ANNUAL TECHNICAL REPORT

2060/061(2003-2004)

HMG/N
Ministry of Agriculture and Cooperatives
Department of Livestock Services
Directorate of Animal Health

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ANNUAL TECHNICAL REPORT

F.Y. 2060/61
[2003/2004]

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FOREWORD

This issue of annual technical report consists of various activities run in the Central Veterinary Laboratory and Animal Disease Control Section (CVL &ADCS) Tripureshor, Regional Animal Disease Diagnostic Laboratories Biratnagar, Janakpur, Pokhara, Surkhet and Dhangadhi, and National Avian Disease Diagnostic Laboratory Chitwan Bharatpur in the fiscal year 2060/61(2003/2004). Prompt disease diagnosis and timely report to the farmers is the main motto of our laboratories. Various activities are carried out in different laboratories located in different places to provide correct and timely disease diagnosis. The Regional Disease Diagnostic Laboratories plays role as referral laboratories for their respective development region and CVL&ADCS acts as referral laboratory for other district and regional laboratories. National Avian Disease Diagnostic Laboratory plays important role to provide avian disease diagnosis primarily to the poultry farmers of Chitwan district and other parts of the country.

In the very fast changing context of the world and Nepal being a member of World Trade Organization (WTO) and Office des Epizootics (OIE), the role of Veterinary Laboratory Services has increased and will require further strengthening of veterinary laboratory services to deliver disease diagnosis at the international standards. Better laboratory services will support the livestock farmers by providing efficient diagnosis, so that our farmers can compete and trade in the in international market.

The role of laboratory diagnosis and the experts serving in labs are always of immane importance for the effective and economic treatment of livestock ailments and to control epidemics. At present the challenge is to provide quality service, which is the main aim of our laboratory group and wish to further improve in coming days. The laboratory group of experts is also providing service from village level animal health, infertility, and vaccination campaign organized by District Livestock Services Offices (DLSO) to special disease investigation programme conducted by respective laboratories.

I would like to express thanks to all the five Regional Animal Disease Investigation Laboratories and National Avian Disease Investigation Laboratory for providing the progress reports of their respective laboratories & disease investigation related technical articles in time. I would also like to thank Dr. Poornima Manandhar, Dr. Banshi Sharma, Dr. Kedar Bahadur Karki, Dr. Vinaya Kumar Karna and all the staffs of CVL for contributing to prepare and publish this report. Special thanks goes to Dr. Ganesh Raj Pant and Dr. Rajesh Yadav for compiling & editing the report in this form.

Suggestions are highly appreciated.

Dr. Rebati Man Shrestha
Chief Veterinary Officer
Central Veterinary Laboratory

Table of Content

INTRODUCTION

Central Veterinary Laboratory (CVL) was established in 1995, under the Department of Livestock Services, His Majesty's Government of Nepal to provide laboratory based reliable diagnostic services to the livestock & poultry farmers of the country. CVL has several functional units, such as Pathology, Parasitology, Microbiology, Serology, Haematology, Biochemistry and Molecular biology. Microbiology unit has been providing diagnosis services of Rabies since the fiscal year 057/58. Serology unit has been performing the most important role by performing sero surveillance to detect the antibodies against Rinderpest and Goat plague (PPR). In addition to these units, CVL is also involving in several investigations activities to study the major animal health problem and to recommend effective and cheap control strategy of animal disease. The role of CVL is highly enthusiastic for the development of skilled & competent manpower according to the need of the nation.

Central Veterinary Laboratory leads five Regional Veterinary Laboratories (RVL) established in Biratnagar, Janakpur, Pokhara, Surkhet and Dhangadhi as well as one National Avian Disease Investigation Laboratory in Chitawn. CVL is also providing technical support to 15 Basic laboratories of the countries for routine bacteriological examination.

Objectives:

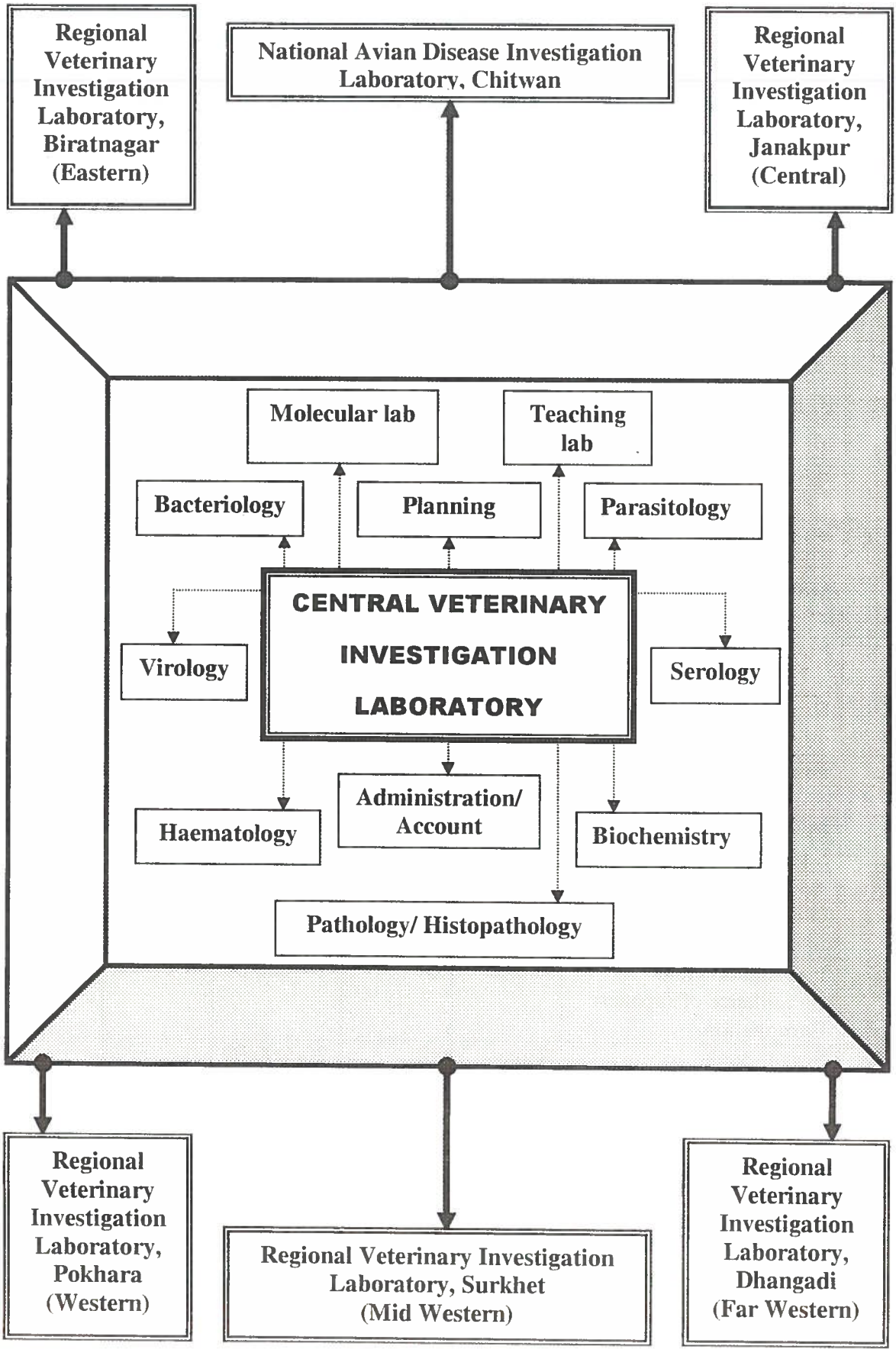
To support the livestock development efforts through efficient disease diagnostic and control services having the major objective of the sections, following are the specific objectives:

- To support national disease control and surveillance programme.
- To collaborate national research and international reference laboratory for the diagnosis, investigation and research according to need of the nation.

- To acquire, adopt, update and disseminate new/ different diagnostic tests for animal and poultry diseases.
- To act as a national referral diagnostic laboratory.
- To assist and advise on control of disease epidemics within the country.
- To organize training on laboratory technology for veterinarians and JT/ JTAs.
- To support regional and district laboratories in order to strengthen their technical capabilities.
- To collate, analyze and maintain national livestock disease database and strengthen Animal Health Information System.
- To disseminate information concerning animal disease to national and international organizations.

To achieve the above objectives, there are series of planned activities carried out by different units of the laboratory and the five regional laboratories.

Organization chart



Annual Work Programme & Annual Progress Of CVL (2060/061)

S.N.	Activities	Unit	Target	Budget allocated	Achieve ment	Progress %
1.	**Diagnostic Services**					
1.1	Parasitology	Number	1600	80000.00	2108	**100**
1.2	Microbiology	Number	2000	290000.00	3398	**100**
1.3	Pathology	Number	1200	228000.00	1346	**100**
1.4	Serology	Number	4500	787000.00	13165	**100**
1.5	Haematology	Number	350	82000.00	480	**100**
1.6	Biochemistry	Number	800	113000.00	876	**100**
1.7	Molecular Diagnosis	Number	18	195000.00	21	**100**
1.8	Rabies Diagnosis	Number	15	85000.00	16	**100**
1.9	Dispatch of samples to other laboratories	Number	500	42000.00	503	**100**
2.	**Disease Investigation and Surveillance Program.**					
2.1	Investigation & Surveillance on Infertility in Cattle & Buffalo	Times	12	265000.00	13	**100**
2.2	Outbreak Investigation	Times	12	265000.00	13	**100**
2.3	Investigation and surveillance of Japanese Encephalitis	Times	6	180000.00	8	**100**
2.4	Serum Bank Management	Times	12	200000.00	12	**100**
2.5	Investigation and surveillance of Sarcosistosis in Buffalo Meat	Times	3	75000.00	3	**100**
2.6	Pen Side test of PPR	Times	6	0.00	6	**100**
2.7	Sero Epidemiological Surveillance of PPR	Times	6	0.00	6	**100**

3.	**Teaching Lab Program**					
3.1	Teaching Lab Management	Times	12	175000.00	12	**100**
4.	**Supervision and Monitoring Program**	Times				
4.1	Follow-up & Reporting of Laboratories	Times	12	100000.00	12	**100**
4.2	Supervision & Monitoring of Lab Technician Training	Times	6	60000.00	6	**100**
5.	**Workshop Program**					
5.1	Workshop on disease investigation in Nepal	Times	1	50000.00	1	**100**
5.2	Participation in regional workshops (5 regions)	Times	5	40000.00	6	**100**
5.3	Workshop on Prog. & Budget of the next F/Y	Times	1	60000.00	1	**100**
6.	**Publication**					
6.1	Annual Technical Report	Times	1	60000.00	1	**100**
7.	**Contract Service**					
7.1	Sweeper & Gardener	Times	3	25000.00	3	**100**
8.	**Improvement of Physical facilities**					
8.1	Lab Improvement	Times	1	300000.00	1	**100**
9.	**Purchase**					
9.1	Technical Books & Journals	Times	3	40000.00	3	**100**
9.2	Microscope	Set	1	250000.00	0	
9.3	Elisa Reader	Set	1	750000.00	0	
9.4	Automatic Tissue Processor	Set	1	250000.00	0	
9.5	Computer	Set	1	100000.00	0	
9.6	Refrigerator	Set	1	75000.00	0	
Total				**5204000.00**		***84.00***
Administrative Expense				**3765000.00**		
Grand Total				**8969000.00**		

Man Power Situation of Central Veterinary Laboratory and Animal Disease Control Section (F/Y 060-61)

S.N.	Type of the Post	Class	Number	Fullfilled	Vacant	Remarks
A.	*Technical*					
1.	Chief Veterinary Officer	G I	1	1	-	
2.	Senior Veterinary Officer	G II	4	4	-	
3.	Veterinary Officer	G III	5	5	-	
4.	Junior Technician (JT)	NG I	9	9	-	
5.	Junior Technical Assistant	NG II	1	-	1	
6.	Stock Man	NG III	8	7	1	
Total Technical			**28**	**26**	**2**	
B.	*Administration/Account*					
1.	Nayab Subba	NG I	1	1	-	
2.	Typist	As per efficiency	1	1	-	
3.	Accountant	NG I	2	1	1	
4.	Kharidar	NG II	1	1	-	
5.	Mukhiya	NG III	1	1	-	
6.	Driver	Light Vehicle	1	1	-	
7.	Peon	-	6	6	-	
Total Administration			**13**	**12**	**1**	
Grand Total			**41**	**38**	**3**	

Staff of Central Veterinary Laboratory and Animal Disease Control Section

(At the end of F/Y 2060/061)

S.N.	Name of Staff	Post	Class	Starting from	Remarks
1.	Dr. Rebati Man Shrestha	CVO	G.I	058.11.02	
2.	Dr. Gyanendra Nath Gongal	SVO	G.II	059.03.01	
3.	Dr. Ganesh Raj Pant	SVO	G.II	055.09.23	
4.	Dr. Poornima Manandhar	SVO	G.II	057.12.22	
5.	Dr. Pusp Prasad Shrestha	SVO	G.II	060.04.14	
6.	Dr. Banshi Sarma	VO	G.III	056.11.01	
7.	Dr. Rajesh Yadav	VO	G.III	058.06.01	
8.	Dr. Kedar Bahadur Karki	VO	G.III	060.10.21	
9.	Dr. Vinaya Kumar Karna	VO	G.III	059.01.22	
10.	Dr. Binu Shrestha	VO	G.III	059.09.29	
11.	Mr. Asal Bahadur Tamang	JT	NG.I	052.04.01	
12.	Mr. Ashok Pd. Shrestha	JT	NG.I	052.04.01	
13.	Mr. Prakash Devkota	JT	NG.I	060.08.01	
14.	Mr. Bal Bdr. Kunwar	JT	NG.I	053.02.24	
15.	Mr. Tek Bahadur Air	JT	NG.I	058.09.04	
16.	Mr. Dhan Raj Rai	JT	NG.I	057.03.09	
17.	Mrs. Sila Pant	JT	NG.I	057.04.30	
18.	Mr. Brai Kishor Thakur	JT	NG.I	060.07.24	
19.	Mr. Prem Kumar Lama	JT	NG.I	058.01.09	
20.		JTA	NG.II		

S.N.	Name of Staff	Post	Class	Starting from	Remarks
21.	Mr. Sri Ram Pande	S.Man	NG.III	059.04.01	
22.	Mr. Damodhar Pandey	S.Man	NG.III	052.11.13	
23.	Mr. Purna Maharjan	S.Man	NG.III	053.12.20	
24.	Mr. Hari Pd. Pyakurel	S.Man	NG.III	054.12.02	
25.	Mr. Prahlad Basnet	S.Man	NG.III	057.12.01	
26.	Mr. Hari Bhakta Karki	S.Man	NG.III	059.01.01	
27.	Mr. Bhimsen Adhikari	S.Man	NG.III	057.08.01	
28.		S.Man	NG.III		
Administration/Account					
29.	Mr. Madhav Prasad Neupane	N.Subba	NG.I	059.01.26	
30.	Mrs. Kamala Shrestha	Typist	NG.I	055.07.11	
31.		Account.	NG.I		
32.	Mr. Nara Hari Luitel	Account.	NG.I	057.10.01	
33.	Mr Krishna Hari Dongol	Kharidar	NG.II	052.11.06	
34.	Mrs. Amala Devi Khadka	Mukhiya	NG.III	053.08.05	
35.	Mr. Macha Kaji Maharjan	Driver	L.V.	055.07.01	
36.	Mrs. Chiri Maya Maharjan	Peon	Lo.lev.	055.10.01	
37.	Mr. Santa Raj Budathoki	Peon	Lo.lev.	059.11.01	
38.	Mrs. Bhima Acharya	Peon	Lo.lev.	055.04.01	
39.	Mr. Hari Gobinda Shrestha	Peon	Lo.lev.	059.11.06	
40.	Mr. Chandra Bdr. Rana	Peon	Lo.lev.	056.08.23	
41.	Mr. Anoj Bajracharya	Peon	Lo.lev.	058.11.01	

Microbiology unit

This microbiology unit is responsible for routine diagnosis of microbial infection and laboratory examination of samples submitted to this unit under investigation launched by CVL. The Unit is leaded by Dr. Poornima Manandhar, Senior Veterinary Officer and assisted by two Senior Technicians Mr. Tek Bahadur Air and Mr. Bal Bahadur Kunwar and one Junior technician Mr. Bhimsen Adhikari, This unit (Bacteriology Lab) started to produce Salmonella Antigen for the Plate Agglutination Test since 2003 and supplying needed amount of this antigen to different regional labs as well as to the privet practicing Vets. This unit is also developing the Test procedure and for standardizing the Pen Side Test for the diagnosis of PPR on the spot/field since 2002.

Microbiology Unit of Central Veterinary Investigation Laboratory (CVIL) comprises mainly of 4 sub units. They are as follow

- Bacteriology and Mycology
- Virology
- Rabies Diagnosis
- Media preparation and Sterilization

Bacteriology and Mycology Lab

This lab is involved in routine diagnosis of bacterial and fungal diseases. So, the main work of this unit is isolation and identification of organism as well as to perform drug sensitivity test to isolated organisms. This lab also involves in Research work for the postgraduate as well as undergraduate students. The main research area till date dealt by this lab is on isolation of *Salmonella sps.* in Poultry meat, Goat meat, Buffalo meat and Pork. This unit also involved in research work of Salmonella in Poultry litter of Chitawan district conducted by Tuft University, America. This lab has facilities and developed its own standard protocol for isolation of *Salmonella sps.* from different sample.

It receives samples from field, veterinary hospitals as well as from the postmortem section of CVL itself. This lab also receives samples/primary isolates from different regional labs as well as from avian lab. The major samples receives by this section are the milk, tissues, blood and swabs. Antibiotic sensitivity test is one of the important works that facilitates the proper treatment of Mastitis in time.

Progress

A total of 3030 samples were received from different species of animals. Among 3030 samples, organisms could isolate from 1065 samples. Out of this 135 isolates were fungus. Out of 1065 samples, only 475 samples were processed for the Drug Sensitivity Test (DST) as shown in Table 1.

Table 1. ANNUAL WORK RECORD OF BATERIOLOGY UNIT (2060/061)

Month	Total Sample Culture	Isolation	DST	Fungal Test	Media Preparation	Sterilization	Others	Sample sent to other lab.	Remarks
Shrawan	340	169	119	40	9 times	9 times	12	-	
Bhadra	466	112	70	18	7 times	6 times	266	-	
Ashwin	167	51	31	14	8 times	6 times	71	-	
Kartik	194	49	43	4	7 times	7 times	98	-	
Marg	265	134	32	3	5 times	6 times	96	-	
Paush	139	30	20	4	8 times	7 times	85	-	
Magh	315	99	22	4	7 times	6 times	190	-	
Falgun	185	56	26	13	9 times	8 times	90	-	
Chaitra	534	215	31	16	8 times	6 times	272	-	
Baisakh	113	49	25	11	6 times	5 times	28	-	
Jestha	131	41	19	6	7 times	5 times	65	-	
Ashad	181	60	37	2	9 times	8 times	82	-	
Total	**3030**	**1065**	**475**	**135**	**90**	**79**	**1355**	**-**	

A total of 367 milk samples were received from the field and 293 samples showed positive to the California Mastitis Test (CMT). The major isolated organisms from milk were Staphylococcus, Streptococcus, and *E.coli.* Few *Pseudomonas sps*, *Klebsiela sps* and fungus (*Candida sps*) were also isolated organisms from milk samples.

As mentioned earlier this lab receives postmortem samples from different species. Among them poultry is the main species as there are many commercial poultry farms in the country.

In poultry main problem according to this section seems *E.coli.* and *Salmonella sps.*

The other important work of this unit is the antibiotic sensitivity test in milk samples and rest in poultry samples. Enrofloxacin and Gentamycine and Kanamycine were found most sensitive antibiotics according to our findings (Table 2).

Table 2. Drug Sensitivity Test

Month		Tested Antibiotics								Isolated Organisms
		G	EX	C	K	NF	T/O	CX	P	
Shrawan	S	108	119	90	85	50	60	30	-	E. coli, Staph., Strepto., Proteus, Bacillus, Klebsiella, Salmonella
	W/S	9	-	20	30	25	40	25	-	
	R	2	-	9	4	44	19	64	119	
Bhadra	S	65	70	40	50	37	25	32	-	Staph.,E.coli,Pseudomonas, Penicillinium.
	W/S	5	-	20	15	20	28	15	-	
	R	-	-	10	5	13	17	23	70	
Ashwin	S	25	31	28	25	20	15	10	-	Staph.,Candida, Microsporum, E. coli, Bacillus, Salmonella
	W/S	5	-	2	4	10	7	15	-	
	R	1	-	1	2	1	9	6	31	
Kartik	S	40	43	35	33	28	20	18	-	Penicilli., Strepto, Staph+, E. coli.
	W/S	3	-	6	5	8	15	15	-	
	R	-	-	2	5	7	8	10	43	
Mangsir	S	30	32	20	23	20	25	29	-	Proteus, Bacillus, Pseudomonas, Staph., E. coli
	W/S	2	-	8	7	10	5	2	-	
	R	-	-	4	2	2	2	1	32	
Paush	S	18	20	15	18	15	10	23	-	Strepto., E. coli, Pasteurella, Salmonella
	W/S	2	-	5	1	4	5	15	-	
	R	-	-	-	1	1	5	3	20	
Magh	S	20	22	18	20	10	15	8	-	Shagella spp., E. coli, Microsporum, Salmonella
	W/S	2	-	3	1	6	5	12	-	
	R	-	-	1	1	6	2	2	22	
Falgun	S	20	26	18	20	15	17	10	-	Klebsiella, Staph., E.coli, Fungus, Strepto. sps.
	W/S	3	-	6	5	5	3	15	-	
	R	3	-	2	1	6	6	1	26	
Chaitra	S	26	31	20	28	15	17	19	-	Candida, Staph., E.coli, Microsporum
	W/S	4	-	5	2	6	6	5	-	
	R	1	-	6	1	10	8	7	31	
Baishakh	S	20	25	15	21	10	12	10	-	Haemophillus, Blastomycas, Candida, E.coli
	W/S	5	-	8	3	8	8	12	-	
	R	-	-	2	1	7	5	3	25	
Jestha	S	15	19	13	16	13	15	14	-	Penicillinium, E.coli, Staph., Strepto.
	W/S	4	-	4	2	3	2	4	-	
	R	-	-	2	1	3	2	1	19	
Ashad	S	30	37	34	35	15	20	22	-	Staph., Strepto, Fungus, Salmonella
	W/S	7	-	2	1	10	15	8	-	
	R	-	-	1	1	12	2	7	37	

Virology section

This section is involved in the diagnosis of the viral diseases. Though this section has no tissue culture facility and other modern facilities, it is doing isolation of virus in embryonated chicken eggs, HA/HI test and Agar gel diffusion tests are the main tests conducted in this lab. It receives samples mainly from Postmortem of CVL and from the field.

This section is also involved in PPR diagnosis by doing rapid test called Pen side test in ocular and nasal swabs of goats. This test can confirm the disease within few minutes and can be tested in the field on the spot. It need 1% piglet RBCs.

Table 3. Examination of ND by egg inoculation method (2060 Shrawan - 2061 Ashad)

S. N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Chick	ND	Egg inoculation	13	7	
2	Lalitpur	,,	,,	,,	4	1	
3	Bhaktapur	,,	,,	,,	2	-	
4	Nuwakot	,,	,,	,,	4	2	
5	Dhading	,,	,,	,,	2	-	
6	Kaski	,,	,,	,,	2	-	

This lab received 27 samples suspected for Ranikhet disease and turned positive only 10 (37%) (Table 3). A total of 32 samples were tested for Marex virus. None of them turned positive in both AGID and Egg Inoculation (Table 4,5).

Table 4. Examination of MAREK'S disease by egg inoculation (2060 Shrawan - 2061 Ashad)

S. N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Chick	Marek's	Egg inoculation	7	-	
2	Bhaktapur	,,	,,	,,	3	-	
3	Dhading	,,	,,	,,	3	-	

Table 5. Examination of MAREK'S disease by AGID method (2060 Shrawan - 2061 Ashad)

S.N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Chick	Marex	AGID	13	-	
2	Bhaktapur	,,	,,	,,	3	-	
3	Dhading	,,	,,	,,	3	-	

Attempts were made to diagnose Inclusion body hepatitis (Lichi heart disease) in poultry, which was an emerging problem in last year. Twenty samples were examined by conducting AGID. None of them were found positive on AGID. Similarly 24 samples were tested by conducting egg inoculation test. Out of 24 tested samples, 15 were found positive on egg inoculation test.

