part of pasture is base of sward (http://vein.library.usyd.edu.au/links/pact/facialeczema.html).

Austrailian worker reported that outbreaks typically occur when weather conditions suitable for rapid fungus growth and spore production are combined with abundant dead, recently killed plant material, which favours fungal growth. The fungus requires warm, humid weather and light rain (or irrigation) for growth. This is most likely to be a problem in autumn when the summer has been hot and dry, the pasture well eaten back, and rains fall when the ground is still warm. In such conditions both pasture and grass grow rapidly.

Field veterinarians in Australia are in opinion that the fungus producing sporidesmin is normally not visible to the naked eye. It multiplies by producing millions of spores which are coated with the toxin sporidesmin. Freshly produced spores are the most toxic; if fungal growth stops after a change in the weather, the residual spores on the pasture lose their toxicity within one or two weeks.The fungus will grow on most pasture plants, but it grows best on perennial ryegrass. It grows in the dead pasture litter at the base of the plants. When the fungus reaches toxic levels, animals grazing short pasture at high stocking rates are at greatest risk (http:// vein.library.usyd.edu.au/links/pact/facialeczema.html) 1 fran

Objective of Investigation:

A. Ascertain the exact cause of the Syndrome.

B. Evaluation of Mycobiota of rice straw fed to cattle in the area.

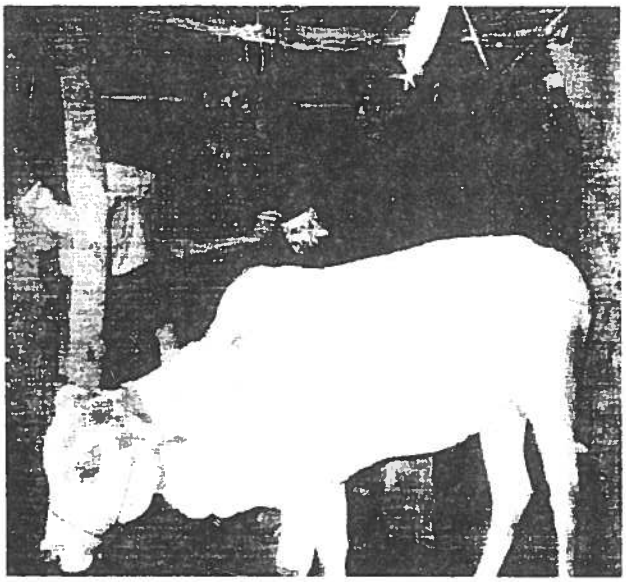
C. Evaluation of Hematological parameters of clinical and post-treated animal.

D. Evaluation of the treatment with Antidegnala liquor/penta sulphate for systemic mycoses

Clinical Pathology: clinical picture

Dry skin with moist lesion in thigh region of cattle (1)

Generalized hyperemic moist eczema (2)



Hyperemic moist eczematous lesion on face and neck (3)

Hematological findings of samples from clinical case pre-treatment:

Species of animal	RBC	WBC	PCV%	HB
OX	4*10 millionmmc	7.2*10 mm3	23	7.6
C.calf	4.6*10 millionmmc	8.2*10 mm3	28	9.3
C.calf	4*10 millionmmc	7.8*10 mm3	24	8
C.calf	4.5*10 millionmmc	8.2*10 mm3	27	9
Normal	5*10millionmmc	4-12*10mm3	28-42	8.5-13.5

Hematological findings of samples from clinical case post treatment:

Species of animal	RBC	WBC	PCV%	HB
Ox	7.2*10 millionmmc	4.6*10mm3	28	9.3
Ox	8.5*10 millionmmc	5*10mm3	30	10
Calf	9*10 millionmmc	5.5*10mm3	33	11
Calf	8.6*10 millionmmc	5*10mm3	30	10
Ox	7.9*10 millionmmc	4.8*10mm3	29	9.6
B.bull	9.5*10 millionmmc	6*10mm3	36	12
Normal	5*10millionmmc	4-12*10mm3	28-42	8.5-13.5

Mycobiota of Straw and fodder forage:

Revealed the growth of fungus Penicillium spp in mycological medium on laboratory culture

Treatment provided

Use of 5% of Anti-Degnala liquor 5-10ml s/c or i/m alternate day 4 times a week has been found to be successful. Alternately orally Penta-sulphate and liquid toxin binder like toxolivum ,toxol was given.

Result and Discussion:

All animal which were treated with above preparation the eczematous lesion were disappeared within week and animal returned to normal health. Low land marshy landscape of area, moist hot humid tropical climatic condition traditional husbandry practice of feeding rice straw seems to be the source of opportunistic fungal invasion which was evident from the fungal growth in laboratory mycomedia.The increase of total WBC count and decrease in PCV,Hb in blood of clinically ill animal and treatment response indicates that in any incriminated change in weather condition new disease condition is likely too occur in livestock need to be assesed

Conclusion:

The disease is strongly associated with the feeding of rice straw containing multiple dark specks. This observation is concurs with the findings of earlier researchers (Irfan and Maqbool 1986) who reported that fungal infested straw and fungal mats of various species in different combinations, when mixed with fresh non-infested rice straw, produced the disease. Saprophytic fungi infesting rice straw produce mycotoxin possessing vasoconstriction, which produces the lesions of the disease (Irfan et al., 1984). The greater severity of the disease in buffaloes as compared to that in cows may partly be due to the high susceptibility of this species. Therapeutic trials with an antidote (a penta-sulphate mixture) given orally, and a vasodilator (nitroglycerin ointment) applied locally on the lesions effected the highest percentage (90%) cure rate. This cure rate was in a broad agreement with the findings of Schontal (1980) who reported a cure rate of 80% with a penta-sulphate mixture. Same way in this observation it was observed the entire animal which was treated with injection of anti Degnala liquor followed by penta sulphate recovered completely. Same way there was marked increase in total WBC count ,and decrease in PCV and Hb during clinical phase of syndrome on treatment there was marked increase of both PCV and Hb and increase in RBC count and normal WBC count also support that this syndrome was attributed by infestation of fungus on rice straw which was fed to these animals. If proper management of dry forage during rainy season carried out it can minimize the loss due to endemic moist eczematous syndrome. Further more if timely treatment of animal is if initiated with use of anti Degnala liquor or with penta sulphate will minimize the losses need to be looked into.