Table 6. Examination of IBH disease by AGID Method (2060 Shrawan - 2061 Ashad)

S. N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Chick	IBH	AGID	13	-	
2	Chitwan	,,	,,	,,	4	-	
3	Nuwakot	,,	,,	,,	3	-	

Table 7. Examination of IBH disease by Egg Innoculation Method (2060 Shrawan - 2061 Ashad)

S. N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Chick	IBH	Egg inoculation	12	10	Typical Dwarfing of Embryos observed in Positive cases
2	Lalitpur	,,	,,	,,	2	-	
3	Nuwakot	,,	,,	,,	10	6	

Eight pox suspected samples were received from different districts. Only 3 samples were found positive for the Pigeon pox and none of the chicken samples found positive for the fowl pox (Table 8).

Table 8. Examination of PIGEON POX by egg inoculation method

S.N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Pigeon	Pigeon Pox	Egg inoculation	2	2	
2	Kaski	Pigeon	,,	,,	2	1	
3	Kathmandu	Chick	IBV	,,	2	-	
4	Chitwan	,,	,,	,,	2	-	

This virology section also received 75 PPR suspected samples from Kathmandu, Dolakha, Tanahun, and Nawalparasi districts. The samples were analyzed by Pen Side Test and found 45 samples positive. Out of that 7 samples were analyzed by HA/HI test and found all positive (Table 9 and 10).

Table 9. Examination of PPR by Pen side method (2060 Shrawan - 2061 Ashad)

S. N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Goat	PPR	PEN SIDE	45	30	
2	Tanahun	,,	,,	,,	10	-	
3	Dolakha	,,	,,	,,	5	-	
4	Nawalparasi	,,	,,	,,	15	15	
	Total				75	45	

Table 10. Examination of PPR by HA/HI method (2060 Shrawan - 2061 Ashad)

S. N.	District	Animal sp.	Suspected disease	Test Conducted	No. of sample tested	No. of sample positive	Remarks
1	Kathmandu	Goat	PPR	HA/HI	7	7	

Rabies diagnosis section

Microbiology unit has responsibility for the diagnosis of Rabies, too. Although this works belongs to Virology section it is kept separately as it is directly linked with human life or public health importance. The section has facility for all the 3 different tests, namely Negri body test, Fluorescence antibody test and Biological test. The section received samples from dog, buffalo, goat and human. A total of 15 cases (1 human, 10 dog, 3 buffalo, and I goat) only 9 cases were found positive from all the tests. Among that one human case from Patan hospital was also found positive (Table 11).

Table 11. RABIES diagnosis by different tests (2060 Shrawan - 2061 Ashad)

S. N.	Test Conducted	Animal Species	Suspected Disease	Number of Sample tested	No. of Sample Positive	Remarks
1	Biological Test	Human	Rabies	1	1	
2	Negribody test	,,	,,	1	1	
3	FAT	,,	,,	1	1	
4	Biological test	Canine (Dog)	,,	10	1	
5	Negribody test	,,	,,	10	1	
6	FAT	,,	,,	10	1	
7	Biological Test	Bovine (Buff)	,,	3	1	
8	Negribody test	,,	,,	3	1	
9	FAT	,,	,,	3	1	
10	Biological test	Ovine (Goat)	,,	1	-	
11	Negribody test	,,	,,	1	-	
12	FAT	,,	,,	1	-	

Media preparation and sterilization

This section is the backbone of Microbiology unit. It provides clean glassware for the media preparation and reagent preparation. This section is equally important to other units, too.

Pathology unit

Introduction

Pathology unit of Central veterinary laboratory (CVL) has two important disease diagnostic branches: Gross pathology and Histopathology. Being a referral veterinary laboratory of the nation, CVL receives a wide variety of samples from Regional veterinary laboratories (RVL), District livestock service offices (DOLS), private veterinary practitioners, hatcheries and directly from farmers.

Post-mortem examination

The total post-mortem samples received during the fiscal year 2060/061 were 771. Seven hundred and fifty-two (97.5%) of the total samples received were of poultry and rest nineteen samples (2.5%) were received from a wide variety of domesticated animals and birds. This figure indicates how much popular the poultry farming is in the capital valley and its around. This figure also reflects the level of awareness of the farmers for varied species of livestock. The annual disease occurrence pattern of the poultry diseases and a brief discussion about them has been presented in tabular form in table 1.

The nineteen samples include duck-6, pig-4, rabbit-3, dog-2, goat-2 sheep-1 and pigeon-1. Varied disease conditions were tentatively diagnosed for ducks. Diseases that were diagnosed during postmortem examination were Mycosis, Colisepticaemia, and Coccidiosis. The three post-mortem cases of pigs were diagnosed as bacterial infections of which two of them were the cases of Pasteurellosis and one diagnosed simply as bacterial infection. The rest one case of pig was diagnosed as poisoning.

The post-mortem samples of rabbit were all delivered from other diagnostic & production laboratories to CVL, P.M. unit. These laboratories keep rabbits as experiment animals. Enteritis of bacterial origin was found to be the cause for the

death of a rabbit while other was diagnosed to be died of pneumonia and No Abnormality Detected (NAD) in the last one.

Post-mortem examination of dogs revealed that one of each died of pneumonia and per acute food poisoning while for goat, one died of Peste des petit ruminants and No Abnormality Detected (NAD) in other. The sheep was died of pneumonia while the pigeon was suffering from heavy Ascarid (*Psittacula* Spp.) infestation.

Histopathology

Histopathology unit received a total of 159 samples from different animals during the fiscal year 2060/061. Poultry contributes a total of 106 (67%) samples followed by 13 (8%) from goat, 11 (7%) from pig and buffalo each, five (3%) from dog, three (2%) from cattle, two (1%) from horse, (1%) from rabbit, sheep and Llama and the number of samples could not be processed were six (4%).

Among poultry, seventy-seven samples of broilers, 24 samples of layers and five samples of parent stocks were received. Of the 77 samples, five samples could not be properly processed. Thirty-four samples were provisionally diagnosed as the case of Inclusion body hepatitis- Hydropericardium syndrome (IBH-HPS) whereas 15 samples were found to be negative for the same. Similarly, eight cases of Aflatoxicosis, seven cases of Gumboro disease, five cases of hemorrhage, inflammation and necrosis and one case each of Avian encephalomyelitis, Ranikhet disease and Bacterial infection were diagnosed. Among layer birds, nine cases were found positive for the occurrence of Marek's disease while four cases were found negative for the occurrence of either Marek's disease or Avian Leukosis Complex. Four cases of Avian Leukosis Complex, two cases of hemorrhages, inflammation and necrosis and one case each of IBH-HPS and Mycosis were diagnosed. Of the remaining three samples, one was found negative for IBH-HPS while two samples were improperly processed with no interpretation. Among parent stocks, two cases of hemorrhages and necrosis, one case each of IBH-HPS and Avian Encephalomyelitis were found. The rest one could not be properly processed.

Among 13 samples of goat, five cases were positive for the occurrence of Sarcosporidiosis while three cases were negative for the same and one case each for PPR, toxin/poisoning and hemorrhages, necrosis and inflammation were diagnosed. The rest two samples were of tapeworm cysts that need not be processed.

In case of pig, three samples were positive for the occurrence of Sarcosporidiosis and two were found negative for the same. Two samples of Sarcosporidiosis could not be processed during the fiscal year. Of the remaining four samples, one sample was provisionally diagnosed as the case of viral disease, two samples with hemorrhages, inflammation and necrosis and one sample could not be processed nicely. In case of buffalo, one sample showed the general pathological condition; hemorrhages, inflammation and necrosis, four samples positive for Sarcosporidiosis while two samples negative for the same and four different samples suspected for Sarcosporidiosis could not be processed.

Of the five samples of dogs, each one was diagnosed as the cases of Rhabdomyomma, cystadenoma, fibroma, chronic wound and late pneumonia. In case of cattle, each one was found as the case of Hemorrhagic inflammation (Probably Hemorrhagic Septicemia) and the case of general pathological condition like hemorrhage, inflammation and necrosis. The rest one intended to check for the occurrence of Cerebral Babesiosis was found negative for the same.

The cases of horse were each diagnosed as acute toxicity and Chronic Obstructive Pulmonary Disease (COPD) with esophageal neoplasia. The case of sheep was diagnosed as hemorrhages, inflammation and necrosis whilst the case of rabbit was diagnosed as acute serum sickness (Hypersensitivity type III reaction) and that of Llama as hepatic carcinoma.

Table 1.

S.N.	Disease diagnosed	1	2	3	4	5	6	7	8	9	10	11	12	Sub-Total			Tota
														B	L	P	l
1.	Colibacillosis	17	9	13	12	15	9	14	8	15	32	20	20	172	11	1	184
2.	IBH-HPS	6	20	8	3	5	8	10	14	8	8	10	9	96	8	5	109
2.1	IBH+ Colibacillosis	1	2	0	1	8	0	1	2	1	3	0	5	23	1	0	24
2.2	IBH+IBD	1	2	1	0	1	1	0	2	0	3	3	0	13	1	0	14
2.3	IBH+ Coccidiosis	0	0	0	1	0	0	2	1	0	0	2	1	7	0	0	7
2.4	IBH+ Salmonellosis	0	0	0	1	0	0	0	1	1	0	0	0	0	3	0	3
2.5	IBH+IBD+ Colibacillosis	0	0	0	0	1	0	0	0	0	0	1	0	2	0	0	2
2.6	IBH + Chronic Respiratory Disease	0	0	0	0	0	0	1	1	0	0	0	0	2	0	0	2
2.7	IBH + CRD + Coccidiosis	0	0	0	0	0	0	3	0	0	0	0	0	3	0	0	3
2.8	IBH+ Visceral Gout	0	0	0	0	0	0	0	1	2	0	1	0	0	4		4
2.9	IBH + IBD + Coccidiosis	0	0	0	0	0	0	0	1	0	1	0	0	2	0	0	2
2.10	IBH+RD	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1
2.11	IBH + RD + Coccidiosis	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1
3.	Undiagnosed	3	3	2	6	7	4	11	12	4	8	5	2	47	12	8	67
4.	Gumboro disease	12	7	2	1	3	4	4	4	2	4	4	7	48	5	1	54
4.1	IBD + Colibacillosis	0	0	2	0	3	4	2	0	1	3	3	1	19	0	0	19
4.2	IBD + Coccidiosis	2	1	0	1	0	3	1	0	0	0	0	1	8	1	0	9
4.3	IBD+CRD	0	0	0	0	0	1	1	0	3	0	0	0	5	0	0	5
4.4	IBD + RD	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1
4.5	IBD+ Salmonellosis/ Pasteurellosis	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1
4.6	IBD+ IBH+ RD+ Colibacillosis	0	0	0	1	0	0	0	0	0	1	0	0	2	0	0	2
5.	CRD	6	0	0	1	3	6	10	1	3	1	2	2	28	2	5	35
5.1	CRD+ Coccidiosis	0	0	0	1	0	1	1	0	1	3	0	0	7	0	0	7
6.	Salmonellosis	4	3	2	1	1	1	0	2	2	5	4	7	11	12	9	32
7.	Caecal coccidiosis	2	2	0	4	3	1	5	0	0	2	1	7	19	4	4	27
7.1	Coccidiosis+ Colibacillosis	0	0	0	0	0	0	1	0	1	2	1	2	7	0	0	7
7.2	Coccidiosis + Salmonellosis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1
7.3	Caecal + Intestinal Coccidiosis	0	1	0	1	1	1	2	0	0	0	1	0	6	1	0	7
8.	Respiratory Disease Complex	0	0	1	1	2	3	4	2	4	3	1	4	22	3	0	25
9.	Ranikhet Disease	2	0	2	3	0	3	3	4	1	0	3	2	14	8	1	23
9.1	RD+Colibacillosis	0	0	0	0	0	0	0	0	1	0	1	1	2	1	0	3
9.2	RD+Coccidiosis	0	0	0	0	0	0	1	1	0	0	1	0	3	0	0	3
10.	Visceral gout	1	0	0	0	2	1	5	4	1	1	1	3	14	1	4	19
11.	Stress	0	0	0	2	1	1	1	0	0	2	4	2	12	1	0	13
12.	Marek's Disease	2	3	2	0	0	1	0	0	1	0	0	1	0	8	2	10
12.1	Marek's Disease+ Colibacillosis	0	0	0	0	1	0	0	0	1	0	0	0	0	2	0	2
13.	Mineral+ Vitamin deficiency	2	0	0	0	0	0	0	0	0	1	1	6	9	1	0	10
14.	Aflatoxicosis	0	0	0	0	1	0	1	0	1	0	1	0	3	1	0	4
15.	Avian Leukosis Complex	2	0	0	0	0	0	1	0	0	0	0	0	0	1	2	3
16.	Ascaridia Infection	1	0	0	0	0	0	1	0	0	0	0	1	0	3	0	3
17.	Necrotic enteritis	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	2
18.	Physical Injury	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1
19.	Fowl Pox	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	Total	64	54	35	43	59	53	87	62	55	81	73	85	609	98	44	751

Note: The numerical used in the table represents Nepalese months indicating the first month of the new fiscal year as 1 and likely the same up to the last month as 12.

Serology

Serology unit of Central Veterinary Laboratory (CVL) is headed by Dr. Ganesh Raj Pant, Senior Veterinary Officer and assisted by Mr. Ashok Prasad Shrestha, Senior Laboratory Technician. This unit performs different serological tests at CVL for diagnosis, monitoring and surveillance of animal diseases mainly associated with viral and bacterial infection. Most of the samples are submitted to this unit by Regional Veterinary laboratories, Districts Livestock Services Offices, Quarantine check posts, farmers and staff of CVL during disease outbreak investigations well as routine diagnosis. This unit possesses capacity and facility of Competitive enzyme-linked immunosorbent assay (ELISA), Indirect ELISA, Tube agglutination test, Agar gel immunodiffusion (AGID) test, Plate agglutination test (PAT) and A solid phase immuno assay (Immuno comb).

Serology unit had tested 76800 animal sera collected from year 1998 to 2000 covering 600 Village Development Committee wards of the 75 districts of the country for the surveillance of Rinderpest (RP) disease supported by Strengthening of Veterinary Service for Livestock Disease Control (SVSLDC) Project. The contribution of this unit has been realized to fullfill the main objective of this SVSLDC project to eradicate Rinderpest from Nepal. Serology unit has been providing scientific evidence for maintaining "Rinderpest Infection Free" status to the nation by conducting regular sero-surveillance of this disease since 2002. In fiscal year 060/061, 280 serum samples collected from Nawalparashi quarantine check post was examined for RP and found negative on C-ELISA test.

Similarly this unit is supporting to National Peste des petits ruminants (PPR) Control Program by testing sera collected from sheep and goat to detect antibodies against infection and to monitor antibodies in vaccinated goats. This unit has tested 16675 sera collected from fiscal year 056/057 to 060/061. In fiscal year 060/61, 10463 samples were tested to monitor antibody in vaccinated flock and 1542 samples were tested to detect antibodies due to infection during PPR outbreaks in different districts of the country. Out of 12005 tested sera, 8962 (75%) were found positive for the presence of antibodies against PPR on C-ELISA test.

In fiscal year 2060/061 this unit tested 1101 sera collected from poultry to monitor the antibodies against Infectious Bursal disease, New Castle disease and Infectious bronchitis disease in vaccinated flocks by using Immunocomb. The percentage of positive result on Immunocomb test for these three diseases was nearly 100%.

In addition to above activities, a total number of 1712 serum samples were tested by performing Plate Agglutination Test (PAT) and, Immuno comb assay for the diagnosis and, screening of Salmonellosis, Mycoplasmosis and, Egg drop syndrome in poultry and, Brucellosis in cattle, buffaloes, goats, dogs and human. Only 3.43% of tested samples collected from poultry were found positive on PAT

by using *Salmonella pullorum* crude antigen. Laboratory diagnostic result for *Mycoplasma* infection was found variable in PAT and Immunocomb tests.

All the tested (157) samples were found positive in PAT and only 96 samples out of 652 were found positive on Immunocomb for *Mycoplasma* infection. Similarly 8 (66.66%) samples out of 12 were found positive for EDS on ELISA test. A single cow was tested for bovine tuberculosis by performing tuberculin test and found negative. Serum samples collected from cattle, buffaloes goat, dog and human were tested for Brucellosis however only one dog serum was found positive providing 0.51% of positive. In this way a total number of 15069 samples were tested at serology unit in fiscal year 060/061 in comparison to total number of samples 7244 tested in fiscal year 059/060 to provide diagnosis, monitoring and surveillance service to the farmers of the country.

Although this unit tested highest number of samples at Central Veterinary Laboratory, several problems and were being faced. C-ELISA EDI software program is not working to validate and to calculate the test result. Therefore percentage of inhibition is calculated manually which is time consuming and tedious work. This unit has limitation to test other several important infectious diseases of different species of animals due to lack of reagents and fund.

Serum bank

Sera collected from different species of animals such as cattle, yak, buffaloes, sheep, goats, pigs, horses, dog and poultry covering 75 districts and three ecological zone of Nepal in previous years for disease surveillance, monitoring and investigation have been recorded and stored at –20°C in serum bank of CVL. Until the end of fiscal year 060/061 total number of sera stored in serum bank was about 130, thousand. In this way serum bank provides facility and significant number of serum samples for retrospective as well as research study of any disease in Nepal. In fiscal year 060/061, number of serum collected for PPR sero-monitoring were 11, 108 and number of serum collected for RP were 280. Total numbers of untested sera for PPR were 36,162 until the end of fiscal year 2060/61.

Storage of large quantity of serum for longer time is problem for serum bank. Number of sera is increasing every year but storage capacity is limited. Therefore this is right time to think to remove and dispose the old sera to provide enough space for newly collected samples.

Central Veterinary Laboratory

Annual Serological Test Report

F.Y.: 060/061

S.N	Disease	Unit	Animal Species	Total numbers of sample tested	Diagnostic test method	Test Results		Remarks Positive %
						Positive	Negative	
1	PPR	Nos	Goat & Sheep	12005	C-ELISA	8962	3043	75%
2	RP	Nos	Buffaloes	280	C-ELISA	0	280	0.00%
3	Salmonella Pullorum	Nos	Poultry	698	PAT	24	674	3.43%
4	Mycoplasma	Nos	Poultry	157	PAT	157	0	100%
5	Brucellosis	Nos	C,B,G,D & Men	193	PAT	1 (Dog)	192	0.51%
6	Poultry IBD (Bursal)	Nos	Poultry	357	Immuno Comb Elisa Antibody Test	357	0	100%
7	Poultry ND (Newcastle)	Nos	Poultry	357	Immuno Comb Elisa Antibody Test	357	0	100%
8	Poultry IB (Bronchitis)	Nos	Poultry	357	Immuno Comb Elisa Antibody Test	354	3	99.15%
9	PoultryMG/MS	Nos	Poultry	652	Immuno Comb Elisa Antibody Test	96	556	14.72%
10	Poultry EDS	Nos	Poultry	12	Elisa	8	4	66.66%
11	Bovine Tuberculosis	Nos	Cow	1	Bovine Tuberculin Test	0	1	0.00%
Total				15069		10316	4753	

CENTRAL VETERINARY LABORATORY
RESULT OF PPR TEST DURING OUTBREAK

F.Y. 060/061

S.N.	Regions	Animal Species	No of Tested Samples	No of Positive Samples	Positive %
1	Eastern	Goat	374	293	78%
2	Central	Goat	244	93	38%
3	Western	Sheep & Goat	318	42	13%
4	Mid-Western	Sheep & Goat	283	134	47%
5	Far-Western	Goat	323	216	67%
Total			1542	778	

Central Veterinary Laboratory

Suspected & Immunized Serum Samples Test of Poultry Disease

F.Y. : 2060/061

Districts	IBD Ab. Elisa Test		NDV Ab. Elisa Test		IBV Ab. Elisa Test		MG. Ab. ElisaTest		MS. Ab. Elisa Test		Salmonella (PAT)		Mycoplasma (PAT)		EDS Ab. Elisa Test	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Dhankuta	3	-	3	-	-	3	-	3	-	3				-		
Sunsari	30	-	30	-	30	-	-	30	-	30	7	48	55	-		
Sindhuli	-	-	-	-	-	-	-	-	-	-	-	20	20	-		
Dhanusha	-	-	-	-	-	-	-	-	-	-	-	140	-	-		
Mahottari												25		-		
Kathmandu	10	-	10	-	10	-	10	-	-	10	-	23	23	-		
Lalitpur	10	-	10	-	10	-	10	-	10	-	-	10	10	-		
Bhaktapur	19	-	19	-	19	-					2	36	19	-		
Chitwan	273	-	273	-	273	-	33	240	33	240	15	342	-	-		
Kaski	12	-	12	-	12	-									8	4
Nuwakot												30	30	-		
Total	357	-	357	-	354	3	53	273	43	283	24	674	157	-	8	4

CENTRAL VETERINARY LABORATORY

RP C- ELISA TEST

F.Y. : 2060/061

S.N.	Sample Collected from Quarentine Check Post	Animal Species	Total no. of tested sample	No.of		Date of sample Test
				Positive	Negative	
1	Nawalparasi	Buffaloes	40	0	40	2060/5/9
2	"	"	40	0	40	2060/6/28
3	"	"	40	0	40	2060/8/9
4	"	"	40	0	40	2060/10/24
5	"	"	40	0	40	2060/10/2
6	"	"	80	0	80	2060/11/21
Total			280	0	280	

Central Veterinary Laboratory

Samples Tested For Brucellosis

F.Y. : 2060/061

S.N.	District	Animal Species	Total no. of tested sample	Number of		Remark
				Pos(+)	Neg(-)	
1	Saptari	Cow	8	-	8	
2	Ilam	Cow	2	-	2	
3	Sunsari	Buffaloes	18	-	18	
4	Siraha	Goat	16	-	16	
5	Kathmandu	Cattle Dog Goat	46	1(Dog)	45	
6	Lalitpur	Cow	11	-	11	
7	Rauthat	Buffaloes	18	-	18	
8	Kaski	Cow, Goat	67	-	67	
9	Gorakha	Goat	6	-	6	
10	Bara(Kalaya)	Man	1	-	1	
Total			**193**	**1**	**192**	

Central Veterinary Laboratory
RP C- ELISA TEST

Disease	PY3	PY4	PY5	G.Total
	Number of serum Test	Number of serum Test	Number of serum Test	
RP C-ELISA	18214	33454	25132	76800

RP C- ELISA TEST
Samples Referred By : Quarentine Check Posts

Disease	PY3 2058/059	PY4 059/060	PY5 060/061	G.Total
	Number of serum Test	Number of serum Test	Number of serum Test	
RP C-ELISA	408	260	948	1616

CENTRAL VETERINARY LABORATORY
RESULT OF PPR C-ELISA OF LAST FIVE YEARS

S.N.	F.Y.	Animal Species	Total No of Tested Samples	Number of		Positive %
				Pos(+)	Neg(-)	
1	056/057	Goat/Sheep	951	398	553	42%
2	057/058	"	1460	530	930	36%
3	058/059	"	1436	802	634	56%
4	059/060	"	823	220	603	27%
5	060/061	"	12005	8962	3043	75%
Total			16675	10912	5763	

Central Veterinary Laboratory
Progress Report of PPR Sero-Monitoring Program
F.Y. : 060/061

S.N.	Districts	Animal Species	Numbers of Serum		
			Tested	Positive	Negative
1	Chitwan	Goat	530	425	105
2	Makwanpur	"	225	159	66
3	Kavre	"	540	456	84
4	Sindhupalchowk	"	405	295	110
5	Saptari	"	270	193	77
6	Jhapa	"	360	252	108
7	Dhading	"	370	275	95
8	Bara	"	138	102	36
9	Parsa	"	215	165	50
10	Kathmandu	"	155	92	63
11	Bhaktapur	"	114	91	23
12	Lalitpur	"	162	158	4
	Total		**3484**	**2663**	**821**
1	Udayapur	"	310	279	31
2	Sunsari	"	481	431	50
3	Morang	"	372	342	30
4	Siraha	"	284	247	37
	Total		**1447**	**1299**	**148**
1	Kapilbastu	"	275	242	33
2	Rupandehi	"	292	265	27
3	Nawalparasi	"	288	267	21
4	Kaski	"	375	349	26
	Total		**1230**	**1123**	**107**
1	Dhanusha	"	308	212	96
2	Mahotari	"	295	197	98
3	Sarlahi	"	417	257	160
4	Rauthat	"	358	232	126
5	Sindhuli	"	356	212	144
	Total		**1734**	**1110**	**624**
1	Banke	"	361	286	75
2	Bardiya	"	354	260	94
3	Surkhet	"	463	299	164

4	Dang	"	417	332	85
	Total		**1595**	**1177**	**418**
1	Dadeldhura	"	67	66	1
2	Kanchanpur	"	264	238	26
3	Kailali	"	445	366	79
4	Doti	"	197	142	55
	Total		**973**	**812**	**161**
G. Total(Serum Tested)			**10463**	**8184**	**2279**

Central Veterinary Laboratory
Progress Report of PPR Sero-Monitoring Programme
(2nd Phase) 059/060

S.N.	Districts	Animal species	Target	Total no. of Serum sample collected	Total no. of tested sample	Total no. of	
						+v	-ve
1	CVL Serum Collected From Chitwan	Goat	880	891			
2	Makwanpur	"	1000	1039			
3	Kavre	"	700	832			
4	Sindhupalchowk	"	640	616			
5	Dolakha	"	400	528			
6	Nuwakot	"	400	403			
7	Dhading	"	1000	954			
8	Gorkha	"	300	314			
9	Bara	"	700	697			
10	Parsa	"	700	700			
11	Rauthat	"	900	1023			
12	Ramechhap	"	400	393			
13	Kathmandu	"	120	122			
14	Bhaktapur	"	110	119			
15	Lalitpur	"	110	113			
Total			**8360**	**8744**			
1	RVL Pokhara Serum Collected from Tanhun	Goat		**202**			
2	Palpa	"		300			
3	Lamjung	"		200			
4	Shyangja	"		200			
Total				**902**			
1	RVL Janakpur Serum Collected From Dhanusha	Goat		768			
2	Mahotari	"		1024			
3	Saralahi	"		1048			
4	Sindhuli	"		960			
Total				**3800**			
G.Total				**13446**			

Central Veterinary Laboratory
Progress Report of PPR Sero-Monitoring Programme
(2nd Phase) 059/060

S. N.	Districts	Animal Species	Target	Total No. of Serum sample Collected	Total No. of tested sample	Total No. of	
						+ve	-ve
	RVL Biratnagar						
1	Sunsari	Goat		422			
2	Jhapa	"		418			
3	Morang	"		354			
4	Udayapur	"		315			
5	Saptari	"		419			
6	Siraha	"		290			
Total				**2218**			
	RVL Surkhet Serum Collected From						
1	Banke	"		920			
2	Bardiya	"		900			
3	Surkhet	"		400			
4	Dang	"		1000			
Total				**3220**			
	RVL Dhangadhi Serum Collected From						
1	Kailali	"		1082			
2	Kanchanpur	"		809			
3	Dadeldhura	"		584			
4	Doti	"		373			
Total				**2848**			
	RVL Pokhara Serum Collected From						
1	Kaski			**302**			
2	**Kapilbastu**			**1020**			
3	**Nawalparasi**			**1000**			
4	**Rupamdehi**			**1000**			
Total				**3322**			
G. Total				**(13446)+(11608)=25054**			

CENTRAL VETERINARY LABORATORY
NATIONAL PPR CONTROL PROGRAM
(Sero -Monitoring)
3rd phase (F.Y. 060/061)
(SERUM COLLECTION & TARGET)

S.N.	DISTRICT	ANIMAL SPECIES		TARGET	COLLECTION DATE	TOT. NO. OF SERUM COLLECTION	REM.
		GOAT	SHEEP				
1	KARVE	"	-	1000	2061/2/26	1000	
2	CHITWAN	"	-	700	2061/02/29	700	
3	SINDHUPALCHOWK	"	-	400	2061/02/15	452	
4	DOKHALA	"	-	600	2061/03/21	588	
5	RAUTHAT	"	-	700	2061/03/02	375	
6	LALITPUR	"	-	300	2061/03/16	194	
7	RAMECHAP	"	-	600	2061/03/01	605	
8	GORAKHA	"	-	400	2061/03/20	341	
9	RASUWA	"	-	300	2061/03/22	293	
10	NUWAKOT	"	-	600	2061/03/25	300	
11	MAKWANPUR	"	-	700	2061/04/02	398	
12	DHADING	"	-	600	2061/04/07	603	
13	RVL.SURKHET SALYAN	"	-	500	2061/04/03	500	
14	DAILEKH	"	-	600	2061/04/03	599	
15	RVL.BIRATNAGAR SIRAHA	"	-		2061/05/30	351	
16	SUNSARI	"	-	-	2061/05/30	350	
17	JHAPA	"	-	-	2061/05/30	302	
18	DHANKUTA	"	-	-	2061/05/30	305	
19	MORANG	"	-	-	2061/05/30	351	
20	SAPTARI	"	-	-	2061/05/30	356	
21	UDAYPUR	"	-	-	2061/05/30	350	
22	ILAM	"	-	-	2061/05/30	395	
23	RVL, JANAKPUR(1)DHANUSHA (2)MAHOTTARI	"	-	-	2061/08/24	1400	
TOTAL						11108	

Biochemistry Unit

The function of this unit became vital due to improved livestock farming systems in Nepal. The samples were collected from farmer's group or individual farmhouse or from disease outbreak area. There was disease investigation program in CVIL, which focused on sample collection from different parts of the country.

There was investigation on infertility and abortion in cattle and buffalo based on micro- elements content of serum under Nepalese systems. The program will give ample opportunities for collecting samples. These samples are processed as per the standard operating protocol (SOP) in this unit.

The unit performs the urinalysis and biochemical analysis of the serum samples.

1. Biochemistry

a) Calcium estimation
b) Phosphorus estimation
c) SGOT, SGPT
d) Protein and albumin estimation
e) Bilirubin estimation

2. Urinalysis

a) Dipstick (Multistick/Unistick)
b) Microscopic examination

Infertility is complex physiological phenomenon in livestock involving microbes or deficient in trace elements. In the F/Y 059/60 there was collection of 584 serum samples from infertile and aborted cattle and buffalo. The detailed investigation of the trace elements (Copper, Magnesium and Zinc) in animals was done before identifying the actual cause of infertility and abortion. The copper deficient in infertility case is 35.57% in cattle, whereas severe deficient is observed in 6.04% of cattle. The Magnesium deficient is 34.98% in infertile cattle. The zinc deficient is 11.18% in infertile cattle and buffalo.

In the F/Y 060/61, there was examination of 706 serum samples from infertile and aborted cattle and buffalo. The investigation revealed that there was Zinc deficiency in 13.41%, Calcium deficiency in 12.04%, Phosphorus in 7.69%, Magnesium deficiency in 3.84% of infertile and aborted cattle and buffalo. There was 15.55% of hyperglycemic cases in dog.

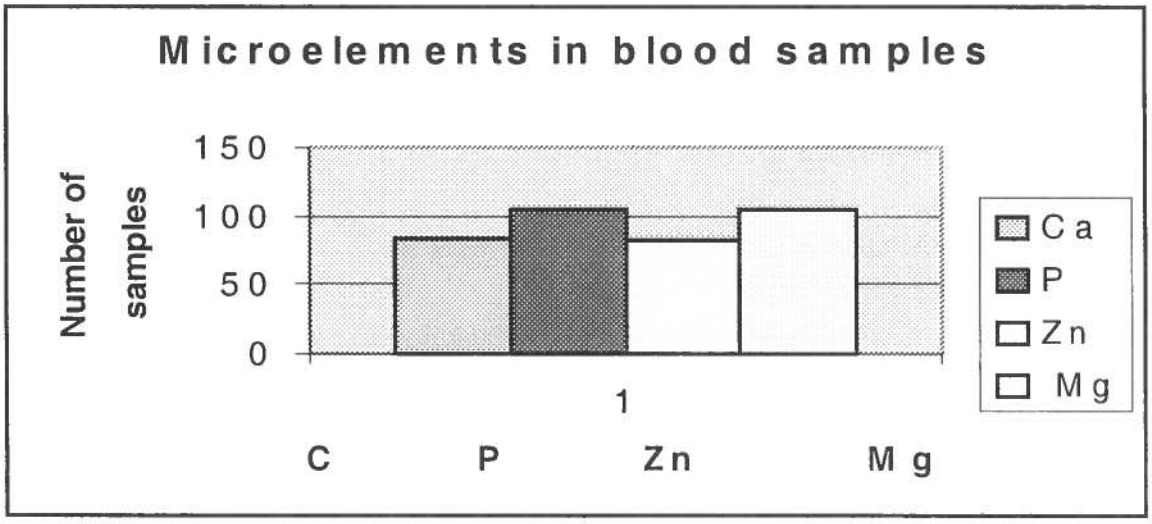
Dipstick (Unistick/Multistick) and microscopic method examine urine samples. Urine is examined for specific gravity, phosphorus, sugar, ketone bodies, albumin, total protein, billirubin, triple phosphate, calcium oxalate, RBC, pus cell etc. A total of 41 urine samples (25 dog and 16 cattle) species were tested during the fiscal year 2060/2061.

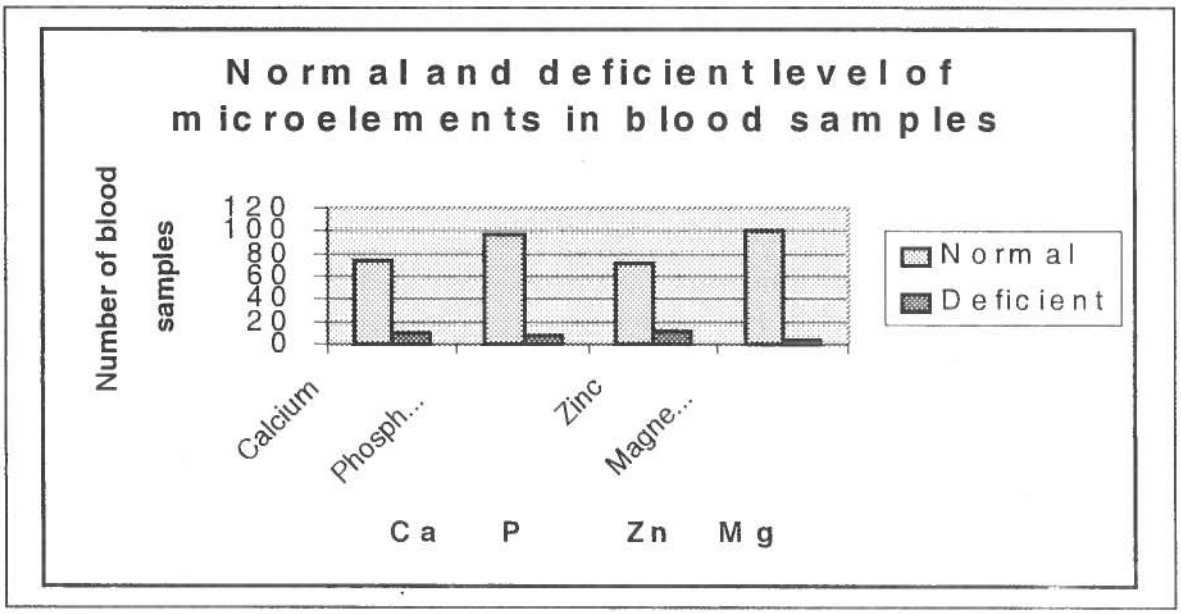
The examinations of urine are indicated in renal diseases, diabetes, urinary calculi, kidney function impairment, jaundice, ascites, haematuria, and haemoglobinurea. The examination of urine is helpful for diagnosis of disease. It will eventually help in assessing the prognosis of the condition.

The serum samples are tested for Calcium, Phosphorus, Magnesium, Zinc, SGOT, SGPT, Protein, Albumin, Blood sugar and Urea. A total number of 751 serum samples of different species were examined in the F/Y 2060/061 for different tests intended.

Biochemistry unit Progress:

S. No.	Sample tested	No. of Sample	Remark
1	**Urinalysis**	41	25 dog and 16 cattle samples
2	Calcium estimation	83	10 deficient
3	Phosphorus estimation	104	8 deficient
4	Glucose estimation	45	7 hyperglycemic
5	Zinc estimation	82	11 deficient
6	Magnesium estimation	104	4 deficient
	Total	792	





Hematology Unit

The hematological parameters are vital in disease diagnosis aspect and its importance has been increased due to improve livestock farming systems in Nepal. The blood samples are collected from farmer's group or individual farmhouse or disease outbreak area. Samples also be obtained from Regional Veterinary Investigation Laboratory (RVIL). There is disease investigation programme in Central Veterinary Investigation Laboratory (CVIL), which focus on sample collection from different part of the country. The area is specified in term of programme activity.

The disease investigation programme deals with problems in particular type of livestock of disease pattern. The programme will give ample opportunities for collecting samples. The sample was processed as per the standard operating protocol (SOP) in this unit. The SOP was prepared in this section with consultation of techniques from different lab and its relevance in Nepalese context.

Primary responsibility of this unit consists of finding out of general blood parameter from whole blood collected in EDTA and blood smear received from different District Livestock Services Office (DLSO) and RVL. These activities are as follow:

1.Haematology:

a) ESR (Erythrocyte Sedimentation Rate)
b) PCV (Packed cell Volume)
c) Hb (Hemoglobin)
d) Total RBC Count
e) Total Platelets count.
f) TLC (Total Leukocyte Count)
g) DC (Differential Count)

Samples (whole blood, blood smear) were obtained from farmers, DLSO, RVL, Central Veterinary Hospital. Some samples were collected by CVIL under different disease investigation programme such as investigation on infertility and abortion in cattle and buffalo program. The blood parameter tests include Hb, DC, PCV, ESR etc. A total number of 126 samples of different species were tested during fiscal year 2060/2061. It also carries out tests to detect blood protozoan parasites by Giemsa stain. There were 136 blood samples that had examined for blood protozoan parasites.

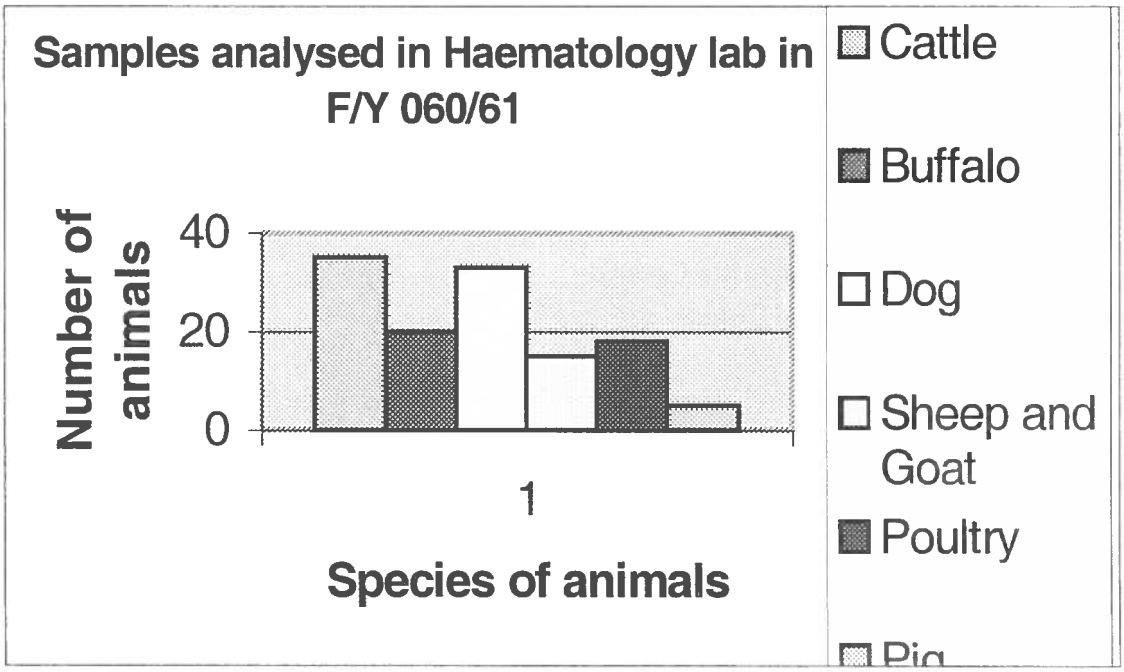
Samples analyzed in hematology are given in the table below.

Annual Report of Fiscal year 060/061

Hematology

S.N.	Species	Hb	PCV	ESR	Total cell count	Differential Cell count	Blood Protoza	Total Test
1	Cattle	35	35	35	35	35	40	215
2	Buffalo	20	20	20	20	20	20	120
3	Dog	33	33	33	33	33	35	200
4	Sheep and Goat	15	15	10	15	15	18	88
5	Poultry	18	18	18	18	18	18	108
6	Pig	5	5	-	5	5	5	25
Grand Total		126	126	121	126	126	136	756

There is compilation of unit's function on monthly basis in the laboratory. It does not necessarily reflect number of problem in livestock species. The flow of samples from farmers, DLSO and RVL are limited due to certain constraints. The haematology unit gives information, which is essential for diagnosis of disease based on blood parameter. There are more number of samples from cattle followed by dog, buffalo, poultry, sheep, goat and pig. The blood parameter gives diagnosis of anemia, blood borne protozoa parasites, parasites, parasitic infestation and debilitated condition in livestock and poultry birds.



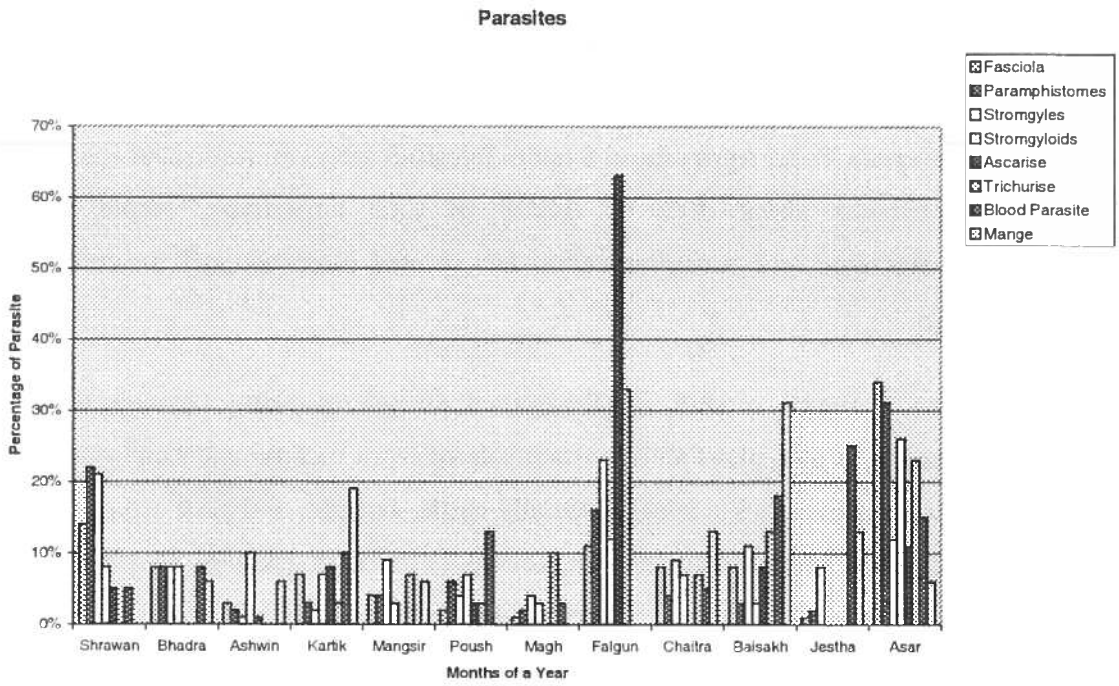
Parasitology Unit

In Nepal's Total Agricultural System livestock plays an important role. The sector contributes about 30% of AGDP of total agriculture. Rapid increase in urbanization and periurbanization has created huge demand for milk meat and eggs.

Now it seems a shift in Traditional Crop/Vegetables farming to Livestock farming. It also plays an important role of extra income and employment in rural area. But livestock raising especially cattle, buffalo, and goat seems to be still in traditional way by unplanned grazing in indirect pasture. Due to this animal although healthy but are bearing different parasite. Their magnitude of infection has been found under different climatic condition of weather, these facts are indicated by the coprological examination of fecal samples being examined in this laboratory. On the basis of this finding, it is suggested that parasitism shouldn't be considered as a disease but should be included in the whole livestock management system, so that we can rise and increase the livestock product and productivity and minimize the cost too. Mainly researcher in this regard has suggested the relation of fodder pasture and parasitic infection (Fassciola 2000).

Annual Retrospective Parasitic Prevalence In Kathmandu Valley.

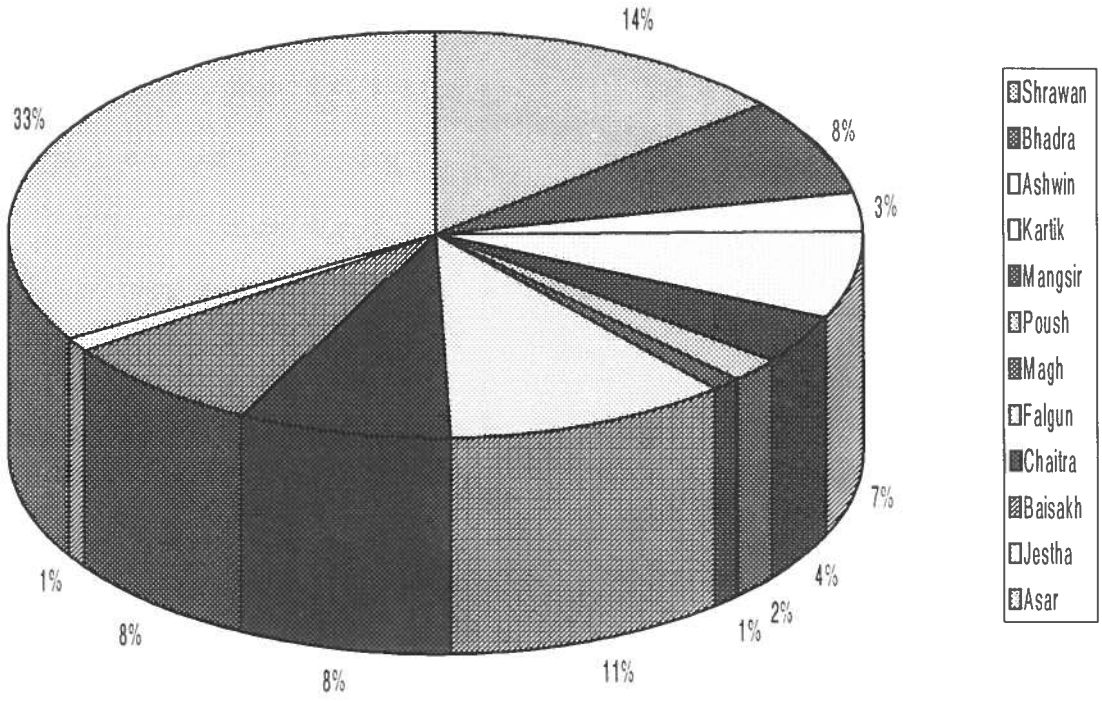
S.N.	Type of Parasites	Sawan	Bhadra	Asbin	Kartic	Mangsir	Paush	Magh	Phalgun	Chaitra	Baisakh	Jestha	Aasadh	Grand Total
1.	Fasciola	57	33	13	27	17	9	5	44	33	21	3	137	399
2.	Paramphistomes	117	42	9	15	21	31	10	88	19	14	9	135	540
3.	Stromgyles	61	25	2	7	27	12	-	67	27	31	-	36	295
4.	Stromgyloids	5	7	6	4	2	4	-	11	5	2	-	19	72
5.	Ascarise	4	-	1	7	-	3	-	55	-	7	-	10	87
6.	Trichurise	-	-	-	1	2	1	3	10	2	4	-	7	30
7.	Blood Parasite	2	3	-	4	-	5	1	-	2	7	10	6	40
8.	Mange		1	1	3	1	-	-	-	2	5	2	1	16
9.	Negative	36	23	9	20	38	31	3	65	45	51	5	249	575
10	Grand Total	277	147	35	88	108	96	22	340	135	142	29	630	2036



The annual Fecal sample examines for different parasite which has been tabulate above and its graphic presentation gives some idea for launching parasite control programme. As per the aim of building the laboratory capability as now Lab. has given specialized manpower, from this year Lab. has started the Larvae culture itself.