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REQUIREMENT OF BIO-SAFETY LEVEL-3 LABORATORY IN NEPAL FROM EMERGING THREAT OF GLOBAL OUTBREAK OF BIRD FLU

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SUMMARY

The guidelines presented in this articles is based on quality standard and special practices, safety equipment recommendations, and performance standards for the facilities that, should be considered as optimal for most laboratory situations in developing and developed countries. There may be instances where unique needs, unknown hazards associated with unknown pathogens, or other contributing requirements will cause supervisors or biosafety professionals to seek higher biosafety requirements. These can be established after appropriate risk assessments have been conducted. At present in Nepal, there is a high risk of bird flu due to subsequent outbreak in neighbouring countries such as India and China. Nepal depends upon both of the neighbours for poultry and livestock industry, livestock trade and commerce. Avian Influenza Control Project (AICP) funded by World Bank (WB) is responsible for construction of Biosafety level-3 (BSL-3) lab in Nepal.

INTRODUCTION

From the earliest days of microbiological research, laboratories have recognized that acquiring infections from the agents they manipulated was a recognized occupational hazard. The most commonly-acquired lab infections were caused by bacterial agents; as microbiologists learned to culture animal viruses, they also found ways to become infected with these agents. Sulkin and Pike said that a significant number of these infections were fatal and that most infections were of unknown origin. Exposures to infectious aerosols were implicated in about eighty per cent of the reported infections.

Guidelines evolved as a means of protecting microbiological workers based on these data and an understanding of the risks associated with various manipulations of many agents transmissible by different routes. These guidelines work from the premise that safe work sites result from a combination of engineering controls, management policies, work practices ,procedures, and occasionally, medical interventions. The different biosafety levels developed for microbiological and biomedical laboratories provide increasing levels of personnel and environmental protection.

There is a definite hierarchy of administrative controls that need to be in effect. Upper level management must set the general tone that safety is a high priority at their institute. Though this is often expressed in broad policy statements, it must be supported by resource allocation decisions: financial, personnel staffing, training, a safety performance reward structure, etc. For each biosafety level there are also specific supervisory qualifications as assurance that the laboratorians are provided appropriate role models and knowledgeable mentors. Crucial to safe working conditions are the various types of specialized equipment available to serve as primary barriers between the micro-organism and the laboratorian. These range from simple gloves and other personnel protective equipment to simple (sealed centrifuge heads) or complex (biosafety cabinets) containment devices.

In Nepal, Central Veterinary Laboratory (CVL) acts as a referral laboratory. Good Laboratory Practices (GLP) has been practiced here. For detecting bird flu or other exotic diseases, Nepal needs BSL-3 lab and it shall be established by WB/AICP project in Budhanilkantha, Kathmandu.

BIOSAFETY LEVEL 1 (BSL-1):

BSL-1 is appropriate for working with micro-organisms that are not known to cause diseases in healthy humans. This is the type of laboratory found in community water-testing laboratories and in some community colleges teaching introductory microbiology classes, where the agents are not supposed to be hazardous for human being.

There is a door that can be closed to keep visitors out of the lab while work with the agents is in progress. Hazard warning signs may be posted on the door indicating any hazards that may be present, including radioactive materials, laser lights, high noise emitting equipment, or toxic chemicals. There is a hand-washing sink available, preferably near the door. Waste materials are segregated according to hazard type, and there is an appropriate chemical decontamination tray for collecting contaminated implements. Work is done on the open bench, and plastic-backed absorbent pads can be placed on the work surface to collect splatter or droplets associated with the work. The bench tops should be impervious to acid and all furniture should be sturdy. If there are openable windows in the lab, they should be fitted with screens.

The lab should be constructed in such a manner that it can be easily cleaned and decontaminated. At BSL-1 there is no specific recommendation that the laboratory be isolated from other parts of the building. Although there is no specific biological safety reason for having more than six air changes per hour in a BSL-1 laboratory, it may be necessary if there are volatile or toxic chemicals in use. In general, inward directional airflow is the ideal.

At BSL-1, standard microbiological practices include the use of mechanical pipetting devices, having a prohibition on eating, drinking and smoking in the lab, and requiring hand washing by all persons when they finish their work or when exiting the laboratory. Persons working in the lab should wear a lab coat to protect their street clothes. It is a recommended practice to wear gloves while manipulating the agents. Additional protective equipment may include working behind a splatter shield or wearing eye or face protection. At BSL-1, no special precautions are needed.

Hand washing is one of the most important procedures that can be used by laboratorians to prevent removal of unwanted microbiological agents, radioactive materials, or chemicals from the laboratory environment. Use of liquid soap is generally preferable to bar soap; twenty seconds of vigorous lathering will remove most of these materials very effectively. After drying your hands with a paper towel, you can use the towel to turn off the faucets and thus prevent re-contaminating your hands.

The scientist who provides overall supervision to a BSL-1 laboratory needs to have general training in microbiology or a related science. The supervisor is responsible for establishing the general lab safety procedures and for ensuring that each laboratorian is properly educated in these procedures. Lab personnel, on the other hand, need to accept such training and follow the Standard Operating Protocol (SOP) of laboratory.

The work space is constructed with sealed seams and a crevasse-free surface, to lessen the chances that micro-organisms will pool in a hard-to-reach location and grow. Personnel in a BSL-1 laboratory are trained in the techniques necessary to prevent contamination of the experiment or themselves.

BIOSAFETY LEVEL- 2(BSL- 2):

A BSL-2 laboratory is similar in design and operation to a BSL-1 lab. A research technician conducts experiments that include challenging insects with a virus mixed with blood at the U.S. Department of Agriculture Arthropod-Borne Animal Diseases Research Laboratory, a bio-containment laboratory that specializes in animal diseases that are transmitted by insects, including plague, West Nile, and tularaemia.

The facility, the containment devices, the administrative controls, and the practices and procedures that constitute BSL-2 are designed to maximize safe working conditions for laboratorians working with agents of moderate risk to personnel and the environment. The agents manipulated at BSL-2 are often ones to which the workers have had exposure to in the community, often as children, and to which they have already experienced an immune response. Unlike the guidelines for BSL-1, there are a number of immunizations recommended before working with specific agents. Most notable is Hepatitis B virus immunization which is recommended by the Occupational Safety and Health Administration for persons, including laboratorians, at high risk of exposure to blood and blood products. These agents are generally transmissible following ingestion, exposure of mucous membranes, or intra-dermal exposure. Eating, drinking and smoking are prohibited in BSL-2 laboratories, and extreme precautions are taken while handling needles and other sharp instruments.

Access to the laboratory is restricted by the supervisor, who establishes the biosafety level, the need for specified personal protective equipment (PPE), the need for training, or other appropriate requirements. The door to the laboratory is kept closed to minimize unnecessary access by casual visitors, vendors, or persons not needing to be in the laboratory. There is no requirement for directional inward air flow in a BSL-2 laboratory, except as may be required for chemical odour control; however, many BSL-2 laboratories opt for this feature.