Name of parasite	Shrawan	Bhadra	Ashwin	Kartik	Mangsir	Poush	Magh	Falgun	Chaitra	Baisakh	Jestha	Asar
Fasciola	14%	8%	3%	7%	4%	2%	1%	11%	8%	8%	1%	34%
Paramphistomes	22%	8%	2%	3%	4%	6%	2%	16%	4%	3%	2%	31%
Stromgyles	21%	8%	1%	2%	9%	4%	4%	23%	9%	11%	8%	12%
Stromgyloids	8%	8%	10%	7%	3%	7%	3%	12%	7%	3%	0%	26%
Ascarise	5%	0%	1%	8%	0%	3%	0%	63%	0%	8%	0%	11%
Trichurise	0%	0%	0%	3%	7%	3%	10%	33%	7%	13%	0%	23%
Blood Parasite	5%	8%	0%	10%	0%	13%	3%	0%	5%	18%	25%	15%
Mange	0%	6%	6%	19%	6%	0%	0%	0%	13%	31%	13%	6%
Negative	28%											

Fasciola


14%
8%
3%
7%
4%
2%
1%
11%
8%
8%
1%
33%
Shrawan
Bhadra
Ashwin
Kartik
Mangsir
Poush
Magh
Falgun
Chaitra
Baisakh
Jestha
Asar

NATIONAL AVIAN DISEASE INVESTIGATION LABORATORY

NATIONAL AVIAN DISEASE INVESTIGATION LABORATORY
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NATIONAL AVIAN DISEASE INVESTIGATION LABORATORY, CHITWAN

With the decision of His Majesty's Government of Nepal, National Avian disease Investigation Laboratory Chitwan has been established on 1st Baishakha 2061.This is the first species specific laboratory in country's veterinary laboratory system. The laboratory has been functioning with limited available resources since the beginning of year 2003. The new establishment was proposed in ninth five year plan to address the problems related to privately evolved poultry industry of the nation. The industry has emerged as a fast growing sector in agriculture with an investment of around Rs1600 millions. The sector is also important from point of view of its contribution to agriculture GDP and employment generated by it.

The establishment of avian laboratory started with an initial objective of delivering all the services related to health, nutrition, management and marketing of poultry. Initially it was proposed to develop as a National Avian Center. Later on poultry diseases were considered the most important sector to be serviced and finally the National Avian Disease Investigation Laboratory is now in picture. The construction works started in fiscal year 055/056. Initially, it was proposed to build a 2-story main laboratory building with accessory structures like lab animal house, biological pit, and link road and drainage system and staff quarters. By the end of 2002, the first story of the building was completed partially. The department of livestock services decided to run the laboratory with whatever facilities available mobilizing a Veterinary Officer and a JTA from adjoining DLSO, Chitwan. With the registration of first sample on 12th Feb. 2003, National Avian disease investigation laboratory began working.

Now, facilities of biological pit, road around the main building and incomplete building structure of second story have been completed. Most of the required equipments are now available and ready to be operated. The main sections of the laboratory is under the process of organization and will run in its optimum capacity very soon.

Objectives

The National Center for Avian Disease Investigation started with a final goal of minimizing the risk associated with poultry industry so as to secure it as a profitable and sustainable business.

The overall objective of this center is prioritized as follows:

Immediate objectives

i. To develop facilities to make available accurate and reliable diagnostic services for avian diseases of epidemiological importance inside the country.
ii. Develop and recommend effective vaccination schedule based on epidemiological evidences and seromonitoring of the flock.
iii. surveillance of avian diseases of zoonotic importance to formulate effective control strategy

Mid-term objectives

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

Long-term objectives

i. To develop different types of antigens, antisera antibodies from field isolates.
ii. To develop different types of test kits for poultry diseases.
iii. To assure easy and steady supply of biologicals needed for poultry disease diagnosis.
iv. To develop vaccines based on local strains.
v. To develop it as center of research and development for all aspects of poultry industry.

Main units of NADIL

Sample Collection Unit

- Registration of Samples
- Recording of History
- Labeling of samples with Sample Number
- Delivering of samples to appropriate unit
- Distribution of final report
- General Inquiry

Post – Mortem Unit

- PM of birds
- Making smear from suspected materials and lesions, their staining and microscopy
- Fecal examination
- Sample collection and forwarding to appropriate unit

Histopathology Unit

- Tissue trimming
- Tissue processing and sectioning
- Staining
- Microscopy
- Immunohistochemistry
- FAT

Microbiology

- Culture, isolation and identification of bacteria and fungi and viruses
- Sensitivity testing

Serology unit

- AGID test
- Plate and tube agglutination test

ELISA
FAT

Accessory Unit

- Washing, Cleaning & Sterilization Unit
- Refreshment Room
- Changing Room

Manpower

NADIL started functioning partially since February 2003. At the time of starting its operation, there were no staffs of its own. 1 veterinarian and 1 JTA from adjoining District Livestock Service office, Chitwan were assigned to do initial establishment works of this laboratory in addition to working in the concerned office. His Majesty's Government of Nepal has approved the official status of this laboratory with required staffing. The status of manpower till date is as follows:

S.N.	Designation	Class	Employee
1	Senior Veterinary Officer	Gz.II	Vaccant
2	Veterinary Officer	Gz.III	Dr.P.S.Kushwaha on part time deputation
3	Veterinary Officer	Gz.III	Vaccant
4	Veterinary Assistant (VA)	Non Gz.I	Mr. Shailendra Bhandari
5	Veterinary Assistant (VA)	Non Gz.I	Vacant
6.	Junior Veterinary Assistant (JVA)	Non Gz.II	Vacant
7	Junior Veterinary Assistant (JVA)	Non Gz.II	Vacant
8	Assistant Accountant	Non Gz.II	Vacant
9	Mukhiya	Non Gz.III	Rishee Ram Acharya
10	Peon	-	Chunni Lal Yadav
11	Peon	-	Vaccant
	Total - 11		

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY

BIRATNAGAR

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY
[Eastern Region, Biratnagar]

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Fax: +977-21-523027
E-mail: rvlbrt@ntc.net.np

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, BIRATNAGAR (Eastern Region)

Situated in sub-metropolitan city-17, Regional Animal Disease Investigation Laboratory (RADIL) was established in the fiscal year 1988/1989 AD. But until 1990/1991, the laboratory was non-functional and could not perform its activities as per objectives due to lack of manpower, necessary equipments and time to time changes in organizational structure. From fiscal year 1991/1992, the RADIL has its separate identity. There was provision of manpower, essential equipments and other logistics. Programme was lunched as per objective.

The working area of this RADIL is all the districts of Eastern Development Region.In the eastern region there are three zones namely Mechi, Koshi and Sagaramatha and 16 districts. Geographically the region is divided into three ecozones :-

High hills: This eco-zone lies in the northern part of the region covering Taplejung district of Mechi zone, Sankhuwasabha district of Koshi zone and Solukhumbu district of Sagaramatha 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. Districts comes in this region are Panchthar and Ilam of Mechi zone, Dhankuta, Tehrathum and Bhojpur of Koshi zone, and Okhaldhunga,Khotang and Udayapur of Sagaramatha zone. Mixed farming system and agro-based livestock industries are the main occupations of the farmers of this region. Mainly cattle, buffalo, goat, swine are reared in this region. Poultry and rabbit industry have become popular in recent years.

Tarai: Jhapa district of Mechi zone, Morang and Sunsari districts of Koshi zone and Saptari and Siraha districts of Sagaramatha zone comes in this region. Though cereal crop farming is the main occupation of the farmers in this region, as a secondary occupation, they rear cattle, buffalo, goat and poultry. Though traditional system of livestock rearing is followed in this region, in the recent year's poultry farming, dairy industries and piggery has become commercialized,

especially in town areas where facilities like road, market, and electricity are available.

To provide proper laboratory diagnosis and improve in the quality of veterinary services, the government has established five regional laboratories, one in each developmental 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:

The main objective of Regional Animal Disease Investigation Laboratory is to provide prompt and efficient disease diagnostic services and effective control measures for the development of livestock. In addition, the regional veterinary laboratory has following objectives:

- To investigate and diagnose epidemics in the region.
- Assist and support DLSO in disease diagnosis and epidemic control.
- To upgrade DLSOs disease diagnosis capacities by technical back stopping.
- To collate, analyze and predict animal disease situate in the region.
- To develop human resources for field level veterinary laboratory services.
- Coordinate and support national animal disease control and eradication programme.
- Support and facilitate national regulatory veterinary services and other veterinary service providers.
- Active participation in collaborative and Coordinated research programme related in animal health and production in the region

Annual Progress Report (2060/061)

S.N.	Programme	Unit	Annual target	Annual progress	Progress %
1	Laboratory services				
1.1	Parasitological examination	Units	1200	1654	100 %
1.2	Microbiological examination	Units	480	542	100 %
1.3	Pathological examination	Units	150	166	100 %
1.4	Serological examination	Units	400	411	100 %
1.5	Haematological examination	Units	275	341	100 %
1.6	Biochemical examination	Units	500	819	100 %
1.7	Sample to be sent to other lab.	Units	200	298	100 %
2	Investigation & surveillance programme				
2.1	Study of infertility in cow	Times	6	6	100 %
2.2	Sero-epidemiological disease investigation (Theileriosis)	units	200	403	
2.3	Investigation of epidemic disease	Times	6	6	100 %
3	Supervision & monitoring programme				
3.1	Supervision & monitoring of district lab.	Times	6	6	100 %
4	Veterinary disease investigation workshop	Times	1	1	100%
5	Training programme				
5.1	Computer training	Member	2	2	100%
6	Publication programme				
6.1	Publication of quaternary epidemiological bulletin	Times	4	4	100 %
6.2	Publication of annual technical report	Times	1	1	100 %
7	Purchase				
7.1	Technical books	Times	1	1	100 %
7.2	UPS & Printer	Units	2	2	100 %

Total progress report 100%

Annual Progress Report (2060/061)

Programme- Garibi Nibaran Byabsayonmuk Bakharapalan Karyakram

S.N.	Programme	Unit	Annual target	Annual progress	Annual weight	Progress %	Remarks
1	sero-monitoring & reporting	Times	3	3	14.46	100	
2	Serosurveillance & reporting (150 goats)	Times	3	3	14.46	100	
3	Epidemic disease investigation & control	Times	3	3	28.92	100	
4	Faecal collection & examination (150 goats)	Times	3	3	3.61	100	
5	Training /workshop	Times	7	7	16.87	100	
6	Medicine trial & recommendation (150 goats)	Units	3	3	18.07	100	
7	Monthly, quaternary & annual progress report	Times	16	14	3.61	88	
8	Average progress percentage	98.28 %					

Laboratory services

Laboratory tests involve are examination of samples of faeces, skin scraping, urine, milk, blood etc. Tests may be used to determine the various bio-chemical constituent of the sample or to detect the presence of bacteria. Samples (usually serum) may be used to detect the presence of antibodies to various infective agents.

Parasitological examination:

Routine faecal examination of clinical cases is done in the laboratory. The samples are being sent either by the different DLSOs or brought directly by the farmers. Samples are also collected during survey and investigation of disease from the field. Mostly the faecal samples are examined by sedimentation and floatation techniques to identify the eggs of gastrointestinal parasites. However, in certain cases Mc Master Technique is followed to quantify the eggs per gram of faeces.

In the fiscal year 2060/061, altogether 1495 faecal samples from different species of animal such as cattle, buffalo, goat, dog etc. were received and examined in this laboratory in which 888 samples (59.39%) found positive whereas 607 samples (40.60%) were negative for parasitic eggs. The result of the faecal test revealed that fascioliasis is the most commonly occuring parasitic infection in cattle and buffaloes followed by nematodiasis and paramphistomiasis.

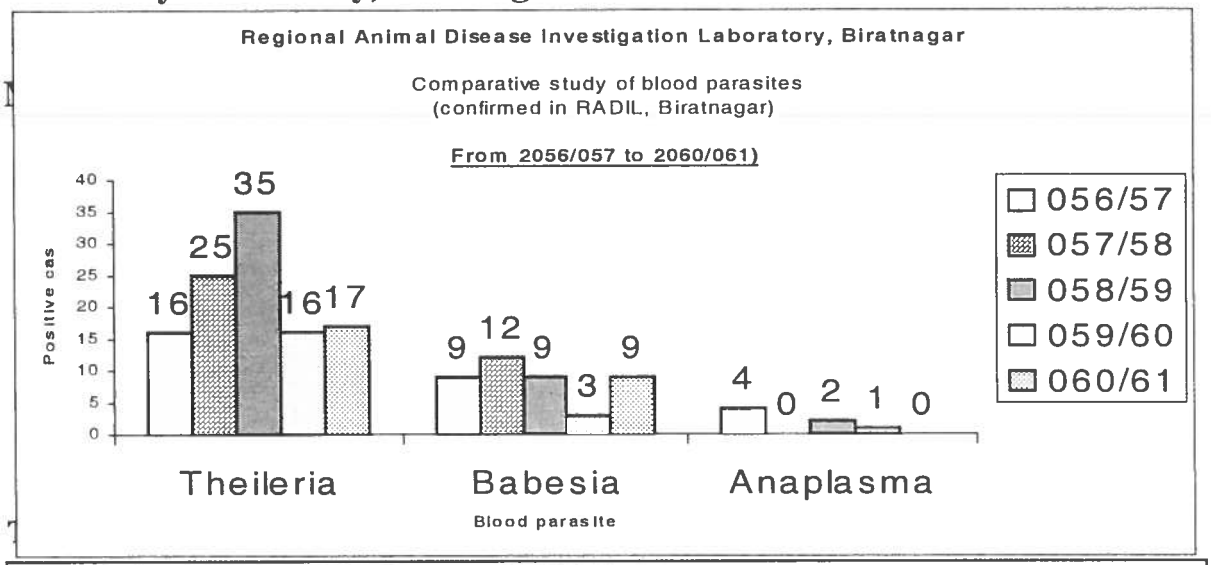
The figures of faecal samples examined and types of Parasites revealed in the tests are as follows –

Months	(+)ve sample	(-)ve sample	Fasciola	Paramphistome	Trichuris	Haemonchus	Ascaris	Others	**Total**
July	85	78	42	13	5	6	3	16	163
August	95	43	63	15	5	6	0	6	138
Sept.	64	22	22	27	2	4	1	8	86
Oct.	152	20	60	18	12	18	1	43	172
Nov.	158	70	45	9	4	13	8	78	228
Dec.	44	88	26	5	4	2	2	5	132
Jan.	54	43	30	3	4	1	5	11	97
Feb.	23	73	10	1	2	1	0	9	96
March	62	36	46	3	3	4	0	6	98
April	69	92	56	2	5	3	1	2	161
May	49	39	32	5	2	6	0	4	88
June	33	3	27	1	1	3	0	2	36
Total	**888**	**607**	459	162	49	66	21	190	**1495**
	59.39	40.60		11.48					

Skin scrapings are collected from animals suffering from skin lesion and examined for identification of mites. Collected samples are treated with 10% KOH in a test tube and gently heated without boiling, cooled & centrifuged at 2500 RPM for 2 minutes. The supernatant fluid is discarded and a drop of sediment is placed on a clean glass slide, covered with a coverslip. It is then examined under low power microscope for the presence of parasites. Altogether 23 samples from different species of animals were received and examined in this laboratory. Mostly *Sarcoptes* mites were identified in skin scraping examination.

Blood samples received from different districts of Eastern Development Region were examined for blood parasites. A total number of 144 blood samples were examined for blood parasites, in which 26 samples were found positive. Out of the 26 positive samples, the number of *Theileria, Babesia and Anaplasma* confirmed were 17, 9, and 0 respectively.

Comparative study of different blood parasites confirmed at Regional Veterinary Laboratory, Biratnagar

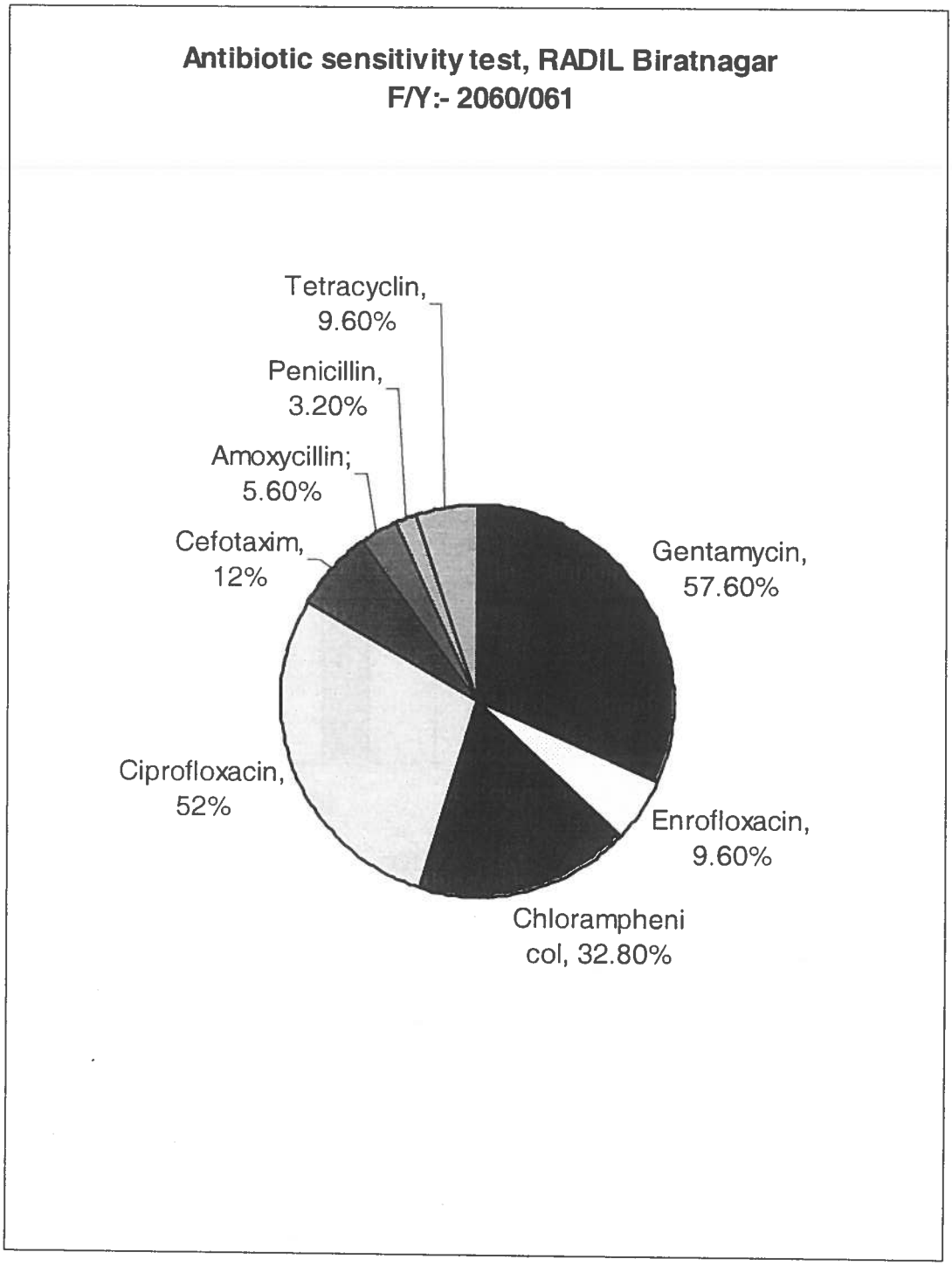


Months	Total sample	Positive	Negative	% of positive
July	61	33	28	54%
August	78	50	28	64%
Sept.	42	18	24	42.85%
Oct.	33	19	14	57.57%
Nov.	79	46	33	58.22%
Dec.	23	11	12	47.82%
Jan.	43	21	22	48.83%
Feb.	20	23	7	65%
March	35	17	18	49%
April	57	33	24	58%
May	30	18	12	60%
June	41	16	25	39%
Total	542	295	247	54.42%

Altogether 542 milk samples were presented to this laboratory in the fiscal year 2060/061, of which 295 found positive .The most common bacteria isolated from milk culture were *Streptococcus, Staphylococcus, E.coli, Pseudomonas* etc.

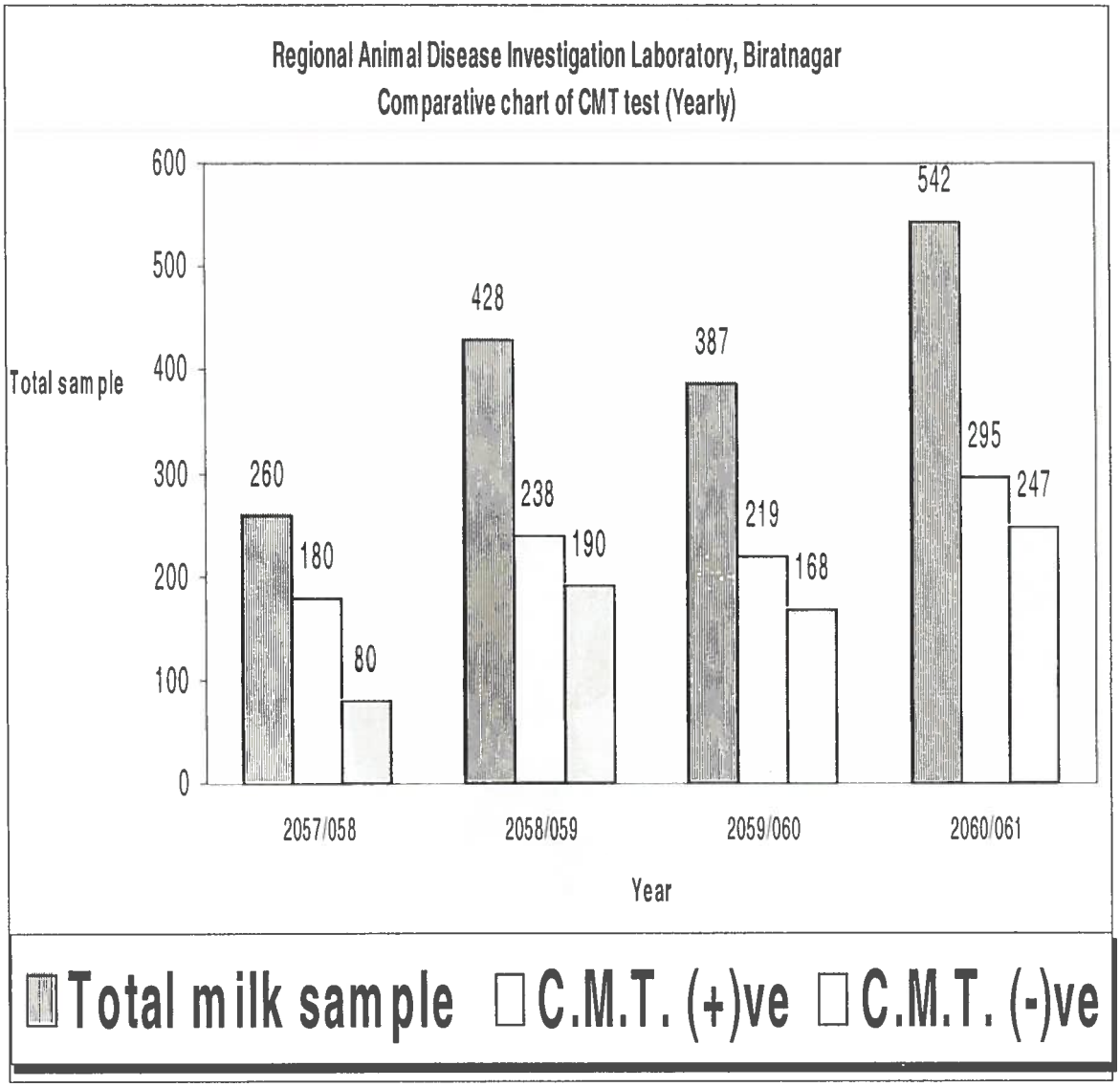
Sensitivity of different antibiotic with bacteria isolated from milk samples:-

Antibiotic sensitivity test:-



Year wise comparative chart of California Mastitis Test:-

Year wise comparative chart of California Mastitis Test:-



Pathological examination:

Mostly post-mortem examinations of dead birds and occasionally of dead animals are done in the laboratory or in field condition under pathological examination. During post-mortem examination, specimens such as impression smears, swab, and tissues are collected for required test. Tissues that are collected for histopathological examination are being sent to the Central Veterinary Laboratory, Kathmandu. Among the diseases diagnosed after PM examination, Gumboro disease ranked the most common followed by CRD, Coccidiosis and Ranikhet disease.