Some work may be done on the open bench by persons wearing appropriate protective clothing or gear. Any work that may produce splatters or aerosols of infectious materials should be done inside a biological safety cabinet (BSC) or other containment device, such as aerosol-containing centrifuge cups. Waste materials need to be segregated into chemical, radioactive, bio-hazardous, or general waste streams. Infectious waste should be decontaminated (by treating with chemical disinfectants or by steam autoclaving). The most common device is the biological safety cabinet, and the most common cabinet in use is referred to as a Class II, type A BSC. The typical HEPA filter removes 99.97% of all particles that are 0.3 micron or larger in size, which means that all microbial agents will be trapped in the filter. The air returned to the laboratory and delivered to the work surface is virtually sterile, which means that an open flame (Bunsen burner) is not needed within the BSC.

Before materials are introduced into the BSC, they should be wiped with 70% alcohol to remove any external contaminants. Experience has shown that clean materials should be kept to one side of the work surface, dirty items on the other. Management of workflow within the BSC is crucial to preventing cross-contamination. Rapid air movement outside the cabinet (caused by co-workers walking past, air supply vents directed across the face of the BSC, etc.) will interrupt the rather fragile air curtain, which may cause air-borne contaminants in the cabinet

to be drawn into the lap of the worker. The chair should be adjusted so that the lower portion of the sash is even with the worker's armpits.

Any paper or plastic materials introduced into the BSC should not be allowed to interfere with air flow through the front or rear grilles. The downward airflow from the supply filter "splits" about one third of the way into the cabinet; in the front third, air moves to the front grille, with the remainder of the air flowing to the rear. This means that aerosol-generating activities should be performed towards the rear of the cabinet to provide further worker protection.

As the biosafety level increases, all those microbiological practices and procedures delineated for the lower level(s) are carried forward to the next higher level. Thus, the standard microbiological practices found at BSL-1 are still in effect at BSL-2, with emphasis on wearing gloves, using mechanical pipetting devices, and attention to handling sharp objects. In any situation, do not break or bend needles; in most situations it is prudent to use single-use needles and syringes. Do not recap needles. Needles and syringes, butterfly needles and associated tubing, and similar devices should be discarded intact into a puncture- and leak-proof container. Other sharps items (such as broken glass, should not be handled by hand. Consider substituting plastic ware for glass laboratory items.

Specific policies and procedures regarding access to the BSL-2 laboratory should be developed and posted. On the one hand, it is prudent to allow entry to repair technicians or engineers only if they are very familiar with the activities of the lab or are escorted by a laboratory man. On the other hand, it needs to be emphasized that the posting of a BSL-2 biohazard sign on the door does not mean that the agent is everywhere in the room; rather, the agent is normally confined to the BSC. an incubator or refrigerator or freezer. It is prudent to schedule entry by non-laboratorians to times when there is no active work with the agent being conducted.

A leak proof box, preferably equipped with a gasket seal lid, should be used for transport of infectious materials from one location to another. This is particularly important when moving samples from animal care areas to laboratories, or from an off-site collection centre to the lab.

Storing a base-line serum sample may be required prior to working in certain laboratories. This sample can be used at to compare with future serum samples to determine any changes in immunological response to the agents used in the laboratory. Alternatively, a base-line serum sample may be drawn at the time of a possible exposure, and then compared to a future sample for possible rising antibody titre.

Other special practices include: decontaminating work surfaces after completing the work with the infectious materials, keeping non-research animals out of the laboratory, and reporting all spills and accidents. An incident log book is a useful means for recording events that have gone wrong; it is important to document these events, not for punitive action, but to be able to better understand what happened with an eye to preventing similar events in the future.

Infectious waste materials should be chemically disinfected or, preferably, decontaminated in a steam autoclave. Infectious waste materials to be removed from a BSC should be placed in a pan or tray that can be covered during transport to the autoclave, or placed in a biohazard autoclave bag. By placing an inch or two of water in the bag before sealing it for transport, steam will be generated within the bag during the autoclave cycle.

The supervisor of a BSL-2 laboratory should be a competent scientist who has a technical understanding of the risks associated with the microbiological agents in use. The supervisor limits access to those persons who have received the appropriate immunizations and establishes

the personal protective standards for the laboratory; he/she is also responsible for developing the lab's biological safety manual. Laboratory personnel should be aware of the potential hazards associated with the work and be proficient in the specified practices and procedures.

Procedures like blending and centrifugation create the opportunity for organisms to become airborne. Special protective clothing such as a facemask is worn, and biological safety cabinets are present. The location of the specialized equipment must be approved (i.e., a safety cabinet is not allowed to be by an open window or the door to a hallway). Air enters and exits the lab via the building's ventilation system. If windows are present, they can be opened.

BIOSAFETY LEVEL 3

This facility is designed for work with micro-organisms that can easily become airborne and that carry a great risk of infection. Often a BSL-3 laboratory is in a hospital or an infectious disease research facility.

One distinguishing characteristic of a BSL-3 laboratory, compared to BSL-1 and BSL-2 labs, is the requirement that work with the micro-organisms be done within biological safety cabinets or other containment equipment, or by personnel wearing protective clothing (i.e., wrap-around gowns, scrub suits, coveralls, gloves that are changed frequently). Another characteristic is increased restrictions for access to the lab. For example, newer facilities must have double doors, which are sealed around their edges. The first door that connects to the outside of the lab must be fully closed before the door to the BSL-3 lab is opened. BSL-3 is suitable for work with infectious agents which may cause serious or potentially lethal diseases as a result of exposure by the inhalation route. BSL-3 laboratories should be located away from high-traffic areas. Examples of agents that should be manipulated at BSL-3 are *M. tuberculosis* (research activities), St. Louis encephalitis virus, and *Coxiella burnetii, Highly Pathogenic Avian Influenza (HPAI)*.

There are some specific secondary barriers needed at BSL-3, which tend to set these laboratories apart from BSL-2. At CDC the current main BSL-3 laboratories are located in a unique high containment building that also houses the BSL-4 laboratory. These laboratories are characterized by having a double-door entry (an ante-room; other configurations are also used). Because the agents manipulated at BSL-3 are transmissible by the aerosol route, particular attention is given to air movement in these labs. Air moves from areas of lesser contamination to areas of higher contamination, such as from the corridor into the laboratory. Air movement is also single pass; exhaust air is not recirculated to other rooms. Exhaust air does not have to be HEPA filtered, unless local conditions are such that re-entering into building air supply systems is unavoidable. Ventilation systems in the BSL-3 lab are independent from the rest of the building's ventilation system. The air from the laboratory is exhausted directly to the outside and not into the general building circulation. The exhaust air is also filtered to remove micro-organisms. Also, ideally the airflow through the laboratory should be balanced (i.e., the air flow into the room is the same as the air flow out of the room) and should flow from areas that are not used for experimental work such as office space to areas containing the micro-organisms.

All work that may create aerosols or splatter is done inside a biological safety cabinet. Wall, ceiling and floor penetrations are sealed to keep aerosols in and to keep gaseous decontaminants in. The floor is monolithic, and there are continuous cove mouldings that extend at least 4" up the wall. Acoustic tiles are not used in BSL-3 laboratories; ceilings should be waterproof for ease of cleaning Centrifuge tubes are placed into containment cups or heads in the BSC, transferred to the centrifuge, spun, and then returned to the BSC to be unloaded. In

some laboratories the centrifuges themselves are enclosed in a vented area to minimize possible aerosol exposures created in the event of a centrifuge failure. Vacuum lines are protected with HEPA filters so that maintenance personnel are not exposed to infectious aerosols .Standard microbiological practices are the same as for BSL-1 and BSL-2 laboratories. Class II type A biological safety cabinets are suitable in BSL-3 laboratories. Sometimes Class II type B3 cabinets are installed, requiring thimble connection to the building exhaust systems. Depending on the nature of the work being done in the BSL-3 laboratory, additional personnel protective devices may be worn, such as respirators. When pulmonary protection is required, the laboratorians need to have appropriate medical evaluations and be trained in proper fit testing and care of their respirators.

The floors and walls of a BSL-3 laboratory are designed to be free of cracks, impermeable to fluids, and chemical resistant. While windows are permitted, they cannot be opened.

Supervisors of BSL-3 laboratories should be competent scientists experienced in working with the agents. They establish criteria for entry into the laboratory, restrict access, develop appropriate practices and procedures, and train the laboratorians. They are also responsible for developing the laboratory safety manual. The lab personnel must rigorously follow the established guidelines, demonstrate proficiency in performing their various procedures, and receive appropriate training. They must participate in specified medical surveillance programs, and report all incidents that constitute potential exposures.

The satisfactory performance of all equipment and personnel in the lab is regularly monitored and recorded for inspection. The lab and the personnel are re-verified each year.

BIO-SAFETY LEVEL-4 LABORATORY (BSL- 4):

The BSL-4 facility is designed for work with micro-organisms that pose a dire health threat. The most infectious micro-organisms (i.e., Ebola virus, *Bacillus anthracis* (the cause of anthrax), the Marburg virus, and Hantavirus) can be handled only in a BSL-4 laboratory. A newly discovered micro-organism that is genetically related to a known extreme pathogen shall be handled in a BSL-4 lab until, when, and if it is demonstrated that the organism does not pose a threat to health or life.

Two hallmarks of the micro-organisms that can be handled only in a BSL-4 laboratory is their ability to be easily transmitted from and to people via the air, and from person to person (they are highly infectious). The design of a BSL-4 laboratory prevents the release of these micro-organisms into the environment and protects the researchers from infection.

An example of a BSL-4 laboratory is the one that is present in the United States Army Research Institute of Infectious Diseases, in Fort Detrick, Maryland. At 10,000 square feet, the USAMRIID BSL-4 facility is the largest highest-level bio-containment laboratory in the United States. As of 2002, three other BSL-4 labs exist in North America. The others are at the Centres for Disease Control and Prevention in Atlanta, Georgia, San Antonio, Texas, and Winnipeg, Manitoba, Canada. There is a BSL-4 lab in Bhopal India. It works on HPAI virus.

The personnel who work in a BSL-4 laboratory have been highly trained and certified. They are experts in microbiological techniques and in the containment of infections. Only these lab personnel are allowed into the laboratory. Entry to the Level 4 area requires passage through several checkpoints and the keying in of a security code that is issued only after the person has been successfully vaccinated against the micro-organism under study.

All work in the level 4 lab is done in a pressurized and ventilated suit. Air for breathing is passed into the suit through a hose and is filtered so as to be free of micro-organisms. Standard operating procedures are in place for every technique and operation in a BSL-4 laboratory (i.e., changing a filter on a reverse osmosis filtration device), and all work done in the laboratory is documented. A BSL-4 laboratory is completely isolated from the rest of the rooms in the building. Ideally, the lab is located in a separate building. The laboratory is designed to be a secure facility with respect to the escape of micro-organisms.

ESTABLISHMENT OF A BSL3 LABORATORY IN CHAPALI KATHMANDU

The world is now more than ever aware of the threat posed by bio-terrorism (anthrax, plague) and emerging diseases (SARS, West Nile) and re-emerging infectious diseases (Mumps, Staphylococcus aureus). No wonder so many bio-safety laboratories are being constructed. But many of these facilities are for owners who may have never before built, operated or maintained a bio-safety laboratory. Although commissioning is a significant part of certification, it is also part of a larger effort and process. Our aim is to describe the certification process in general terms and the specific role of commissioning in that process.

The goal of certification is to prove compliance with the national laboratory Bio-safety in Nepal. Government agencies place the responsibility with the end user to develop a detailed description of the building, its systems, and its methods to safely conduct research using select agents. A small number of consultants provide services to support facility owners to develop a formal process that leads to certification. Conclusive results of system tests must demonstrate that systems and standard operating procedures (SOPs) will provide environmental and biological safety without compromising the research. The role of the commissioning process is best understood by first identifying the purpose of the commissioning process and then describing the desired outcome for typical BSL-3 facility. Nepal will get consultant on this matter from FAO under WB/AICP .The purpose of the commissioning process is to document and verify that the facility and its systems achieve the owner's requirements. In the context of certification, the most important tests include:

- Building automation system, operation and monitoring
- HEPA filter tests
- Primary bio-containment device effectiveness
- Room pressurization control
- Decontamination system integration with facility
- Decontamination system efficacy
- Tissue digester
- Autoclave cycles
- Waste handling (liquid and solids)
- Redundancy tests
- Failure analysis of primary systems (fans, pumps, etc.)
- Standby or emergency power tests

The owner relies on the commissioning authority to provide conclusive documentation that proves compliance with the guidelines relevant to the operation of the facility to conduct its intended research. This information will be reviewed by the government of Nepal or other parties as required by donor community as a part of the certification process. Conclusive results of system tests should demonstrate that all systems and standard operating procedures will provide environmental and biological safety. Training component is vital in this aspect along with documentation process. This is the emerging challenges and Nepal shall face this challenge with the assistance of international community and stakeholder in AICP project.

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ESTIMATION OF NORMAL LEVEL OF CALCIUM, PHOSPHORUS AND TOTAL PROTEIN IN SERUM OF BROILERS, NON LAYING AND LAYING CHICKENS.