Biochemical examination:

Routine examination of urine and analysis of blood serum to assess biochemical constituents is done in this laboratory under biochemical examination. Serum samples are collected from sites/farms selected for investigation of infertility and other disease conditions. Altogether 819 serum samples were collected and analyzed in the fiscal year 2060/061 for the estimation of total protein, glucose, phosphorus, zinc etc. 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, blood etc. Mostly Rothera's test and Robert's test were done to detect ketone bodies and protein respectively.

Haematological examination:

Under haematological examination, total leucocytic count (TLC), total erythrocytic count (TEC), differential leucocytic count (DLC), estimation of packed cell volume (PCV) and haemoglobin (Hb) are done in this laboratory. Estimation of haemoglobin is done by Sahli's haemoglobinometer method, packed cell volume by microhaematocrit method, total count of R.B.C.& W.B.C. by haemocytometer. For differential leucocytic count, blood smears are stained with Giemsa's stain. A total number of 341 blood samples were examined during the fiscal year 2060/061.

The haematological changes regarding Hb and PCV values in haemoparasitic cases are presented in the table below.

Total no. of blood sample examined – 140

No. of sample +ve for blood parasites – 26

Blood Parasite	Total	Avg. Hb gm%	Min. Hb gm%	Avg. PCV	Min. PCV
Theileria (+)	17	6.2	3.2	20	11
Babesia (+)	9	5.2	4.5	16	13
Anaplasma (+)	0	0	0	0	0

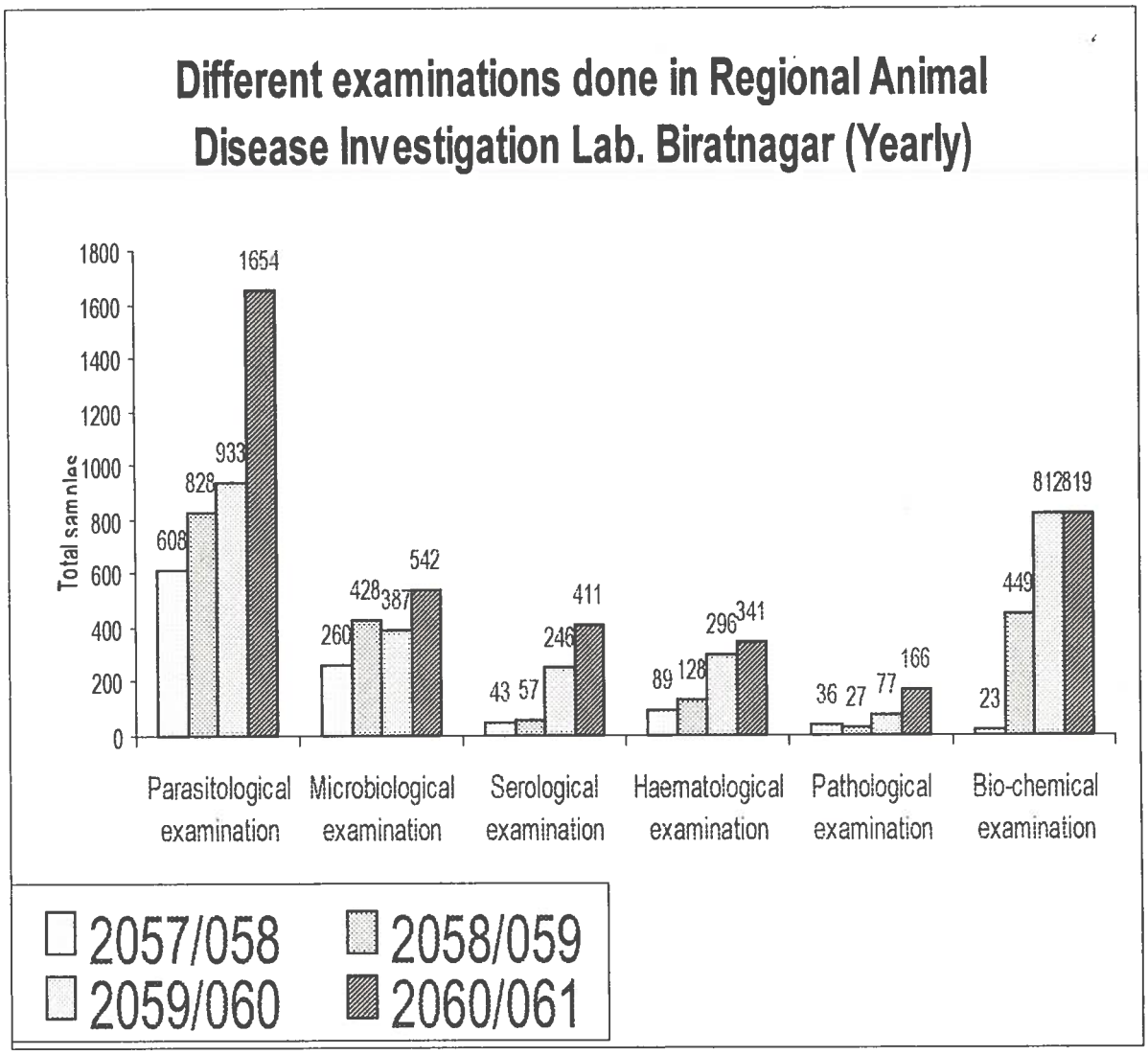
Haematological findings evidenced severe anaemia in piroplasmosis. This is attributed to lysis of erythrocytes by emerging parasites for babesiosis and immune-mediated destruction of unparasitized erythrocytes in addition to parasitized erythrocytes for both babesiosis and theileriosis.

Sample sent to Central Veterinary Laboratory, Kathmandu F/Y – 2060/061

Location	Brucellosis	PPR	FMD	Abortion	Total
Agri. Research Center, Tarahara	6				6
Siraha		138		16	154
Saptari		54	12		66
Dhankuta			7	10	17
Udayapur		52			52
Jhapa			3		3
Total	6	243	22	26	298

National PPR Programme (Seromonitoring) F/Y- 2060/061

DLSO	Udayapur	Sunsari	MOrang	Siraha	Saptari	Dhankuta	Ilam	Jhapa	Total
No. of house hold farmers	35315	43746	77857	65322	65211			74727	362178
Total goat/sheep population	97700	158571	107997	84185	153428			149369	751250
Total vaccination target	50000	60000	60000	60000	60000	60000	70000	60000	480000
Total serum collection	349	350	352	350	352	300	395	302	2750


Different examinations done in Regional Animal Disease Investigation Lab. Biratnagar (Yearly)
Total samples
1800
1600
1400
1200
1000
800
600
400
200
0
608
828
933
1654
260
428
387
542
43
57
246
411
89
128
296
341
36
27
77
166
23
449
812
819
Parasitological examination
Microbiological examination
Serological examination
Haematological examination
Pathological examination
Bio-chemical examination
2057/058
2058/059
2059/060
2060/061

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY

JANAKPUR

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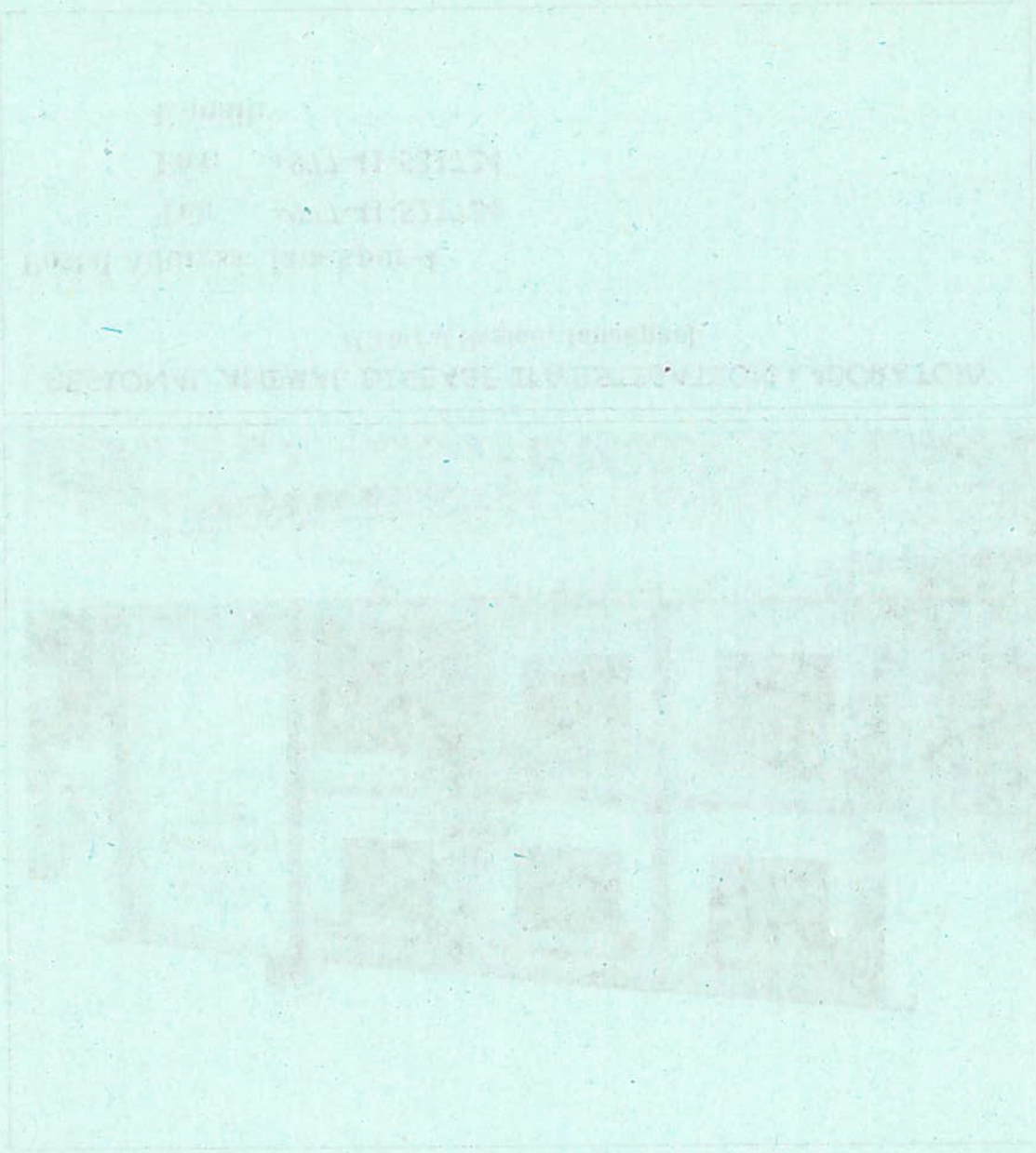

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, JANAKPUR (Central Region)

INTRODUCTION

Central Development Region possesses 1.6 million cattle, 0.8 million buffaloes, 17.8 million goats, 1.1 million sheep, 1.3 million pigs and 77.7 million poultry which contributing 24%, 30%, 13%, 18 & 50% of the total population of the country respectively.

The history of the laboratory diagnosis of animal diseases started at Dhanusa district in year 2045/046 with financial assistance of second Livestock development project. Regional Veterinary laboratory (RVL) was established in year 2049. RVL, Janakpur is providing veterinary diagnostic services to 19 DLSO, 105-service center and 167-service sub-center of this Central Development Region. The objectives of this laboratory are summarized in following points.

Objectives

1. to act as a regional referral diagnostic laboratory.
2. to investigate disease outbreak in any district of the region.
3. to help in epidemiological survey, sero-surveillance & sero-monitoring of the disease of national importance
4. to provide routine laboratory diagnosis services to the farmers
5. to support district laboratories to enhance disease diagnosis capacity and facility
6. to co-ordinate the animal health and infertility camp organized by DLSO and other institution.
7. to co-ordinate national disease eradication and control programmers of the country.

Annual Progress Report – 2060/2061

S. No.	Programme	Unit	Annual Target	Annual progress	Remarks
1.	Laboratory Service	Number			
1.1	Parasitological Examination	,,	700	4901	
1.2	Micrpbiological ,, ,,	,,	250	258	
1.3	Pathological Examination	,,	150	158	
1.4	Serological Examination	,,	250	269	
1.5	Hematological Examination	,,	300	308	
1.6	Biochemical Examination	,,	450	454	Progress
1.7	Sample to be sent to other Lab	,,	150	203	Percentage-
2.	Investigation and surveillance Prog.				100 %
2.1	Infertility Investigation in Buffalo	Times	6	6	
2.2	Investigation of Epidemic Disease	,,	6	6	
3.	Supervision & monitoring Programme				
3.1	Dist. Laboratory	,,	6	6	
3.2	Workshop Meeting	,,	3	3	
4.	Computer Training	Person	2	2	
5.	Publlication programme				
5.1	Quarterly bulleting	Times	4	4	
5.2	Annual Technical report	,,	1	1	
6.	Veterinary Disease investigation workshop	,,	1	1	
7.	Purchege Programme				
7.1	Scientific Books	,,	1	1	
7.2	Electronic weight balance	Number	1	1	
7.3	Cool Box	,,	4	4	

Parasitology

In F/y 2060/061 total 4901 fecal Sample from different species of animals were examined. Samples were received from different sources. A total positive sample was 3508 & negative sample were 1393. The percentage of positive sample & negative sample were 72 & 28 respectively. Among positive sample paramphistomes 35%, fasciola 31%, tapeworm 1% round worm (strongyloids, strongylus, Ascaris, others) 33%. The detail of fecal sample is given quarterly as follows.

Quater	No.of Sample	Paramphistome	Fasciola	Tapeworm	Round worm
First Quater	1545	437	368	9	384
Second ,	1603	418	362	14	392
Third ,,	1753	388	354	16	366
Total	4901	1243	1084	39	1142

Skin scraping are mostly collected from goats of defferent places of this region with clinical symptoms of alopecia, itching. Total 27 sample are examined in laboratory & mostly sarcoptes mites identified 11 samples are found for positive of pseroptic mites.

Blood smear sample of animals for blood parasites were examined where Babesia, Trypanosoma, Theileria & Anaplasma were identified.

Total 176 blood sample were examined in which Anaplasmosis (14) Babesiosis (11) Trypanosomiasis (10) Theileriosis (2) cases were found.

Blood protozoan Parasites on bovine (Cattle & Buffalo)

Quater	Anaplasmosis	Babesiosis	Trypanosomiasis	Theileriosis
First Quater	8	7	4	1
Second ,,	2	-	-	-
Third ,,	4	4	6	1

Haematology Unit

In Veterinary field as human is necessary to diagnose a disease by haematological parameter. Its importance are increassing day by day. The blood sample are received from DLSOs & collected from farmer's house. Responsibility of this unit consists of finding out of general parameter from whole blood collected in EDTA. The activities performed by this unit has been summarized as follows.

A. E.S.R. (Erythrocyte Sedimentation Rate)
B. P.C.V. (Packed Cell Volume)
C. HB. (Haemoglobin)
D. R.B.C. (Red Blood Cell Count)
E. Total platelats Count
F. T.L.C. (Total Leucocyte Count)
G. D.L.C. (Differential Leucocyte Count)
H. M.C.V. (Mean Corpuscular Value)
I. M.C.H. (Mean Corpuscular Haemoglobin)
J. M.C.H.C. (Mean Corpuscular Haemoglobin Concentration)

A Total No. of 308 Samples of Different Species were tested during this fisical year.

Species	Hb	Pcv	ESR	RBC	TLC	DLC	MCV	MCH	MCHC
Cattle	109	109	109	109	85	85	75	28	52
Buffalo	107	107	107	107	72	72	35	10	26
Sheep & Gost	92	92	92	92	38	38	27	8	10
Total	308	308	308	308	95	195	137	46	88

The blood parameter gives diagnosis of anaemia, blood protozoa & helminths infestation & other disease conditions of unknown etiology.

Microbiology Unit

A total 258 milk sample's were screening from mastitis test through CMT test. 198 sample's were found positive for mastitis. The details of CMT & antibiotic drug sensitive test are given as below.

Quarter	No. of Sample	Positive	Negative	Remarks
First Quarter	107	86	21	Percentage of
Second „	64	35	29	Positive Sample
Third „	87	45	42	60 %
Total :	258	156	92	

Result of drug sensitive test :

Type of sample	No. of antibiotic Test	No. of antibiotic sensitive	Percentage of sensitive
Milk	Gentamycin 66	57	**86**
	Enrofloxacin 66	59	89
	Tetracyclin 66	28	42
	Chloramphenicol 56	29	52
	Norfloxacin 50	29	58
	Nitrofurantion 45	16	35
	Kanamycin 48	26	54
	Penicillin 45	18	40
	Cloxacillin 28	21	75
	Ciprofloxacin 56	55	93
	Streptomycin 55	38	69

Pathology Unit

Under this unit mainly the poultry postmorom was performed based on lesions found in different organ. The result of post martom finding are demonstrected below. A total 158 chicken were exmined. The finding of P.M. are as follows.

Diseases	No. of Cases	Percentage	Remarks
Coccidiosis	50	32	
Aflatoxicin	22	14	
Collibacillosis	47	30	
Salmonellosis	9	6	
IBD	7	5	
ND	8	5	
CRD	5	3	
Mixed infection	10	6	

Biochemistry Unit

This unit performed following tests to predict & to interpret the Laboratory finding & co-relates with healthy & disease condition. Under biochemical examination calcium, Phosphorus, Magnesium, Protein estimation performed in this Laboratory. For the estimition of Zink, Copper, Samples are sent to C.V.L.A. total number of 287 serum samples were tested.

The examination of urine also performs by this unit. Urine examination mostly done by dipstick (Unistick, Multistick) & microscopic examination. Urine is examined for specific gravity, phosphorus, Suger, Keton bodies, PH, albumin, total protein, bilirubin, triple phosphorus, calcium oxalate RBC & pus cell. A total of 56 urine samples of cattle & Buffalo were testies. The examination of urine is indicated in renal disease, diabetes, urinarycalculie, Jaundice, Ascitis, Haematuria, Haemoglobinuria, Acidosis & alkalosis. The examination of urine is helpful for diagnosis of disease.

Biochemistry Unit Progress

Sample Tested	No. of Sample	Remarks
Urinalysis	68	Cattle & Buffalo
Calcium estimation	105	15% deficiat
Phusphorus ,,	115	18% ,,
Protein ,,	96	12% ..
Magnesium ,,	58	28% ,,

Salmonella test : Plate agglutination test of salmonella were per formed by salmonella antigen. A total 247 samples were tested an 12 were found positive.

Serology Unit

This unit is responsible for the sero diagnosis & sero monitoring of various infections disease of animals birds. Serum submitted by D.L.S.O. disease outbreak area or different quarantine chekpost. Mostly Goat serum are collected for P.P.R. diagnosis. A total 269 serum samples are recived among them174 were from Goat & rest from Cattle & Buffalo. All the samples were sent to C. V. L. for P.P.R. & brucellosos diagnosis.

Sample collection & dispatch

Mostly Serological & viral typing (F.M.D., P.P.R.) sample & undiagnese sample were sent to C. V. L. for identification & monitoring. A total 203 samples were collected.

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY

POKHARA

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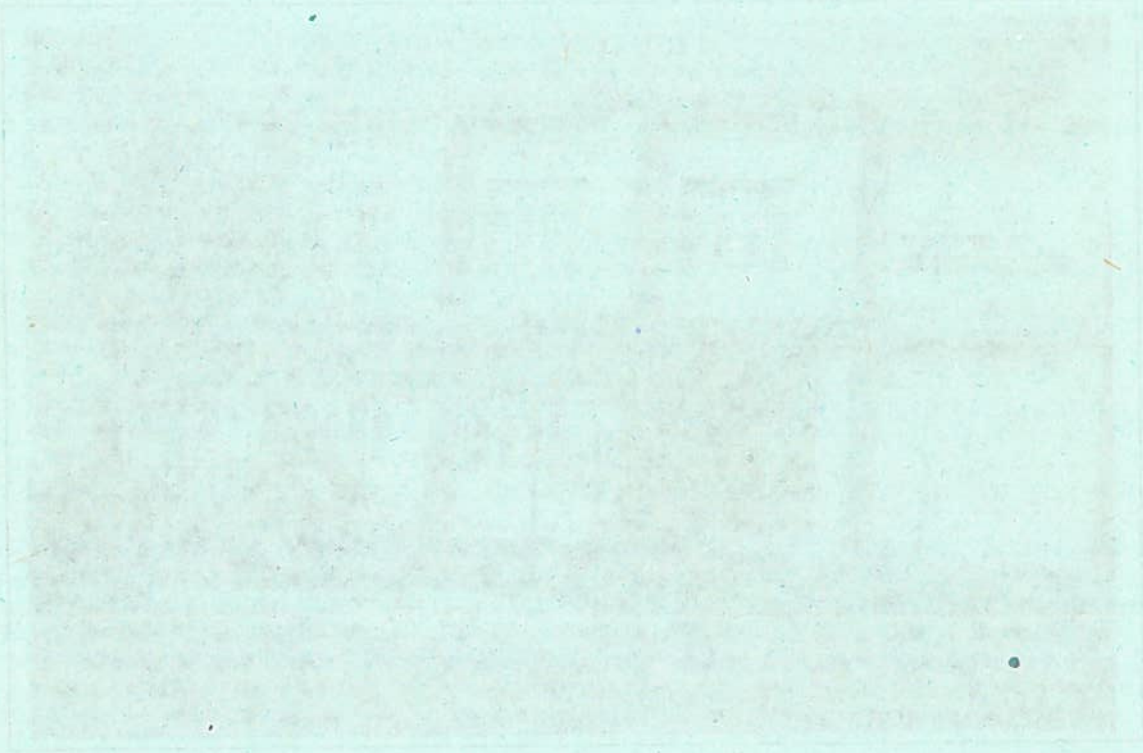


REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, POKHARA (Western Region)

Livestock plays an important role in the agriculture-dominated economy of Nepal and it contributes about 18 per cent to the national GDP and 32 per cent to the agricultural GDP. Contribution of livestock sector in agricultural GDP is expected to increase from 32 percent to 45 per cent in 20 years plan (APP, 1995). Western development region has tremendous scopes for livestock production. Most of the districts in the region have good infrastructures and access to markets, growing tourism and changing food habits of the people favor livestock production to become commercialization.

In Nepal, infectious diseases and parasites are the major constraint for the improvement in livestock production and productivity. Many infectious diseases are also zoonoses, which increase the significance of the disease. A number of exotic diseases such as Infectious bursal disease and Leechi heart disease in poultry and Peste des Petits Ruminants (PPR) in goats have been introduced into the country with the import of live animals or animal products. With the changing context of livestock industry, there are several constraints to cope with to improve livestock production in relation to quantity and quality. Every year, several diseases and parasites are responsible to cause a considerable amount of economic loss to livestock industry of the country, compelling the need of a massive disease control program. Correct diagnosis and epidemiological information of animal diseases and parasites are required to formulate an effective disease control program. For correct diagnosis of the diseases, laboratory services are essential components.

This annual report presents an overview of the programs and activities conducted by the Regional Veterinary laboratory (RVL), Pokhara during the fiscal year 2060-61 (2003-04). This report includes progress and achievements, laboratory findings and investigation works carried out during the period. Epidemiological information on the diseases of livestock in the region is also incorporated. Disease investigation activities in the goats distributed to the farmers under poverty alleviation programme in Nawalparasi, Baglung and Arghakhanchi districts conducted by RVL, Pokhara is also incorporated.

I would like to express my sincere thanks to Dr. D. R. Ratala, Program Director, Directorate of Animal Health, Tripureswor, Kathmandu and Dr. R M Shrestha, Chief, Central Veterinary Laboratory, Tripureswor, Kathmandu for their encouragement and guidance for the effective implementation of programs of this laboratory. Thanks are also due to all DLSOs of the region for regular reporting

of epidemiological reports and the help provided during program implementation. I would also like to extend my thanks to all the staff of RVL, Pokhara for their continuous support and assistance for the successful implementation of programs of this laboratory.

Introduction

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

Himalayan region:

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

Hilly region:

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

Terai region:

Terai region covers Nawalparasi, Rupandehi and Kapilbastu districts. This plain extends from east to west of the region and stretched from 15 to 40 Kilometers in width. Sediments and silt are main constituents of soil deposited by rivers making it more fertile and this belt supplies the food and fibers to other regions of the country. Compared to mountains and hilly regions, this region has relatively better infrastructure and market accessibility. People of this region are motivating 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), Chicken infectious anemia, Coccidiosis, Leechi heart disease and Fowl cholera in chickens are major disease problems.

Objectives of Regional Veterinary Laboratory:

- To provide disease diagnostic services to the livestock and poultry rearing farmers in the region.
- To investigate epidemics of animal diseases in the region and assist, advice and support District Livestock Services Offices (DLSOs) to control them.
- To prepare the epidemiological profile of livestock and poultry diseases and maintain regional epidemiological information database on animal health.
- To investigate the diseases of livestock species relatively important in the region.
- 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 support animal health and infertility camps organized in the region.
- To coordinate national disease control and eradication programs in the region.

The annual work program and progress made by the Regional Veterinary Laboratory,
Pokhara during fiscal year 2060/61 is presented in table 1.

Table 1: Annual work Program and summary of achievements of Regional Veterinary Laboratory, Pokhara (for fiscal year 2060/61)

S. N.	Programs and Activities	Annual Target		Annual Progress	Annual Weightage %	Remarks
		Unit	Weightage (%)			
1	**Laboratory Services**					
1.1	Parasitological Examinations	600	2.26	624	2.26	
1.2	Microbiological Examinations	400	5.37	467	5.37	
1.3	Pathological Examinations	400	6.23	606	6.23	
1.4	Serological Examinations	300	4.19	318	4.19	
1.5	Hematological Examinations	150	3.11	152	3.11	
1.6	Biochemical Examinations	200	3.01	206	3.01	
1.7	Sample collection and dispatch	400	4.30	440	4.30	
2	**Disease Investigation and Surveillance Program**					
2.1	John's disease investigation	1	20.30	13	20.30	
2.2	Investigation of Epidemic	15	24.10	15	24.10	
3	**Monitoring and Supervision**					
3.1	Monitoring and Supervision of district based Laboratories	1	8.70	10	8.70	
4	**Disease Investigation Workshop**	1	4.83	1	4.83	
5	**Training program**					
5.1	Computer training	3	0.86	3	0.86	
6	**Publication Program**					
6.1	Tri-monthly Epidemiological Bulletin publication program	4	2.58	4	2.58	
6.2	Annual Technical Report Publication	1	1.61	1	1.61	
7	**Purchase program**					
7.1	Purchase of equipment	3	7.52	0	0	No release of budget
7.2	Purchase of Scientific books	4	1.07	4	1.07	

1. Laboratory Services

1.1 Parasitological Examinations:

Faecal samples were examined adopting both qualitative and quantitative methods. In the fiscal year 2060/61 altogether 624 faecal samples from different species of animals such as cattle, buffalo, sheep, goats, and dogs were examined. Out of 624 faecal samples examined 351 samples were found to be positive for various internal parasites. Two hundred seventy three samples were negative for any parasites.

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

Table 2: Monthly faecal examination results during 2060/61

				M	O	N	T	H	S				Total	Positive
Parasites	**Shrawan**	**Bhadra**	**Asoj**	**Kartik**	**Mangsir**	**Paush**	**Magh**	**Falgun**	**Chaitra**	**Baishak**	**Jestha**	**Asar**		(%)
Fasciola	8	5	17	13	6	9	5	2	3	3	3	1	75	21.37
Paramphistome	5	4	2	1	7	2	2	2	1	1	4	5	36	10.26
Ascaris	0	0	1	0	0	0	0	0	0	0	0	0	1	0.28
Strongyle	4	1	11	0	38	14	0	0	1	1	5	2	77	21.93
Strongyloides	4	0	8	0	27	14	0	0	3	0	3	0	59	16.81
Trichuris	0	0	0	0	7	5	0	0	0	0	0	0	12	3.41
Moneizia	0	0	1	0	3	9	0	0	0	0	4	0	17	4.85
Coccidia	2	4	3	4	9	12	0	0	2	0	10	4	49	13.97
Mixed infections	2	1	1	1	0	0	0	0	0	0	0	0	5	1.43
Others (B.coli)	1	1	8	1	1	3	3	0	0	2	0	0	20	5.69
Total positive	**26**	**16**	**52**	**20**	**98**	**68**	**10**	**4**	**10**	**7**	**29**	**12**	**351**	**100**

Thirty-seven skin scrapings from goats; cattle and dogs were received for the examination and identification of mites. The eleven positive samples revealed Sarcoptic and Demodectic species of mites in cattle/goat and dogs respectively.

A total of 32 blood samples for the presence of blood parasites were examined. Three samples were found to be positive for for Anaplasma spp.

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 2060/61 a total of 467 pathological samples were examined in microbiology unit of the laboratory. 261 pathological samples from poultry and other species of animal were subjected for the bacteriological examinations. Out of 206 milk samples 151 samples were positive for Sodium Lauryl Sulphate test (SLST). The SLST positive milk samples were subjected for bacteriological culture and the isolates were subjected for antibiotic sensitivity testing. Out of 151 SLST positive milk samples 146 samples resulted bacterial growth. Results of bacteria isolated from milk samples and other pathological samples are presented in Table 3.

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

Table 3: Bacterial species isolated from mastitic milk and other pathological samples

Bacterial Species	Number isolated from mastitis milk samples	Number isolated from other pathological samples
Staphylococcus spp.	47	10
Streptococcus spp.	21	7
Bacillus spp.	35	13
Pasturella multocida		3
Proteus spp.	14	12
Micrococcus spp.	7	4
Enterobacter spp.		3
Salmonella spp		3
Escherichia coli	34	15
Other Enterobacteria	23	2

Results of antibiotic sensitivity testing of bacterial isolates from the 151 SLST positive milk samples are given in Table 4.

Table 4: Results of Antibiotic sensitivity testing of bacterial isolates from milk samples

Antibiotics used	Sensitivity %
Enrofloxacin	98.01
Gentamicin	88.07
Chloramphenicol	80.79
Nitrofurantoin	43.04
Ciprofloxacin	65.31
Co-trimoxazole	25.32
Tetracycline	49.16

Results of antibiotic sensitivity testing revealed that Enrofloxacin followed by Gentamicin and Chloramphenicol was found to be the most sensitive antibiotics against the bacteria isolated from mastitic milk of cattle and buffaloes. It may therefore, be concluded that these antibiotics could be used for the routine treatment of mastitis cases in the district where culture and antibiotic sensitivity testing of milk samples is not possible.

1.3 Pathological Examinations

Pathological examinations mostly consisted of post mortem examination (PM) of animals and poultry. Six hundred and six poultry, 5 piglets, 3 goats and 1 sheep were brought for the postmortem examination. Piglets were found to have died due to pasturellosis. Sheep autopsied was found to have died due to acute fasciolosis. 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 confirmatory diagnosis. Diseases of chickens diagnosed by PM are summarized in Table 5.

Table 5: Diseases of chickens diagnosed by PM and lab examinations

S.No.	Disease diagnosed	No. Of Cases	Percentage of Cases
1	Coccidiosis	164	27.06
2	Infectious Bursal Disease	121	19.96
3	Colibacillosis	117	19.30
4	Haemorrhagic enteritis	44	7.26
5	Chronic Respiratory Disease	27	4.45
6	Ranikhet Disease	25	4.12
7	Salmonellosis	20	3.30
8	Hepatitis	16	2.64
9	Nutritional Deficiency	15	2.47
10	Leechi heart disease	14	2.31
11	Omphalitis	11	1.81
12	Miscellaneous diseases	11	1.81
13	Ascitis	9	1.48
14	Ascariasis	5	0.82
15	Tapeworm	3	0.49
16	Egg drop syndrome	2	0.33
17	Infectious bronchitis	2	0.33
Total		**606**	**100**

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. Agar Gel Precipitation Test (AGPT) was used for the detection of antibody in chicken against avian encephalomyelitis virus. 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 2060/61, the serum samples tested and results are presented in table 6.

Table 6: Results of serum samples tested

Animal/Bird species	Number of serum tested	Tested for	Test applied	Result
Cow	8	Brucellosis	RBPT	All negative
Buffalo	4	Brucellosis	RBPT	All negative
Goat	17	Brucellosis	RBPT	All negative
Poultry	33	Mycoplasmosis	PAT	17 positive
Poultry	26	Salmonellosis	PAT	12 positive
Poultry	5	Avian encephalomyelitis	AGPT	All negative

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 (TEC), 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 152 blood samples from animals were examined for different hematological parameters.

1.6 Biochemical examinations:

Biochemical examinations revealed 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 206 samples were examined in biochemistry unit including 101 urine samples. Out of 101 urine samples examined, 42 were diagnosed to be haematuria and 17 proteinuria cases. Results of biochemical analyses of 10 serum samples collected from buffaloes of Livestock farm, Pokhara is given in table 7.

Table 7: Results of serum biochemistry of buffaloes of livestock farm, Pokhara

S. No.	Animal Species	Calcium mg/dl	Phosphorus mg/dl	Magnesium mg/dl
1	Buffalo	9.197	11.162	1.893
2	Buffalo	12.218	7.906	1.981
3	Buffalo	11.546	3.255	1.488
4	Buffalo	12.352	8.372	1.685
5	Buffalo	12.352	3.72	1.931
6	Buffalo	12.08	5.238	1.916
7	Buffalo	11.681	8.837	2.055
8	Buffalo	11.546	8.837	2.037
9	Buffalo	11.412	3.488	1.376
10	Buffalo	11.949	11.58	1.845

1.7 Sample collection and dispatch:

Due to lack of biological reagents and ELISA reader, RVL, Pokhara is not capable of performing a wide range of serological tests to diagnose the animal diseases occurred in the region. Therefore, the samples not possible to analyze in this laboratory and/or required to be re-confirmed were referred to CVL, Kathmandu. During 2060/61, a total of 440 serum, blood and tissue samples of different animal species and poultry were collected. A total of 240 various samples were dispatched to CVL, Kathmandu and FMD laboratory, Kathmandu for confirmatory disease diagnosis.

The results of samples referred to CVL, Kathmandu and FMD laboratory, Kathmandu for laboratory investigation is presented in table 8.

Table 8: Results of sample dispatched for laboratory investigation during 2060/61

S.N.	Animal/Bird species	District	Type of samples	No. of samples	Investigation requested for	Samples despatched to	Lab. test used	Result
1	Goat	Tanahu	Skin lesion	1	goat pox	CVL, Ktm	Virus isolation	Negative
2	Goat	Arghakhanchi	Serum	32	PPR	CVL, Ktm	ELISA	All negative
3	Poultry	Kaski	Heart, liver	2	Leechi heart disease	CVL, Ktm	Histo-Pathology	Not confirmed
4	Poultry	Kaski	Trachea, lungs, intestine	3	Ranikhet disease	CVL, Ktm	Virus isolation	Not confirmed
5	Goat	Gorkha	Serum	6	PPR	CVL, Ktm	ELISA	4 positive
6	Poultry	Kaski	Serum	11	IB, ND, EDS	CVL, Ktm	ELISA	9 positive for ND, 8 positive for IB, 8 positive for EDS
7	Poultry	Kaski	Heart, trachea, lungs, liver	4	Leechi heart disease	CVL, Ktm	Histo-Pathology	Not confirmed
8	Poultry	Kaski	Sciatic nerve, liver	3	Marek's disease	CVL, Ktm	Histo-Pathology	Not confirmed
9	Cattle, Buffalo	Mustang, Parbat, Lamjung	Buccal and tongue mucosa	12	FMD	FMD Lab., Ktm	ELISA	1 each positive for type O and A FMD virus
10	Goat	Baglung,Nparasi, Argha	Serum	158	PPR	CVL, Ktm	ELISA	57 positive

Establishment of Mycobacterium culture unit at RVL, Pokhara

During 2060/61with the assistance of JICA, culture/isolation of *Mycobacterium spp.* unit has been established at RVL, Pokhara. For the development of culture/isolation facilities of *Mycobacterium spp.*, JICA has provided some equipment such as Laminar flow, Centrifuge, water bath, Vortex mixer and Electric sealer.

Isolation of *Mycobacterium spp.* from milk and feacal samples from tuberculin test positive cows and buffaloes was attempted. The isolates colonies suspected to be *Mycobacterium spp.* were sent to Gunma Prefactural Institute of Public Health and Environmental Sciences, Japan for further confirmation and characterization. So far 3 isolates have been confirmed to be *Mycobacterium spp.* The detailed paper on Isolation of Mycobacterium spp. from tuberculin test positive milking cows and buffaloes in Nepal will be published shortly.

Publication of Quarterly Epidemiological Bulletin

Quarterly epidemiological bulletins are being published with an aim to study the patterns of existing animal diseases at regional levels. During the fiscal year 2060/61, RVL, Pokhara published four issues of epidemiological bulletins. Epidemiological bulletins contained animal disease information of the western development region. The data published in the bulletin were the compilation of monthly epidemiological reports received from District Livestock Service Offices of the region. As compared to past years, there has been excellent improvement in the reporting status of epidemiological reports from DLSOs. The improvements in reporting of epidemiological reports have been made both in quality and the regularity. The epidemiological report, receiving status from the district is presented in Table 10. Although detailed epidemiological information of animal diseases were already published through the bulletins; a brief summary of animal disease profile across the different agro-ecological zones of the region is presented in the following Tables 11, 12 and 13.

Table 10: Epidemiological report reporting status from the districts of WDR during 2060/61.

Districts	Months												Total
	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	
Kaski	√	√	√	√	√	√	√	√	√	√	√	√	12
Tanahun	√	√	√	√	√	√	√	√	√	√	√	√	12
Shangja	√	√	√	√	√	√	√	√	√	√	√	√	12
Parwat	√	√	√	√	√	√	√	√	√	√	√	√	12
Baglung	√	√	√	√	√	√	√	√	√	√	√	√	12
Myagdi	√	√	√	√	√	√	√	√	√	√	X	√	11
Palpa	√	√	√	√	√	√	√	√	√	√	√	√	12
Argha- khanchi	√	√	√	√	√	√	√	√	√	√	√	√	12
Gulmi	√	√	√	√	√	√	√	√	√	√	√	√	12
Lam-jung	√	√	√	√	√	√	√	√	X	√	√	√	11
Manang	√	√	√	√	√	√	√	√	√	√	√	√	12
Mustang	√	√	√	√	√	√	√	√	√	√	√	√	12
Gorkha	√	√	√	√	√	√	√	√	√	√	√	√	12
Kapilwastu	√	√	√	√	√	√	√	√	√	√	√	X	11
Rupandehi	√	√	√	√	√	√	√	√	√	√	√	√	12
Nawalparasi	√	√	√	√	√	√	√	√	√	√	X	X	10

Table 11: Animal/Bird disease profile of mid hills during 2060/61

Mid hill districts																						
Bacterial Diseases	Tanhun		Kaski		Arghakhanchi		Shyanja		Gulmi		Palpa		Parbat		Baglung		Lamjung		Myagdi		Total	
	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death
H.S.	19	0	6	6	0	0	181	7	0	0	17	14	0	0	13	0	0	0	95	24	331	51
Blackquater	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
Mastitis	160	0	0	149	0	74	81	0	120	0	525	0	116	0	25	0	44	0	27	0	1331	0
Anthrax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterrotoxaemia	0	0	0	0	0	0	0	0	6	4	392	3	0	0	0	0	0	0	0	0	398	7
Fowl cholera	50	12	0	0	0	0	0	0	0	0	0	0	525	6	0	0	0	0	0	0	575	18
Fowl typhoid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pullorum diseases	90	3	0	0	0	0	0	0	30	17	0	0	24	3	0	0	0	0	0	0	144	23
Infectious cryza	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150	0	150	0
CRD	799	147	18700	1226	0	0	0	0	0	0	260	40	0	0	0	0	0	0	435	32	20149	1445
Viral diseases																						
Orf	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	17	0
PPR	0	0	0	0	0	0	4	0	21	13	0	0	0	0	0	0	0	0	0	0	25	13
FMD	172	4	423	8	311	1	727	10	75	20	86	0	670	15	68	6	811	21	298	30	3641	165
Ephemeral fever	41	0	0	0	0	0	0	0	0	0	201	0	0	0	0	0	0	0	0	0	242	0
Rabies	0	0	8	8	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	10	10
Fowl pox	55	0	600	0	0	0	126	0	0	0	699	25	206	5	607	5	0	0	966	0	3258	35
MD	0	0	546	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	546	0
RD	0	0	0	0	0	0	200	25	0	0	100	90	0	0	0	0	0	0	62	15	362	130
IBD	911	76	0	0	0	0	0	0	0	0	1615	468	101	0	700	75	0	0	0	0	3327	618

Goat/sheep pox	15	1	32	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47	2
Buffalo pox	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Parvoenteritis	11	0	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	131	0
Swine fever	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Endo-Parasites diseases																							
Fasciolosis	4345	0	4575	0	1055	7	6436	0	4441	6	11345	0	3016	0	1899	0	2935	0	5254	10	45301	23
Paramphistomosis	850	0	3023	0	225	0	1448	0	867	3	6261	0	2260	0	1272	0	562	0	893	0	17661	3
Tape worm	2135	0	1467	0	1905	6	888	0	2996	0	17715	0	101	0	263	0	0	0	179	2	27649	8
Intestinal helminth	310	2	0	0	1015	0	330	0	39	0	6350	15	0	0	0	0	0	0	1373	5	9427	22
Ecto-Parasitic diseases																							
Mange/mites	27	0	2159	0	501	3	425	0	1387	0	94	0	651	0	18	0	40	0	1672	2	6974	5
Ticks/Lices	97	0	1464	0	364	0	2276	0	848	0	6337	0	0	0	130	0	198	0	155	0	11869	0
Protozoan diseases																							
Babesiosis	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0
Coccidiosis	459	16	1187	77	95	0	42	0	768	41	0	0	345	6	1065	40	115	100	303	32	4379	312
Others diseases																							
Diarrhoea	1239	6	2139	0	761	0	388	0	2279	3	8148	0	1074	5	732	0	259	0	2498	8	19517	22
Respiratory signs	173	0	1139	0	98	0	90	0	432	9	1324	0	772	0	0	0	0	0	0	0	4028	9
Infertility	232	0	1351	0	224	0	295	0	255	0	4552	0	48	1	26	0	34	0	244	0	7279	1
Anorexia	200	0	1828	0	0	0	197	0	0	0	0	0	0	0	0	0	0	0	0	0	2225	0
Wound	85	0	398	0	67	0	214	0	712	0	1816	0	0	0	0	0	0	0	6	0	3298	0
Timpany	53	0	1017	0	0	0	78	0	227	1	0	0	0	0	0	0	0	0	66	1	1441	2
Cough	70	0	663	0	0	0	80	0	249	0	0	0	0	0	0	0	0	0	0	0	1062	0
Opacity	14	0	56	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	85	0
Abortion	28	0	244	0	25	0	11	0	55	2	767	0	0	0	0	0	0	0	0	0	1359	1
Nervous Signs	59	0	20	0	0	0	11	1	55	2	696	0	0	0	0	0	0	0	0	0	841	3
Skin lesions	313	0	1464	0	303	0	84	0	513	0	3954	0	224	0	649	0	0	0	0	0	7504	0

Fever	4	0	2547	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	2605	0
Stomatitis	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
Red urine	80	0	118	0	23	0	63	0	0	0	356	0	128	0	22	0	41	0	58	0	889	0
Arthritis	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	17	0
Pneumonia	123	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	123	0
Worble infectation	7	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	0
Asthma	0	0	375	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	375	0
Dystocia	13	0	35	0	0	0	25	0	824	0	424	0	0	0	20	1	0	0	38	0	1379	1
Sudden death	33	33	501	501	20	20	0	0	4	4	40	40	6	6	1	1	7	7	24	24	636	636
Bronchitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Equine influenza	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	6	1
Mycoplasmosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anaplasmosis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Enzootic heamaturia	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45	0
Retained placenta	16	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	93	0	118	0
Uterine prolapse	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	7	35	7
Ruminal dysfunction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	156	0	156	0
Indigestion	0	0	0	0	0	0	0	0	196	0	0	0	0	0	0	0	0	0	182	0	378	0
Retention of urine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	1	28	1
poisoning	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	4	1
Dermatophilosis	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	6	0

Table 12: Animal/ bird disease profile of Terai region of WDR during 2060/61

Tarai districts	Kapilwastu		Rupandehi		Nawalparasi		Total	
Becterial Diseases	Cases	Death	Cases	Death	Cases	Death	Cases	Death
PPR	86	2	0	0	0	0	86	2
FMD	107	0	173	10	674	0	954	10
Ephemeral fever	38	0	173	0	0	0	211	0
Rabies	0	0	0	0	21	9	21	9
Fowl pox	0	0	75	10	545	6	620	16
MD	0	0	0	0	0	0	0	0
RD	0	0	9600	1255	0	0	9600	1255
IBD	0	0	7809	512	0	0	7809	512
Buffalo fox	0	0	0	0	0	0	0	0
Endo-Parasites							0	0
Fasciolosis	1179	6	3850	0	2608	6	7637	12
Paramphistomosis	0	0	1037	0	0	0	1037	0
Tape worm	241	7	1545	0	579	7	2365	14
Intestinal helminth	113	0	1908	25	150	0	2171	25
Ecto-Parasitic							0	0
Mange/mites	141	1	79	0	0	0	220	1
Ticks/Lices	0	0	305	0	10	0	315	0
Protozoan							0	0
Babesiosis	0	0	14	0	1	0	15	0
Coccidiosis	30	0	7079	0	2312	114	9421	114
Others diseases							0	0
Diarrhoea	1364	161	1029	8	2502	53	4895	222
Respiratory signs	29	0	139	0	119	3	287	3
Infertility	52	0	375	0	250	0	677	0
Anorexia	103	0	441	0	91	0	635	0
Wound	118	0	0	0	144	0	262	0
Tympany	42	0	191	0	124	5	357	5
Abortion	18	0	99	0	165	5	282	5
Nervous Signs	10	2	55	0	13	1	78	3
Skin lesions	58	0	300	0	107	0	465	0
Fever	109	0	230	0	186	0	525	0
Stomatitis	0	0	0	0	0	0	0	0
Red urine	0	0	21	0	25	0	46	0

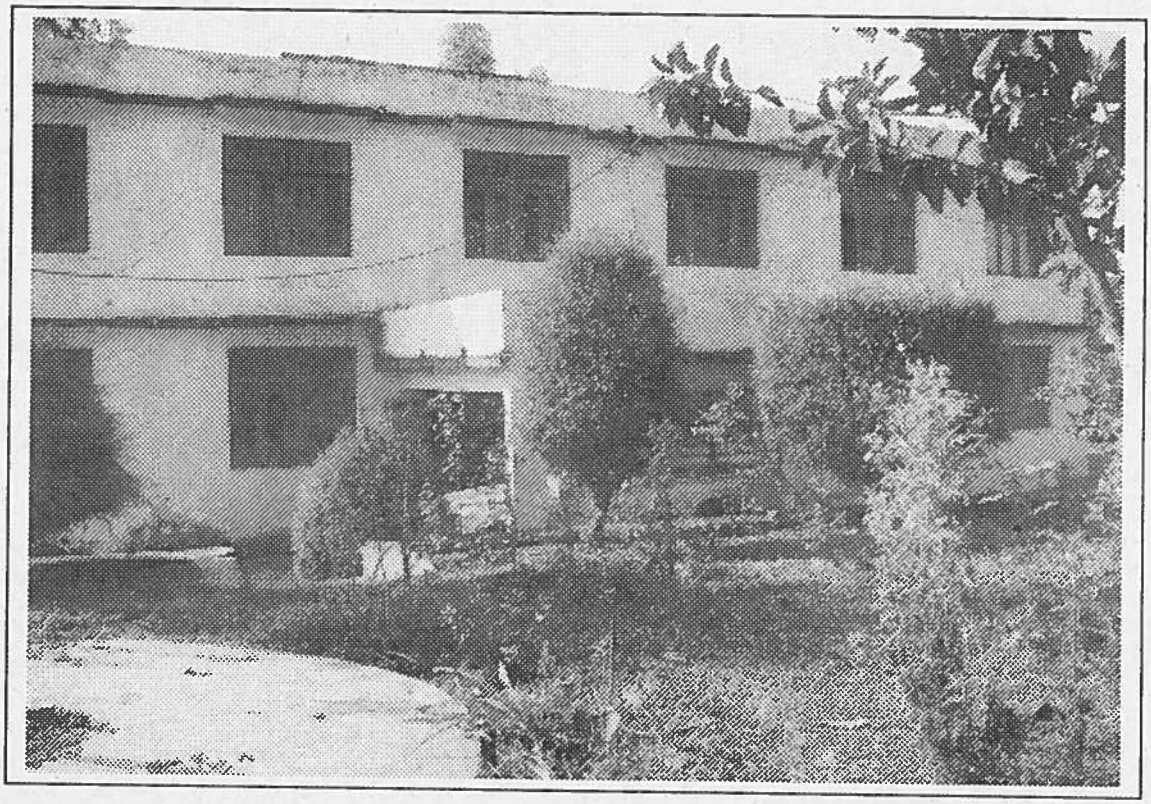
Table 13: Animal/bird disease profile of mountain Region of WDR during 2060/61