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ABSTRACT

This study on Estimation of Normal Level of Calcium, Phosphorus and Total Protein in Serum of Broilers, Non-Laying and Laying Chickens was carried out from February to July 2007 in Chitwan and Makawanpur district

Introduction

1.1. Background

Poultry farming has emerged as a major income generation enterprise in agriculture sector over the last four decades. Chitwan, one of the major poultry pockets in Nepal, shares around 30% of the broiler and 80% of the total layer chicks produced throughout the country (Thapa, 2005). Poultry industry in Chitwan is growing day by day from small scale farmer rearing 500 commercial birds to big farms having up to 50000 birds. Thus the poultry industry has developed as one of the commercial enterprise in private sector of Nepal

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, Hubchix, Avian34, Marshal etc. Similarly the layer breeds in Chitwan are Hyline Brown, Loh man Brown, Babcock, Isa Brown, Keystone Brown etc (Thapa, 2005).

Blood examination is performed for several reasons as a screening procedure to assess general health.Calcium and phosphorus are principal mineral elements in the body.

Almost all calcium in blood is found in the plasma, as erythrocytes contain very little calcium. calcium is present in two forms in the plasma: (1) a non diffusible protein bound form that constitutes 40 to 50 percent of total plasma calcium and (2) a diffusible non protein bound form. diffusible non protein bound calcium exists in two forms: (1) as a complex with citrate and phosphate and (2) as physiologically active ionized calcium. Approximately 5 percent of non protein bound diffusible calcium is in the complex form, with the remainder being the ionized physiologically active form. Ionized calcium is the most important physiologically. A decrease in ionized calcium produces tetany. The hydrogen ion concentration is a major chemical factor influencing the concentration of ionized calcium. An increase in ph (alkalosis) decreases ionized calcium but does not change the total serum calcium concentration.

Phosphorus is present in blood as an organic ester within erythrocytes or as phospholipid and inorganic phosphate in plasma. The inorganic fraction is usually measured to determine serum phosphorus levels. As there is considerable phosphorus in erythrocytes, samples to be analyzed for phosphate must be handles carefully to avoid hemolysis. Phosphoric esters released from erythrocytes may undergo hydrolysis and liberate phosphate, thus increasing serum levels, may be seen in nonhemolyzed samples exposed to high ambient temperatures.

1.2 Objectives

- To estimate the normal Ca, P and TP levels in the serum of Broilers, Non laying and laying Chickens.
- To compare the variation of Ca, P & TP level in blood at different stages of fowl of Chitwan district.
- To recommend the requirement of Ca, P & TP level in feed at different stages of fowl.

1.3. Rationale of the study

- Estimation of Ca, P & TP levels in serum at different stages of fowl hasn't been carried so far in Nepal.
- To diagnose the different disease condition by knowing normal parameters.

1.4. Limitations of the study

- Variation of parameters according to breed could not be performed.
- Comparision of different parameters between male and female chickens could not be done.
- Automatic analyzer was not available.

Review of literature

Serum calcium levels for most normal birds are 8-18 mg/dl. Hypercalcemia occurs with hypervitaminosis D3, or as a normal physiologic occurrence in egg laying hens (Wallach and Flieg, 1969). Hypocalcaemia is associated with advanced nutritional secondary hyperparathyroidism, renal failure, hypoalbuminemia, and excessive fat necrosis (Halliwell, 1981).Hypocalcaemia seizures may occur with serum calcium levels less than 6 mg/dl.

Serum phosphorus levels for most normal birds are 2.0 to 4.5 mg/dl. Elevated serum phosphorus can be associated with renal disease, in which the phosphorus level can be 9.5mg/dl or greater (Altman, 1979). Elevated serum phosphorus can be associated with renal disease is often associated with a hyperuricemia and hyperphosphatemia. Hypervitaminosis D3 will increase serum phosphorus values. Low serum phosphorus levels are seen in enteric diseases when impaired intestinal absorption of phosphorus has occurred (Shane, Young and Lutwak, 1968).starvation and anorexia will produce hypophosphate (Christie and Halliday, 1979).

MATERIALS AND METHOD

Collection of Sample

Blood from 48 broiler chickens of different age (from day old to 42 days) 81 layers of different age and stages was obtained from the wing vein using a 3 ml syring and 23 gauze needle , then immediately transferred into the tube. The samples were kept in an ice box, using icepacks and transferred to the bio-chemical lab for further assays.Samples taken were as follows.

Broiler chickens

Age(day)	No. of samples
1	3
7	6
14	3
21	6
28	6
32	12
42	12

Laying and non laying chickens

Stage	No. of samples
L1	10
L2	14
L3 (stage 1)	31
L3 (stage 2)	12
L3 (stage 3)	12

Serum Separation and Preservation

- Placing the sample containing test tubes at room temperature overnight at slant position.
- Pipetting the separated serum from the test tubes.
- Preserving the serum keeping the serum vials in deep freeze.

Reagent Preparation and Mixing with the Serum.

➢ Reagent preparation for Iron, Phosphorus and Total Protein and mixing those reagents with serum as per the recommendation of the protocol attached with the diagnostic kits.

➢ Principle

Arsenazo 111 specially binds to calcium forming a coloured complex which can be measured at 650 nm.

Ca^{++} +Arsenazo 111 Coloured complex

The amount of calcium present in the sample is directly proportional to the intensity of the coloured complex formed.

PROCEDURE

Wavelength;	650 nm (640nm 660nm)
Cuvette:	1 cm ligh path
Temperature:	25/30/37° C
Measurement	against air

Pipette into cuvate

	Reagent Blank	Calibrator	Sample
Distilled Water	15µ	-	-
Sample	-	-	15 µ
Calibrator	-	5µ	-
Reagent	1.0 ml	1.0 ml	1.0 ml

Mix and read absorbance of the sample (Asample) and Calibrator (Acalibrator) against reagent blank after 5 minutes.

CALCULATION

$$\text{Concentration} = \frac{\text{Asample}}{\text{Acalibrator}} \times \text{calibrator value}$$

Procedure of Phosphorus estimation in serum:

Phosphorus level in the serum is estimated based on Phosphomolybdate method as follows:

Principle: H_2SO_4

norg.Phosphorus + Ammon. Molybdate

Phosphomolybdate

The absorbance is measured at 340 nm.