Mountain region	Gorkha		Manang		Mustang		Total	
Becterial Diseases	Cases	Death	Cases	Death	Cases	Death	Cases	Death
H.S.	0	0	0	0	0	0	0	0
Blackquater	0	0	0	0	0	0	0	0
Mastitis	82	0	0	0	0	0	82	0
Anthrax	0	0	0	0	0	0	0	0
0Enterrotoxaemia	0	0	45	0	0	0	45	0
Fowl cholera	0	0	0	0	0	0	0	0
Fowl typhoid	0	0	0	0	0	0	0	0
Pullorum diseases	3	0	0	0	129	0	132	0
Infectious Coryza	0	0	0	0	0	0	0	0
Strangles	0	0	3	0	0	0	3	0
CRD	0	0	0	0	166	0	166	0
Viral diseases								
PPR	0	0	0	0	0	0	0	0
FMD	79	4	1508	0	363	0	1950	4
Ephemeral fever	0	0	0	0	0	0	0	0
Rabies	6	6	0	0	0	0	6	6
Fowl pox	103	21	0	0	8	0	111	21
MD	0	0	0	0	0	0	0	0
RD	0	0	0	0	24	5	24	5
IBD	575	75	0	0	0	0	575	75
Buffalo fox	0	0	0	0	0	0	0	0
Parasitic Diseases								
Endo-Parasites diseases								
Fasciolosis	4887	0	0	0	1978	0	6865	0
Paramphistomosis	1092	0	0	0	0	0	1092	0
Tape worm	0	0	1799	0	4758	0	6557	0
Intestinal helminth	0	0	279	0	1015	0	1294	0
Ecto-Parasitic diseases								

Mange/mites	1333	0	573	0	2627	0	4533	0
Ticks/Lices	0	0	5002	0	17606	0	22608	0
Protozoan diseases								
Coccidiosis	0	0	0	0	238	10	238	10
Others diseases								
Diarrhoea	3170	56	1106	0	556	0	4834	56
Respiratory signs	0	0	84	0	10	0	94	0
Infertility	18	0	115	0	6	0	139	0
Anorexia	0	0	415	0	0	0	415	0
Wound	0	0	691	0	318	0	1209	0
Tympany	9	0	69	0	3	0	81	0
Cough	1204	0	229	0	0	0	1433	0
Opacity	0	0	3	0	0	0	3	0
Abortion	54	1	47	0	52	0	153	1
Nervous Signs	0	0	0	0	2	0	2	0
Skin lesions	516	0	0	0	557	0	1073	0
Fever	0	0	336	0	0	0	336	0
Stomatitis	0	0	0	0	25	0	25	0
Red urine	38	0	10	0	5	0	53	0
Arthritis	0	0	0	0	0	0	0	0
Pneumonia	0	0	227	0	15	0	192	0
Sudden death	25	25	501	501	20	20	546	546
Worble Infestation	0	0	5	0	0	0	5	0
Dystocia	0	0	2	0	0	0	2	0
Colic	0	0	69	0	0	0	69	0
Retained plecenta	0	0	70	0	0	0	70	0
Indigestion	0	0	80	0	5	0	85	0
Retention of urine	0	0	22	0	0	0	22	0
Foot lesion	0	0	210	0	0	0	210	0

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY

SURKHET



REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY
[Mid-western Region, Surkhet]

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REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, SURKHET (Mid - Western Region)

An Overview of mid-western region

The mid western region is one of the 5 development regions of Nepal and lies between the far-western and western development region. It represents the 3 zone i.e. Bheri, Rapti, Karnali constituted by 15 districts. The topography of this region ranges from 127 mt. in Bankey to 7334.7 mt. in Humla. This vast variation in topography has produced various types of climates in the region based on the topography of the whole region mountain, hill and terai.

Mountain

The five district of Karnali zone makes the high hill mountain. The altitude of this region ranges from 738mt. in kalikot to 7334.7mt. in Humla .Almost all part of Humla and Jumla lies above 4000mt. from the sea level. Other 3 directs of this part has both high and low altitude.

Hill

The hilly part of those region constitute of district of Bheri and Rapti Zone among these districts Surkhet, Dailekh, Salyan, Rolpa and Puythan have about similar altitude while Rukum and Jajarkot is located some what higher to these districts.

Terai :-

West 3 districts make the terai and inner terai of this region. Bankey, Bardia and Deukhuri part of dang make terai and Dang valley lies in terai.

The rest topographical variation of this region can be divided in to five following types.

Temperate: - Humla, Jumla and some of the highest part of Mugu, Dolpa and kalikot

Sub-temperate: - lower part of Mugu, Dolpa and kalikot and throughout Rukum and jajarkot district in general.

Sub-Tropical: - Surkhet, Dailekh, Salyan, Pyuthan, Rolpa and Inner terai of dang district.

Tropical: - Bankey, Bardiya and Deukhuri part of dang districts.

Four districts (Dang, Bankey, Bardiya and Surkhet) of this region are linked to national highway by all weather roads. Rest of district can be approached either by the treaking or aerial route Mugu and kalikot only by means of helicopter other means.

Livestock population of mid- western

S.N.	ANIMAL SPECIES	UNIT	YEAR 2060
1.	Improved breed		
	cow	Num.	6819
	buffalo	,,	102198
	goat	,,	187217
	sheep	,,	19899
	swine	,,	100891
	broiler	,,	290952
	layers	,,	126176
	rabbit	,,	1359
	chauri	,,	-
2.	Local breed	,,	-
	cow	,,	1597822
	buffalo	,,	904219
	goat	,,	1289423
	sheep	,,	343985
	swine	,,	259733
	poultry	,,	737949
	rabbit	,,	565
	chauri	,,	22071
3.	Improved verities grass cultivated area	ha	726
4.	livestock product	-	
	milk	mt.	190598
	meat	ton.	72519
	egg	num.	274429
	wool	mt.	238
	skin(leather)	num.	108991

Introduction

Regional vet laboratory Surkhet represents the regional laboratory for the mid-western region of Nepal. The laboratory was established in the fiscal year 1988/89 i.e. 2048/049 with the view of developing it as a reference veterinary diagnostic and investigation centre for the 15 districts of Bheri, Rapti and Karnali Zone of Nepal. It is located at the Itram of Birendra Nagar municapality; the district and regional headquarter of Mid-western region respectively. Before the actual establishment of this laboratory 1988/89, the pre-requisite infrastructure like reconstruction of building and procurement of equipment were undertaken by the existing regional directorate of livestock servicing (RDLS) through its annual program. The laboratory did not have Its own staffing at the time of establishment and was run by the staff of RDLS up to 1991/1992, in 1991/1992, the laboratory has its own staffing and starting functioning as an independent unit. The present staffing of this laboratory constitute 2 veterinarians, 3-mid level technician and 6 supporting staffs.

The building of this laboratory was constructed by karnali-Bheri integrated rural development project (KBIRD), which also facilitate of with a lot of equipment. Additional equipment and chemicals were made available by the livestock development project to RVDL the early activities of the laboratory.

Further renovation in a more technical manner to care the routine work of the laboratory was done in the year 1997/1998 by the strengthening of veterinary services for livestock disease control (SVSLDC) project. SVSLDC project is continuously supporting for the up liftment of the physical and technical capabilities of this laboratory along with other and RVDL to develop its capability as a reference laboratory specially Surkhet and Dhangadi for goat.

In recent years the laboratory is working in the direction of surveillance, investigation and epidemiological centre for mid-western region.

Though, the improving capabilities of the regional laboratory are slow but some fruitful results and some achievement in the direction of investigation ware made.

Main Units of Laboratory

Parasitology unit

This unit involves in the examination of fecal sample, skins scraping, submitted by farmers directly or referred from different districts livestock offices. The sample is processed for identification of parasitic ova and eggs through direct smear method, floatation method and differential floatation method. The quantative examination of the fecal sample is also done to estimate EPG count. Larvae culture and its identification can be performed through this unit.

This section has worked to fine result of lungworm infestation in goats. In addition to this many fecal sample are collected during diseases outbreak investigation program and also from poverty alleviation goat rearing program.

Haematology unit

This unit provides all the hematological examinations like total RBC & WBC count, differential leucocytes count, and estimation of hemoglobin packed cell volume and examination of blood smear for the identification of blood parasites. Several slides samples collected during visit to other districts and also submitted by district laboratory for reconfirmation are usually examined by this laboratory. Some blood samples are also sent by quarantine check post for further examination.

Serology unit

In this unit the serum samples are collected from different districts during diseases investigation and surveillance program. Most of this serum samples collected from livestock and poultry are dispatched to CVL and other referral laboratories. This unit provides some plate agglutination test salmonella pullorum, micoplasama, brucellosis, and tuberculosis. Seromonitoring of vaccinated goat and poultry also performed by sending the serum sample to CVL.

Biochemistry unit

In this unit samples like urine, blood and milk are submitted urine sample are analyzed using multisticks method. In the blood serum test this unit provides diagnostic services like estimation of serum, protein, albumin, urea, glucose, calcium and phosphorous by spectrophotometer. This unit also provides the test like CMT and SLST for milk.

Microbiology unit

This unit has most of the required equipment to perform the bacteriological examinations. The sample, submitted are usually collected from the outbreak areas and positive milk samples for mastitis. In bacteriology, culture and identification of bacteria as well as antibiotic sensitivity test are carried out. In microbiology, culture and identification of the fungi are performed. However, sample for virological examinations are not processed in the laboratory, in stead those samples are dispatched to the CVL and other laboratories. Laboratory animal (specially sheep, rabbit and poultry) are kept and maintained for the use of media preparation and biochemical tests of bacteriology.

Pathology unit

Activities of pathology unit include the postmortem examination of animal and birds received at the laboratory. During the postmortem, samples like tissues, blood impre ssion smears from various organs and intestinal contents are collected and sent to the respective laboratories for confirmatory diagnosis. This unit has been proved to be useful in providing better diagnosis of poultry diseases. This in turn, enhances the poultry industry and helps farmers by giving them proper suggestion and consultancy

Epidemiology unit

Quarterly epidemiological bulletins are being published with aim to study the patterns of existing animal diseases at regional basis on different climatic ecozones.

The data been published in the bulletin are the compilation of monthly epidemiological reports received from district livestock service office of the region.

Four epidemiological bulletins were published every year distributed in the region to concerned offices.

Supporting unit

***a) Sample collection unit*: -** This unit received the sample brought by farmers or by DLSO. Every sample is properly checked for its labeling and condition of the sample to be processed. Registration is done hare and case register also maintained.

***b) Cleaning and sterilization unit*: -** This unit plays important role in proper functioning and delivering good result in the laboratory.

Disposal unit

This unit is placed outside the laboratory building, it contains as single pit where dead body and other disposable materials disposed.

Objective of regional veterinary laboratory, Surkhet

- To provide diseases diagnostic services to all type of livestock's owners.
- To monitors and assist in diagnostic services to basic and primary laboratory in the region.
- To investigate epidemics of animal diseases in the region and assist DLSO to control them.
- To prepare the epidemiological profile of livestock and poultry diseases
- To investigate the diseases of animal species relatively important in this region
- To study the surveillance of animal disease of economical and zoontic importance
- To monitor and supervision to district laboratories and act as referral laboratory for them.

Approved annual work program of fiscal year 2060/061 and summary of achievement of RVL Surkhet if presented below

S.N.	Program and activities	Unit	Annual target	Annual progress	Progress percentage
1	**Laboratory service Program**				
1.1	Parasitological Examination	Num.	1100	1403	100%
1.2	Microbiological Examination	,,	350	351	100%
1.3	Pathological Examination	,,	150	152	100%
1.4	Serological Examination	,,	400	405	100%
1.5	Hematological Examination	,,	150	213	100%
1.6	Biochemical Examination	,,	200	202	100%
1.7	Sample dispatched	,,	300	482	100%
2	**Disease investigation and surveillance program**				
2.1	Investigation of goat respiratory diseases	Each	12	12	100%
2.2	Investigation of Epidemic Diseases	,,	6	6	100%
3.1	Monitoring and supervision of district labs	,,	6	6	100%
4	Disease investigation workshop	,,	1	1	100%
5	**Training program**				
5.2	Computer training	Person	2	2	100%
6.1	Quarterly epidemiological bulletin publication	Each	4	4	100%
6.2	Annual technical report publication	,,	1	1	100%
7.1	Purchase of scientific books and journals	,,	1	1	100%

Parasitology (060/061)

Month	Fasciola	Strongyles	Moniezia	Protozoa	Paramphistomes	Trichuris	Ascaris	Negative	Total
Shrawan	26	7	-	3	-	1	-	11	48
Bhadra	24	10	-	3	-	2	-	9	48
Ashoj	61	53	-	8	-	20	2	39	183
Kartik	18	45	-	6	-	10	-	48	127
Mangshir	15	35	1	8	2	8	1	58	128
Push	31	69	-	29	3	23	14	121	290
Magh	18	55	1	11	2	13	5	51	156
Falgun	31	22	-	4	2	6	3	28	96
Chaitra	33	16	-	1	1	1	-	15	67
Baishak	21	22	-	2	-	-	5	18	68
Jestha	31	27	2	1	-	2	-	23	86
Ashad	24	13	1	-	-	-	1	12	51
Total	333	374	5	76	10	86	31	433	1346

Hematological examination

Hematological unit of the laboratory mainly engaged in determining the normal and different value of different blood parameters such as total count, differential (blood cell) leukocyte count (DLC) erythrocyte sedimentation rate (ESR), determination of hemoglobin by sahli's hemoglobin meters packed cell volume and staining of blood smear for blood protozoa and bacteria. Altogether 213 samples were examined for blood protozoan and other hematological parameters. Out of which 180 samples were from our regular investigation program of diseases of respiratory system in goat. So, the blood protozoan diseases.

Pcv	Hb	N	E	L	M	B
30-40 gm%	9-11 gm%	40-65	3-8	50-70	2-7	0-2

Serological examinations

Apart from the serological samples collected for PPR, mycoplasma and other test for ovine abortion altogether 252 sample were collected during the fiscal year 2060/061 PPR confirmed by CVL **Pyuthan, Dang, Salyan, Rolpa** Test for mycoplasma and brucellosis and other abortion causes can not be confirmed by the events. Though the sample of respiratory problems dispatch to check apart from the serology.

IP	Ca	Phosphorous	Magnesium	Copper	Total protein

Pathological examinations

Pathological examinations mostly concerned with the post mortem of poultry, which were brought by farmers directly. Apart from poultry 8 cases of goat were postmortem for diagnosis of respiratory diseases. In two cases only pasturella hemolytica found from lungs cultured in the pathology unit, the case of death of chickens were observed either by post-mortem examination and also by investigation of sample collected for culture and sensitivity test. Some sample of serum from poultry also tested for rapid slide test for salmonella and other rapid Elisa for IB, IBD and Ranikhet.

Diseases of the poultry were summarized in the following manner. Altogether 140 cases of poultry were examined during the year 060/061.

The disease confirmed during the period 060/061

S.N.	Diseases diagnosed	Number of cases	Percentage
1.	Colibacillosis.	26	18.87
2.	Coccidiosis.	52	37.14
3.	Salmonellosis.	8	5.71
4.	Litchi heart disease.(Infectious anemia)	8	5.71
5.	Haemoragic enteritis.	12	8.57
6.	Ascitis	10	7.14
7.	Deficiency syndrome.	4	2.85
8.	Chronic respiratory disease.	2	1.42
9.	Marek's diseases.	2	1.42
10.	Mycotoxicosis.	1	.71
11.	Avian leucosis.	2	1.42
12.	NAD (Non-diagnosed)	13	9.28

Microbiology unit

In microbiological examinations the isolation and identification of bacteria from the pathological samples either from postmortem or from the sample collected from site of respiratory disease of goat. During the physical year 2060/061altoghter 351 samples were examined.

The total microbiological samples were 351 .out of 351, 110 samples from pathological i.e. from postmortem mostly from poultry 220 samples were from respiratory nasal swab and rest Mountain

The bacterial species isolated from nasal swab and other pathological samples are as follows 220.

Bacterial species		Number Isolated from nasal swab	% of bacteria isolated
Staphyllococcus epidermis		3	1.36
Staphyllococcus ureus		12	5.45
Pasturella multocida		4	1.81
Protens sps		4	1.81
Pasturella haemolytica		18	8.18
E.coli		21	9.54
	Positive	-62	28.2
	Negative	158-	71.8

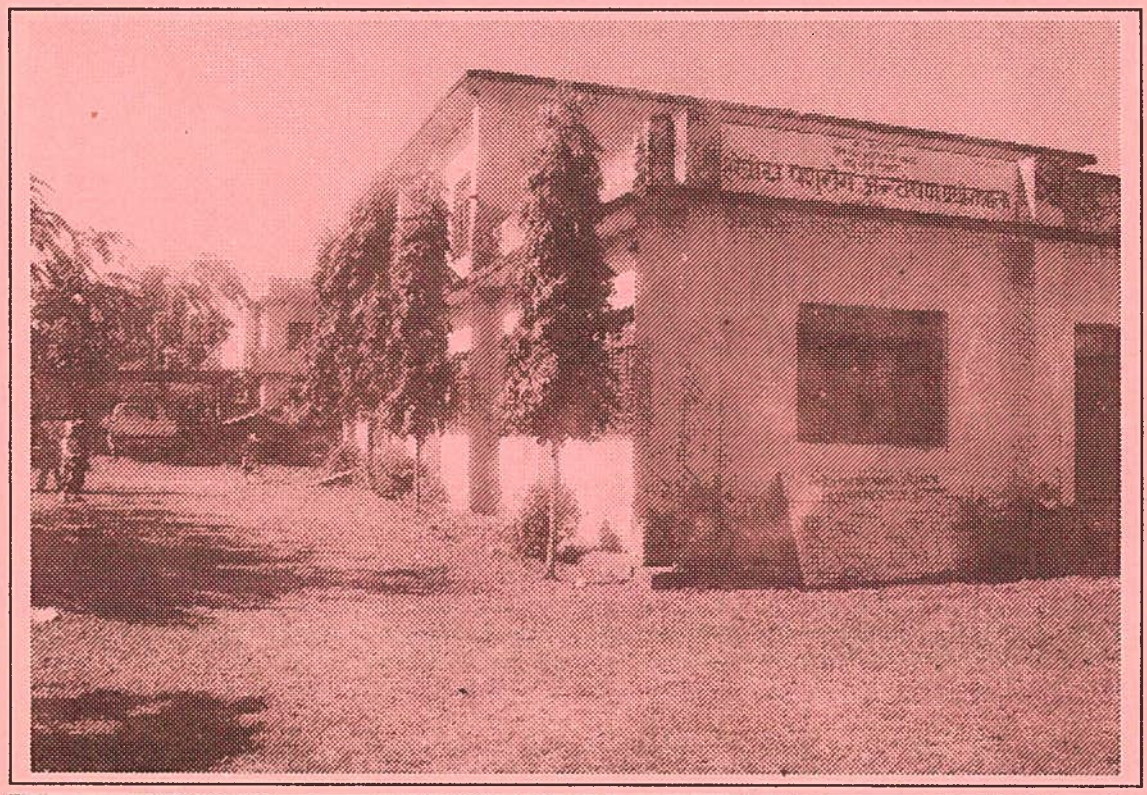
प्रगती प्रतिवेदन (राष्ट्रिय पि.पि.आर. कार्यक्रम सिरोमोनिटरिगं)

कार्यालयको नाम : क्षेत्रीय पशु रोग अन्वेषण प्रयोगशाला सुर्खेत ।

क्र.सं.	सिरोमोनेटोरिगं गर्नु पर्ने जिल्ला	भ्याक्सिनेशन भएको संख्या	सिरम संकलन गर्नु पर्ने लक्ष्य (संख्या)	सिरम संकलन प्रगती	कैफियत
१.	रुकुम	१००००	१००	–	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।
२.	रोल्पा	५००००	५००	–	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।
३.	सल्यान	५००००	५००	५००	–
४.	प्यूठान	६००००	६००	–	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।
५.	दाग	३००००	३००	३००	–
६.	दैलेख	६००००	६००	६००	–
७.	जाजरकोट	१००००	१००	–	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।
८.	सुर्खेत	५००००	५००	५००	–
९.	बाके	४००००	४००	४००	–
१०.	बर्दिया	४००००	४००	४००	–
११.	जुम्ला	१००००	१००	–	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।
१२.	हुम्ला	२००००	२००	–	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।
	जम्मा	४३००००	४३००	२७००	भ्रमण भत्ताको नपुग तथा सिरोमोनेटोरिगं सामान उपलब्ध नभएको कारण ।

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY

DHANGADI



REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY

[Far-western Region, Dhangadi]

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REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, DHANGADI (Far - Western Region)

1. Introduction:

Far Western Development Region is the smallest region among all five regions in Nepal. It shares boundaries with Tibet of China and Uttar Pradesh and Utaranchal of India in the north and southwest sides respectively. To the east is mid western region of Nepal.It comprises two zones viz. Mahakali and Seti and nine districts.

The total area of this region is 19,539-sq.km.and average altitude is around 2500 feet. Maximum and minimum temperature ranges from 2°c to 45°c and the rainfall is around 1800 millimetres. The total population is around 2 millions (projected from B.S. 2048 census).

Agro-ecologically, the region is divided into three main areas viz. Mountains or Himalayan region, Hilly region and Terai region. Bajhang, Darchula and Bajura are three mountainous districts of the region. The upper part of these districts is covered with snow and joins with other high Himalayan ranges. Cattle, Yak, Nak, Chauri, goat, Changra, mules, the people of this part raise horses. Migratory flocks of small ruminants are commonly observed from High Mountain up to Indian states. Ghee, wool, carpet, meat and sweaters are major source of economy through exporting to India. Baitadi, Dadeldhura, Doti and Achham districts are located on the hilly regions. Farmers in these districts commonly keep pig and Buffaloes. People living around the highway are raising crossbred buffaloes and sell their milk in road accessible districts like Dadeldhura and Doti. Achhami cow is popular throughout the kingdom and it requires more attention to conserve such indigenous breed of cattle in Achham.

Terai region of FWR comprises of Kailali and Kanchanpur. Due to better infrastructure and market accessibility, people keep local as well as crossbred livestock for better production. Goat rearing and broiler poultry farming are getting commercialized in these districts. From F/Y 2057/058 on ward, Department of Livestock Services has initiated a new programme of poverty alleviation through poultry farming in nine districts and goat farming in five districts of far western region.