Assay Parameters

Reaction Type:	End point
Wavelength:	340 nm
Flow cell Temp.	30 degree Celsius
Sample Vol.:	0.o1 ml
1-Phosphorus Reagent Vol:	0.5 ml
2-Phosphorus Reagent Vol	0.5 ml
Incubation	5 minute at room temp.
Std. Concentration:	5.0 mg /dl
Zero Setting with:	Reagent Blank
Light path:	1.0 cm

Procedure:

For 1.0 ml cuvate capacity:

Pipette into Tubes	Blank (B)	Standard (Std)	Test (TS)	
1-Phosphoru reagent (ml)	0.5	0.5	0.5	
2-Phosphorus Reagent (ml)	0.5	0.5	0.5	
Sample(ml)	-	-	0.01	
Standard (ml)	-	0.01	-	

Mix well and incubate for 5 minutes at room temperature .Read absorbance of standard (STD) and test (TS) at 340 nm against Reagent blank.

Calculation

Concentration =Asample / Astandard × 5(mg /dl)

Procedure of Total Protein estimation in serum:

Total protein level in the serum is estimated based on the following principle and procedures:

Principle

Wavelength	546 nm
Cuvette	1 cm light path
Temperature	20-25 degree centigrade
Measurement	against reagent blank

	Reagent blank	Standard	Sample
Distilled water	0.02 ml	-	-
Standard	-	0.02 ml	-
Serum	-	-	0.02 ml
Solution	1.0 ml	1.0 ml	1.0 ml

Mix well and incubate for 30 minutes at 20 -25 degree centigrade. Then, measure the absorbance of the sample (A sample) and of the standard (A standard) against the reagent blank .

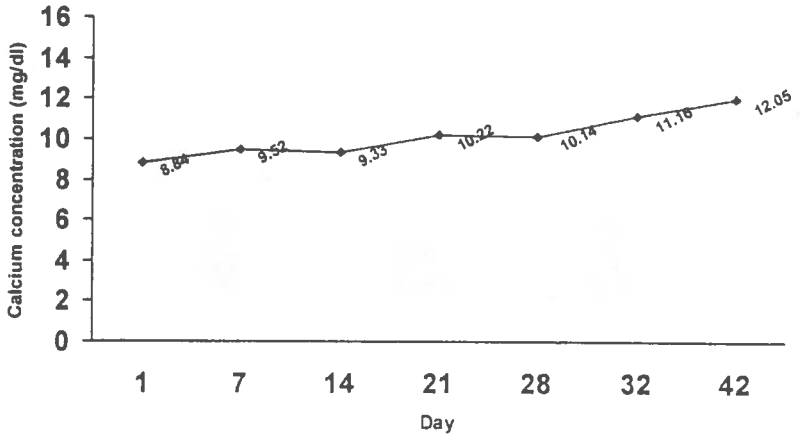
Calculation

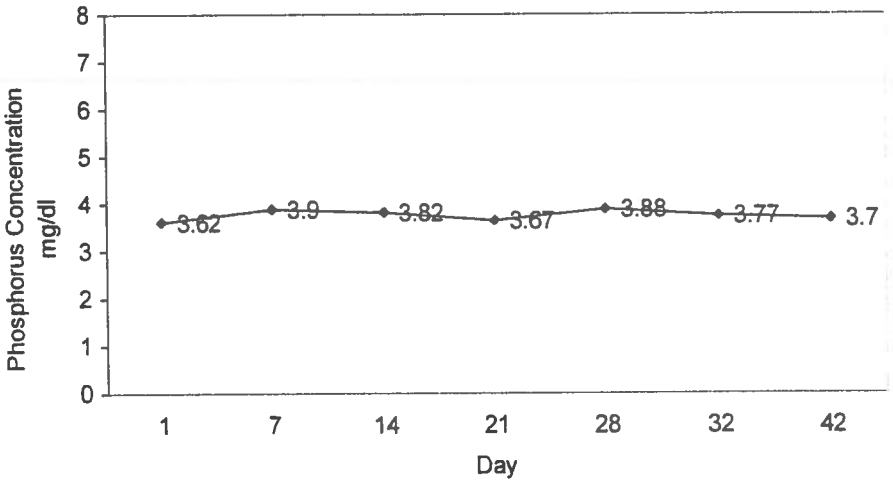
concentration =Asample / Astandard × 6 (mg / dl)

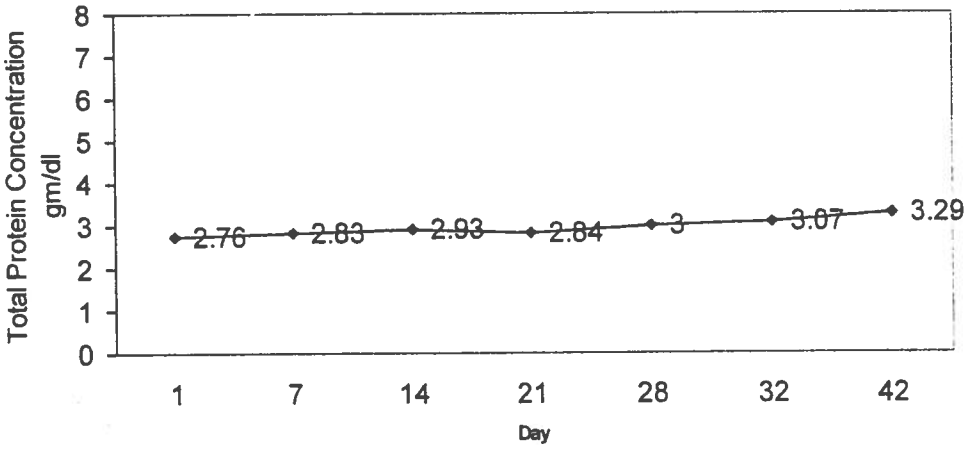
4. RESULT AND DISCUSSION

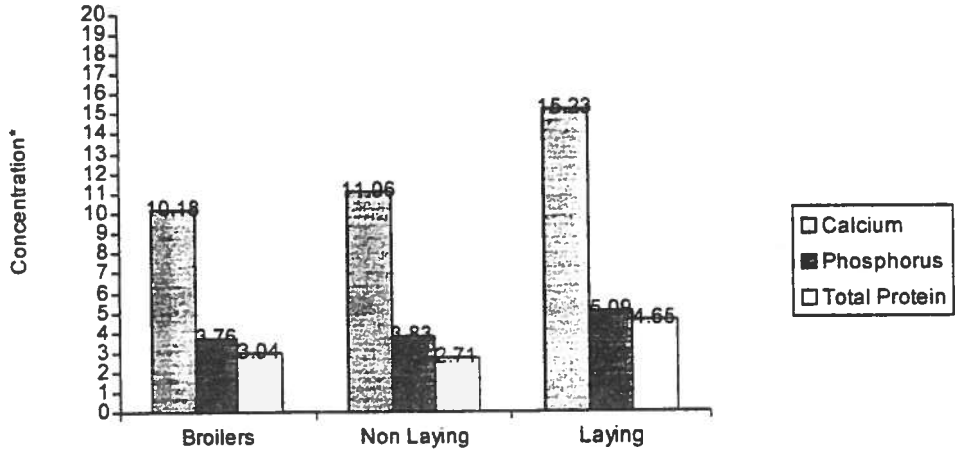
SN	Age	Calcium (mg/dl)	Phosphorus (mg/dl)	Total Protein (gm/dl)
1	1 day	8.84±0.30	3.62±0.29	2.76±0.11
2	7 day	9.52±0.39	3.90±0.17	2.83±0.11
3	14 day	9.33±0.6	3.82±0.71	2.93±0.2
4	21 day	10.22±0.41	3.67±0.13	2.84±0.06
5	28 day	10.14±0.22	3.88±0.41	3.0±0.1
6	32 day	11.16±0.31	3.77±0.88	3.07±0.05
7	42 day	12.05 ±0.29	3.70±0.14	3.29±0.1

SN	Stages	Calcium (mg/dl)	Phosphorus (mg/dl)	Total Protein (gm/dl)
1	L1	10.45±0.3	3.75±0.07	2.52±0.07
2	L2	11.67±0.17	3.91±0.13	2.91±0.09
3	L3 (Stage-1)	13.76±0.29	4.78±0.08	4.01±0.06
4	L3 (Stage-2)	16.45±0.4	5.14±0.15	4.78±0.15
5	L3 (Stage-3)	15.5±0.51	5.35±0.19	5.17±0.1

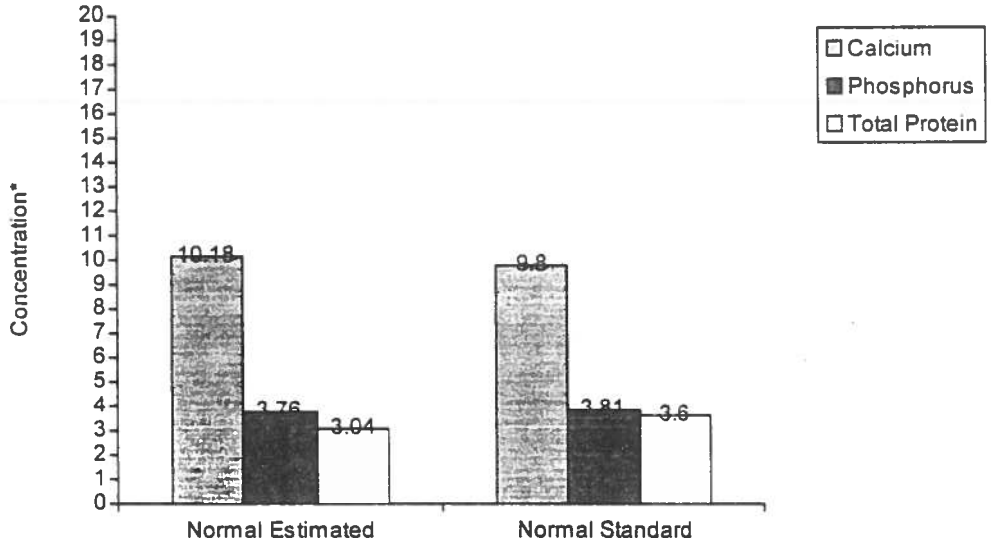








* Ca & P Concentration = mg/dl
Tp Concentration = gm/dl



* Ca & P Concentration = mg/dl
Tp Concentration = gm/dl

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Tp Concentration = gm/dl

4. DISCUSSION

- In broiler Ca, P and TP concentration in serum is found to be within the normal range specified by (Clinical Diagnostic Division, 1990).
- Normal parameters are increasing slightly with the ascending age.
- Ovulating hens have significantly higher Ca level than non reproductive females. This agrees with Kunjarathiyapung et al., (1987) who compared the levels of Serum Ca between laying hens (18.10±2.64 mg/dl) and broilers (6.25-13.75 mg/dl).
- Serum phosphorus level was in the normal range specified by Coles (1986).
- In egg laying chickens, a considerable increase in total protein level occurs, which could be attributed to an estrogen induce increase in globulins. The proteins were the yolk precursors (vitellogenin and lipoproteins), which were synthesized in the liver and transported via the plasma to the ovary where they were incorporated in the oocytes (Ritchie et al., 1994).
- In layers, blood parameters Ca, P and TP were found to be maximum in L3 (Stage-2) which is the peak production stage.
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- In layers, blood parameters Ca, P and TP were found to be maximum in L3 (Stage-2) which is the peak production stage.

CONCLUSION

- In broilers, the average values of Ca, P and TP level were found to be 10.18±0.42, 3.76±0.07 and 3.04±0.04 respectively.
- In non laying chickens, Ca, P and TP levels were found to be within the range of 8.37-12.64, 3.04-4.78 and 2.08-3.31 respectively.
- In laying chickens , Ca, P and TP levels were found to be within the range of 10.99-19.36, 3.82-6.41 and 3.27-6.17 respectively.

5. RECCOMENDATION

- similar research with respect to sex and breed variation could be studied in future.
- On the basis foundation laid by this research, more study could be conducted in this field with the evaluation of new blood biochemical parameters.

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Investigation of Nematodiasis in Goats Under Sedentary Management in a Low Hill Village of Western Nepal

- S P Devkota

Introduction

Goat production is an important component of subsistence agriculture system in Nepal and raised 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 emergencies. The estimated goat population of Nepal has been estimated to be 6.97 million and its meat production has been estimated to be about 39664 mt. in the year 2002/03 (CBS 2004) worth about 134 million US$ at the current market and exchange rates (Rs. 250/Kg of meat, 1 US$ = 74 NCR). However, the national production is unable to fulfi ll the need of the country, hence; a significant number of the goats are imported from neighboring countries. The economic value of sheep and goat manure has been estimated to about 4 million US$ at 1992 prices (Ghimire 1992).

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 mid to lower hills goats are raised under sedentary management system in smaller flocks of 10-50 animals. In some village where cropping intensity is high, pasture and labor availability are lower and fl ock sizes 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 and are housed indoors during night. The grazing area is usually private crop harvesting in winter, grazing is mainly on the follow fields.

Among the various factors affecting productivity, gastrointestinal nematode infection has been regarded as one of the important cause (Shrestha 1994). Thakuri and Mahato (1990) similarly stated that gastrointestinal nematode infection 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 ie. migratory or sedentary system. Joshi (1991, 1994) recorded high mortality due to gastrointestinal nematode infection in sheep and goats under intensive grazing managements in mid and low hilly regions of west Nepal and further stated that parasitic gastroenteritis is one of the major causes of productivity loss in goats in Nepal. Joshi (1996) found that subclinical parasitism was common in goats raised either under the sedentary or migratory management and further stated that the infection was responsible for the reduction in body weight gain by 93 to160 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 confi ned to the wet summer months between Aprils to October with a very low level of infection during the rest of the year. Trichostrongylus spp were the most prevalent species followed by Ostertagia spp, in the migratory and Haemonchus contortus in the sedentary animals. 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 (EPG) of 500-2000 could be considered for subclinical parasitism (Lloyd, 1987 cited by Joshi 1994).

The most important control program for intestinal nematodiasis in ruminants for parasitic gastroenteritis is regular drenching with suitable anthelmentics. Albendazole, Fenbendazole, Levamisole, and Ivermectin are the commonly available anthelmintics in our markets. Albenzole, Fenbenzole, Levamisole and Ivermectine should be used at the dose rate of 5 mg/kg, 5 mg/kg, 7.5 mg/kg and 50-200 ¼g/kg respectively (Soulsby, 1982). Fenbendazole in the given dose rate

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is highly effi cacious in the treatment of adult and immature gastrointestinal nematodes, albendzole is highly efficacious in sheep, Levamisol is also highly efficacious against both mature and immature gastrointestinal nematodes and lungworms, and similarly ivermectine has excellent effi cacy against immature and adult worms of ruminants. Likewise, Armour et al. (1980) reported that subcutaneous injection with 100-200 ¼g/kg removed all adults and inhibited larvae of common gastrointestinal nematodes of cattle in the UK.

The following study was conducted to know the present nematode parasites of goats in mid hills and their burden and identify them . It further aimed to fififi ndout the effective treatment drug for gastrointestinal nematodes in goats in the existing field conditions.

Materials and Methods

1. **Site selection :** For this field study Suraundi Commercial Goat production Group from Pauwegaunde VDC – 8, Syangja District was selected as a study site which is situated in the mid hill of the Western Development Region of Nepal. Here, all goats are raised as a sedentary management system.
2. **Grouping and stratifi cation of the Population :** A team from the RVL, Pokhara visited the site and gathered the name of the farmers and their goats. Goats are selected randomly errespective of the age and feedings. All together 5 groups are made each consisting of 10 goats and given the name as Albendazole group, Fenbendazole group, Levmisol group, Ivermectin group and control group.
3. **Sample collection and examination :** Fecal samples from all the 50 goats are taken on the first visit for epg count and fecal larval culture in a polybags separately with proper identifi action. Again, the fecal samples were collected for epg counts after seven days of drenching and again after 30 days of drenching.

The faecal samples collected from the goats were examined by improved modified McMaster Method (MAFF, 1986) as stated below.

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 centrifuation 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 prepaired 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 fi lled with water and enverted 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 lebelling was kept in a refrigerator for about a night.
5. Next day the supernetent 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.
4. Drenching all the 4 groups are drenched on the second visit and 1 group was left untreated. For Albendazole group the goats are drenched by albendazole (analgon tablet) at the rate of 5 mg/kg body weight, Fenbendazole group by Fenbendazole (Panacure tablet) at the rate of 5 mg/kg, Levamisole (Adzanide-L tablet) at the rate of 7.5 mg/kg and ivermectin group by Ivermectine injection (Kepromec Injection) at the rate of 1 ml/50 kg body weight subcutaneously.

Results

The result of fecal egg count (EPG) of various groups like before and after treatment and within the treatment groups is presented in the table as shown below.

Mean Faecal Egg Count of Goats before and after Treatment

No of animals	Mean EPG of faeces		
	Before Treatment	After Treatment	
		After 7 days	After 30 days
40	1642.5	82.5	500

Mean Fecal egg Count of goats before and after treatment in various groups

Groups (Treatment groups)	Mean EPG of faeces		
	Before Treatment	After Treatment	
		After 7 days	After 30 days
Albendazole	1760	210	470
Fenbendazole	1710	70	320
Levamisole	2010	50	360
Ivermectin	1090	0	100
Control	1833.33	1720	1876.5

The fecal sample before drencning is also subjected for the larval culture gave the following result.

Mean Fecal larval composition of the faecal material examined after larval culture.

Nematodes species	*Mean larvae count*	*Percentage of mean larvae recovered in the faeces*
Trichostrongylus spps.	*22*	*28.95*
Ostertegia spps.	*13*	*17.10*
Oesophagostomum spps.	*7*	*9.21*
Haemonchus spps	*32*	*42.16*
Nematodirus spps.	*2*	*2.60*
Total	*76*	*100*

DISCUSSION

The faecal egg count has been regarded as a crude estimate of the worm burden, it has been suggested (Lloyd 1987 cited by Joshi in 1994) 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 could be considered for subclinical parasitism.In this population of goats the fecal egg count was found to be <2000 indicating these goats are suffering from subclinical helminthic infection.

Though a mixed infection was found in the feacal culture and the epg counts, it can be said that *H. contortus* and *Trichostrongylus spps.* were the main worm species responsible for this infection in the fl ock. *H.contortus* parasite was also reported as a major cause of death in goats in Malaysia (Sani *et al.* 1985 as cited by Joshi 1994). Baxendell (1987) cited by Joshi (1994) also stated that severe haemonchosis in goats occurred when EPG exceeded 2000. Due to high fecundity and short generation interval under optimal environmental conditions, development of this parasite is very rapid and animal become severely infected. The other gastrointestinal helminthes found in the faecal larval culture were *Trichostrongylus spps., Ostertagia spps., Oesophagostomum spps.* and *Nematodirus spps.* The effects of these parasites, as reviewed by Soulsby (1982), could be summarized as loss of appetite, villous atrophy, reduced intestinal effi cacy. These parasites certainly would have contributed to the gradual debility of the animals.

The mean epg of feces before treatment and after treatment as shown by this study indicates that treatment drastically reduced the mean epg form 1642.5 to 82.5 in 7 days and 500 after 30 days of treatment. Among the different drugs compared all are equally effi cient in treating nematodes in goats. Though all drugs are effi cient, Ivermectin is much more effi cient for treating intestinal nematodes, followed by Fenbendazole, Levamisol and then albendazole.

In conclusion, it can be said that productivity losses due to parasitic gastroenteritis can be seen in goats even under small holder sedentary management systems with ocassional mortalities. From the present observations it can be said that if goats are grazed on limited pasture and continuous grazing system a regular anthelmintic treatment with a broad spectrum anthelmintics has to be provided regularly.

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