There is heavy economic loss every year due to infectious diseases like Haemmorhagic Septicemia, Black Quarter, Pestis des petits Ruminants of goat, Foot and Mouth disease, Red urine, Rabies in livestock and coccidiosis, Gumboro, Ranikhet and Fowlpox in poultry. Posterior Lumbar Paralysis (Kumri) and Goat and sheep pox in goat, Classical Swine Fever in Pigs are new emerging diseases in FWR. Chaukhari disease in buffaloes and cattle has already become a serious problem in Baitadi and Darchula districts. The etiology of Chaukhuri and Kumri are still unknown but farmers bear a great loss through these diseases. The

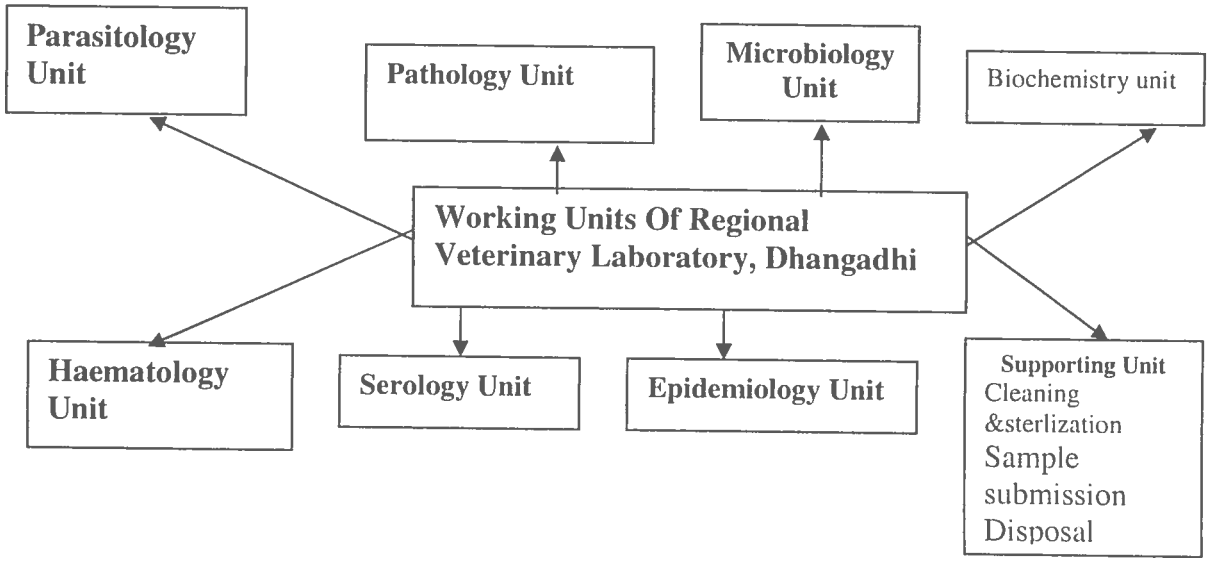
economical loss due to parasitic infestation in this region is very threatening. Rabies and Japanese Encephalitis are the diseases of zoonotic concerns in this region. There were the reports of Leechi Heart Disease and Inclusion body Hepatitis in poultry during last year.

In order to develop the cost-effective disease control programs, proper identification (Diagnosis) and epidemiological pattern of animal diseases for a region with a particular species of animal is needed. Very few diseases have been confirmed through laboratory diagnosis .In turn, majority of diseases, reported to occur in the country are either diagnosed on clinical history reported by the farmers or a combination of clinical examinations and the signs exhibited by the sick animals. Hence, epidemiological patterns of these are not still available. Realizing this fact, His Majesty Government of Nepal established Regional Veterinary Laboratories during fiscal year 2o49/50.

2. Regional Veterinary Laboratory, Dhangadhi: Its Activities

The RVL, Dhangadhi is located in Dhangadhi Municipality, headquarter town of Kailali district. This represents the regional laboratory of far western development region of Nepal.The service of this laboratory covers totally nine districts in Seti and Mahakali zones.

Presently, the routine work of the laboratory is carried out through the following units.



3. Annual Work Program and Summary of Achievement of RVL, Dhangadhi during F/Y-2060/61

3.1 Approved Annual Work Program of fiscal year 2060/61and Summary of Achievement of RVL, Dhangadhi is presented in Table No.4

S. N.	Programs and Activities	Unit	Annual Target		Annual Progress	Progress %	
			Quantity	Weightage			
1.	Laboratory Service programme-						
1.1	Parasitological Examination	No.	2000	7.65	3279	100%	
1.2	Microbiological Examination	No.	300	4.15	323	100%	
1.3	Pathological Examination	No.	400	6.34	480	100%	
1.4	Serological Examination	No.	250	3.50	327	100%	
1.5	Hematological Examination	No.	300	6.23	342	100%	
1.6	Biochemical Examination	No.	350	5.36	458	100%	
1.7	Dispatch of samples (CVL and other Lab	No.	200	2.40	294	100%	
2.	Disease Investigation and Surveillence Programme						
2.1	Investigation and Surveillance of Meat borne Zoonotic Diseases	Time	12	13.44	12	100%	
2.2	Investigation and surveillance of Goat Abortion Disease	Time	12	10.38	12	100%	
2.3	Investigation of Livestock and poultry diseases in coordination with NARC (Kumri and Khari disease)	Time	6	-	-	-	
2.4	Investigation of Epide. Diseases	Time	6	9.95	6	100%	
3.	Inspection and Supervision Programme (poverty Alleviation Goat and Poultry Programme)						
3.1	Inspection and Supervision of District Labs.	Time	6	5.36	6	100%	
4.	Annual workshopAnimal Disease Investigation)	Time	1	2.62	1	100%	
5.	Training Programme:						
5.1	District Level sample collection and dispatch training	Time	1	0.22	1	100%	
5.2	Computer training	Person	1	0.66	1	100%	
6.	Publication Programme:						
6.1	Quarterly Epidemiological bulletin publication	Time	4	2.62	4	100%	
6.2	Annual Technical Book Publication	Time	1	1.67	1	100%	
7.	Improvement in Lab. Infrastructure	Percentage	100	1..64	100	100%	
8.	Purchase Programme						
8.1	Purchase of machinery equipments (Computer printer)	No.	1	4.37	0	0%	Budget not released
8.2	Purchase of photocopy machine	No.	1	10.93	0	0%	Budget not released
8.3	Purchase of scientific Books and Journals	Time	1	1.09	100	100%	
8.4	Purchase of Furniture	Time	1	0.22	100	100%	

3.2. Food Nutrition safety Programme (Poverty alleviation: semicommercial Goat Rearing and Self employment generating poultry farming Programme)

S.N.	Description of activities	Unit	Annual Target		Annual Progress	Percentage	Remarks
			Quantity	Weightage			
1.	Seromonitoring and Reporting	Time	3	17.91	3	100%	
2.	Serosurveillance and Reporting (300goats)	Time	3	17.91	3	100%	
3.	Epidemic disease investigation and control	Time	3	35.82	3	100%	
4.	Faecal Examination and Diagnosis (600)	Time	3	4.48	3	100%	
5.	Participation in workshop, interaction and training programme	Time	7	10.45	7	100%	
6.	Drug trials and recommendation (50 goats)	Time	3	11.19	3	100%	
7.	Dispatch 0f monthly, Quarterly and annual progress report	Time	16	2.24	16	100%	

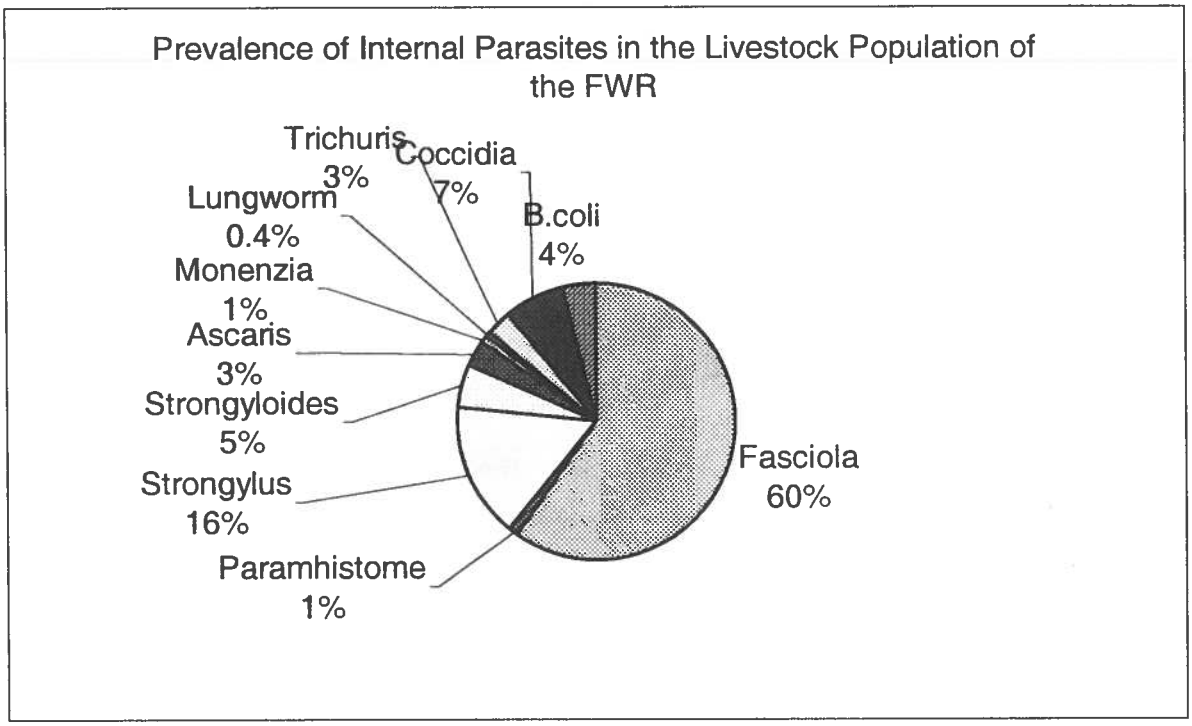
3.3 PPR seromonitoring Programme:

District	No. of Vaccinated Goat and sheep		No. of serum samples		Remarks
	Target	Progress	Target	Progress	
Dadeldhura	50, 000	50,000	500	144	Unavailability of equipments and difficult in access.
Kanchanpur	30,000	30,000	300	205	
Kailali	70,000	70,000	700	104	
Bajura	30,000	30,000	300	301	
Bajhang	40,000	40,000	400	335	
Achham	40,000	40,000	400	270	
Baitadi	40,000	40,000	400	367	
Doti	70,000	68,788	700	244	

Percentage of Progress during f/y 2060/61
Animal Health Services Programme: 90.47%
Weightage of progress: 84.70%
Food Nutrition safetyProgramme: 100%
Weightage of Progress: 100%

4. Laboratory Services: -

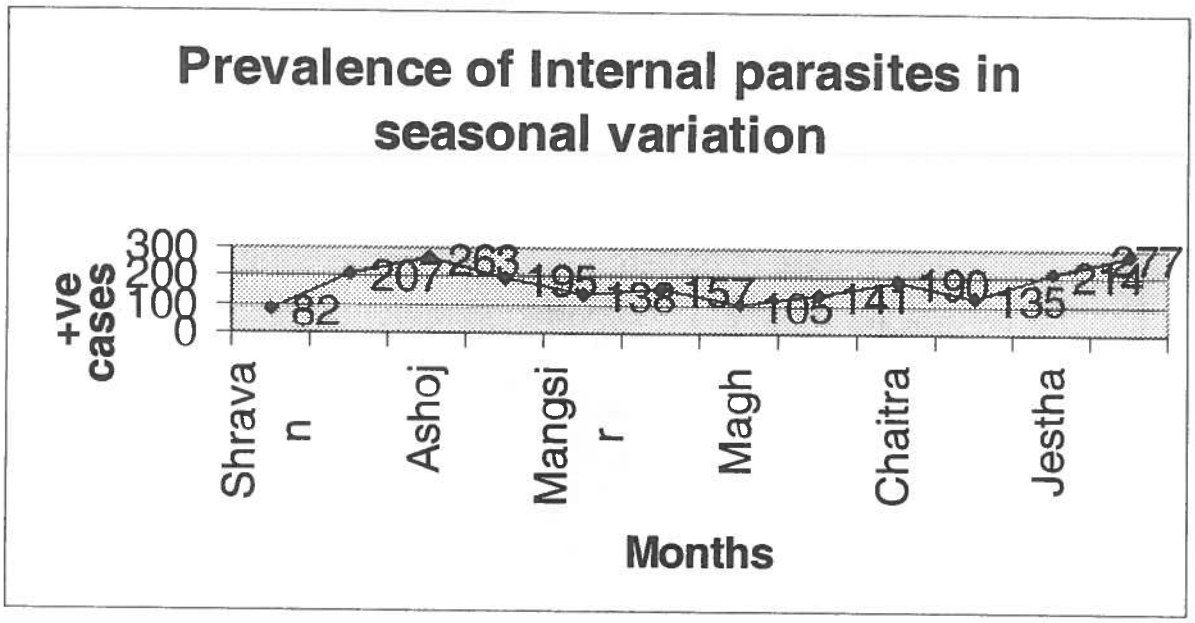
4.1- Parasitological Examinations- Graph-1



Totally 3279 samples were examined during F/Y 2060/61. Among the internal parasites, the highest prevalence was of LF (60%) ollowed by Strongyles (16%) and Cocidiosis (7%) The percentage of strongyloides and B.coli were 5% and 4% respectively. Though examined in less no. Samples, occurance of lungworm infestation in small ruminants particularly goats was also found to be significant. Out of 12 faecal samples tested using Baerman method 8 were found to be positive for lungworm. The percentage of coccidiosis represented in the pie diagram is mostly observed in case of poultry. Sporadic cases of coccidiosis was nevertheless was found to occur in kids.

It has been shown that parasitic infestation is one of the biggest constraint in livestock production and productivity in this region. Though most of the fecal samples were from Kailali district but there were samples, brought from Kanchanpur, Dadeldhura, Doti, Bajura and Baitadi. Occurance of parasitic diseases on seasonal variation has been shown in graph 2, it clearly indicates that parasitic infestation in livestock has occurred through out the year but especially incidence is high during Ashoj onward and again the incidence rises from Chaitra onward and remains high until the monsoon remains. This result also confirms the necessity of anthelmintic drenching program before the onset of monsoon and during Ashoj and Kartik Since, RVL has received large number of faecal samples from all the districts of special poverty alleviation goat farming programme during these months, rationally, the incidence is high but at the same time, it is quite evident that the drenching of anthelmintics to small ruminants before onset and/or during the monsoon is very important, it certainly protects from the loss due to parasitic infestations.

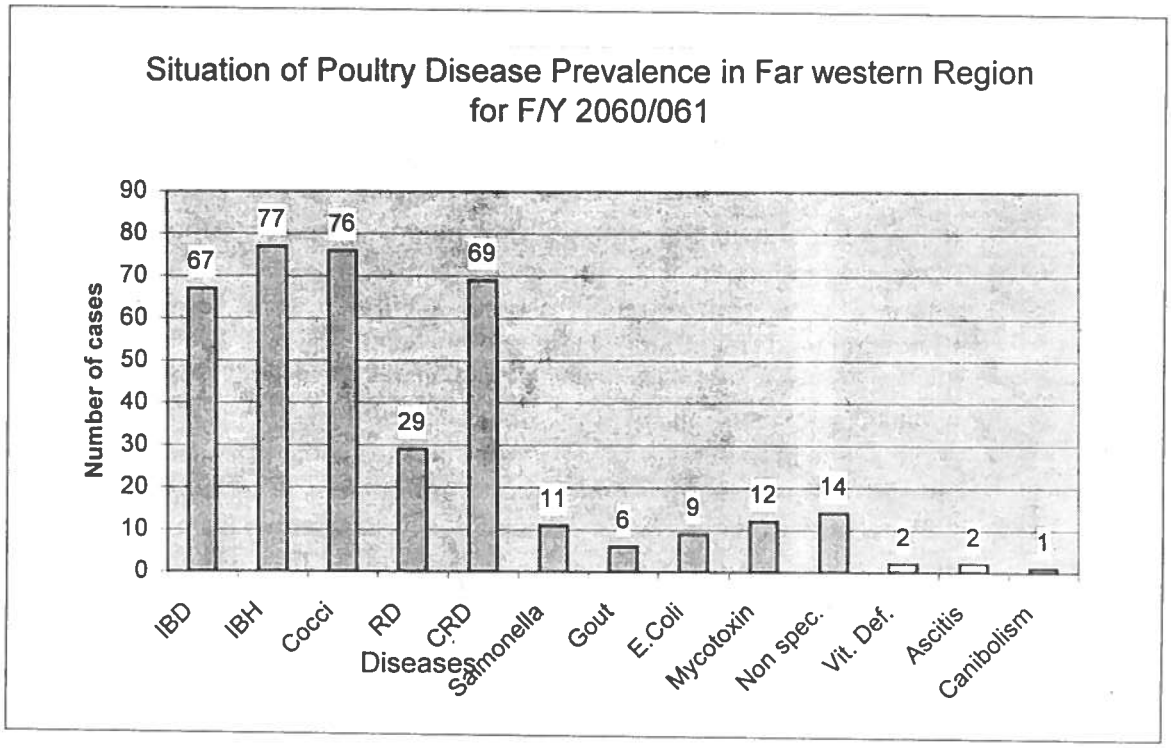
Graph:2



4.2 Pathological Examinations:

Pathological examinations mostly constituted autopsy of dead poultry and few dead goats submitted to the laboratory. These were autopsied and diagnosed on the post mortem lesion with minimum aid of laboratory examinations. Details of the post mortem examination report have been presented through graph below:

4.2.1 Report of Post mortem Examination Of Poultry in RVL:



Out of 375 chickens autopsied, 20.5% .20.26%, 18.4% and 17.8% were diagnosed to be Inclusion body hepatitis, coccidiosis, Chronic Respiratory disease, Gumboro and respectively. Ranikhet contributed 7.7% in total autopsy percentage. Other noticeable diseases were Mycotoxin, Salmonellosis and Colibascillosis.

The incidence of Hydro pericardium and Leechi Heart Disease in Broiler birds is increasing day by day. The cases of Inclusion body hepatitis are threatening problem of broiler industry now adays. There were some cases of vaccine failure in poultry; hence it needs extensive investigation on quality and breakage in maintaining the cold chain of vaccine. Since Kailali and Kanchanpur are forthcoming competent districts of poultry farming, Regional Laboratory, Dhangadhi needs fully equipped diagnostic kits and sufficient biological reagents to diagnose the poultry diseases.

Apart from the dead bodies of goats, kilograms of meats were submitted to the laboratory under legal trials at District Chief Office. Though this laboratory delivered the results regarding the fitness of consumption through physical, chemical and Microbiological examinations, however extensive training is required to the laboratory technicians and veterinarian to bring out best result of laboratory examinations for meat, egg and other livestock products as Animal Health and Animal Services Act has already been implemented and Animal Slaughter and Meat Inspection Act is being ready to implement through out the country. It is very appreciable that all the quarantine check posts in this region have started to submit the sample of live animals and birds and also livestock and poultry products to investigate the quality of the imported commodities.

4.2.2: Result of Post mortem of Livestock:

S.N.	Species	Date	From	Suspected organs	Tentative Diagnosis
1.	Goat		Malakheti, Kailali	Lungs, Liver and Intestines	Necrotic hepatitis Acute Fascioliasis
2.	Swine		Krishnapur	Large intestine, Liver, Rumen, Intestines and other visceral organs	Swine Fever
3.	Goat		Barkunda	Lungs, Urinary Bladder, Kidneys, Intestine and Liver	Poisoning, Bronchitis, Pasteurolosis
4.	Goat		Dhangadhi	Liver, G.I. tract, Urinary Bladder, Respiratory tract	Bacterial septicemia
5.	Goat		Mahphanta, Kanchanpur	Liver, G.I. tract, Urinary Bladder, Respiratory tract	Bacterial septicemia
6.	Kids		Budhitola Goat Farm, Kailali	G.I tract, Liver, lungs, Kidneys	Enterotoxaemia
7.	Kids		Budhitola Goat Farm, Kailali	G.I tract, Liver, lungs, Kidneys	Enterotoxaemia
8.	Goats		Matiyari, Kailali	Liver, G.I.Tract, musculature, Lungs	Paramphistomiasis Jaundice Babesiosis

4.3- Serological Examinations:

Since most of the biological reagents for sero diagnoses of animal and poultry diseases are not available in the country, only Brucellosis, Salmonellosis and Mycoplasma have been diagnosed through serological examinations. Some of the serum samples were dispatched to Central Veterinary Diagnostic laboratory, Tripureswor for further investigation of some diseases. RVL Dhangadhi started to do the seromonitoring of poultry under the Poverty Alleviation Programme, this helps to know the antibody titre level of the body and also the efficacy of Poultry vaccination can be tested.

Most of the tests have been performed in buffalo, Goat, Cattle and poultry. The details of the serological examinations done in the laboratory are given in

4.3.1 –Results of Serological examination, performed in RVL, Dhangadhi

Total no. of serum samples examined	No. of serum samples examined by RBPT		No. of serum samples examined by Salmonella pullorum antigen		No. of serum samples examined by Mycoplasma Gallisepticum Antigen	
327	Negative	Positive	Negative	Positive	Negative	Positive
	61	0	107	4	92	72

4.3.2 Result of Seromonitoring in poultry by Immunocomb Method:

S. N.	Total No. Samples	Breeds	Immuno Score of IBD	Immunocomb score of ND	Immunocomb score of IB	Status
1.	10	Layers	0.3	1.9	1.7	IB, ND and IBD are not in protective level
2.	10	Broilers	1.75	1.6	0.85	IB, ND and IBD are not in protective level
3.	12	Layers	1.0	1.45	0.6	IB, ND and IBD are not in protective level

4.3.3 Results of serum samples, dispatched to CVL and other laboratories.

Name of the Laboratory	Samples Received from	No. of serum samples dispatched		Date of Examination	Method of Examination	Result	
		Species	No.			Positive	Negative
CVL, Kathmandu	AQCp Dhangadhi	Goats	12	2060-4-5	PPR ELISA test	0	12
	Chaumala, Kailali	Goats	3	2060-4-5	PPR ELISA test	2	1
CVL Kathmandu	Amarbasti Kanchanpur	Goats	23	2060-7-7	PPR ELISA test	15	8
	Chaumala, Kailali	Goats	2	2060-7-7	PPR ELISA test	1	1
	AQCP Gaddachauki	Goats	13	2060-7-7	PPR ELISA test	2	11
CVL, kathmandu	Kanchanpur and Dadeldhura	Goats	46	2061-1-10	PPR ELISA test	34	12.
CVL, kathmandu	AQCPs, Dhangadhi Darchula	Goats	27	2061-2-7	PPR ELISA test	0	27
National FMD control Section	Kanchanpur	Bovine	8	04-06-2004	FMD serotyping	'O' strain-2	6 samples Negative

Apart from the above mentioned serum samples, many other serum samples, collected from Chaukhuri affected buffaloes in Baitadi and Darchula districts as well as from some other disease suspected animals were submitted to CVL serum Bank.The suspected meat and visceral samples collected during surveillance programme of Meat borne Zoonoses were submitted to CVL to perform the further Histopathological examinations, Many samples of serum from epidemic goat abortion and from investigation program e were submitted to CVL for serological and Biochemical examination, especially for estimation of micronutrients.

Special Programme of PPR control, conducted under Central program of Animal Health Directorate was done in four districts of far western region and 2848 serum samples were collected by RVL, Dhangadhi and submitted to CVL, Tripureswor to find the efficacy of the vaccine and for seromonitoring. The Result has yet to receive.

4.4 Hematological Examinations:

Under Hematological examinations, blood, blood smear and whole blood collected for the examination of protozoan blood parasites, total blood cell counts (RBCs and WBCs), Packed cell volume (PCV), Hemoglobin (Hb) and Differential counts (DC).

Details of the hematological examinations have been presented in Table No.10

4.4.1 Hematological Examinations done in RVL, Dhangadhi during 2060/061

Total No. of blood samples	No. of samples for Total Red Blood Cell counts	No. of samples for Total White Blood Cell counts	No. of Samples for PCV%	No. of samples for Hb	No. of samples for Differential Counts	No. of samples of Blood Smear for Protozoan Parasites
342	4	4	86	85	133	308

4.4.2Diagnosis made through haematological Examination during 2060/2061:

RBCs		WBCs		Hb		PCV		DLC			Blood Protozoans				
No.of samples	Defective	No. of samples	Defective	No. of samples	defective	No. of samples	defective	Lymphocytosis	Neutrophilic	Eosinophilic	Babesia	Theleria	Anaplasma		
4	4	4	2	85	29	86	15	27	40	45	7	5	6		

* Haematological examinations were performed in the clinically sick animals having pyrexia, Posterior lumbar paralysis, Discolouration in urine and unidentified respiratory complications.

4.5 Biochemical Examinations:

Biochemical Examinations were performed with very little diagnostic facility in this laboratory. This test constituted the examinations of urine, milk and Serum samples. Totally 41 urine examinations were performed. Using multistix, which indicated the results of contents of urobilinogen, protein, PH, blood, specific gravity, ketone, bilirubin Glucose urine tests were done. Upon suspect cases, microscopic tests were performed to find the presence of Blood cells, pus cell and other cells present abnormally in urine. Totally milk samples were examined by using Calfornia mastitis test reagent, Sodium hydroxide and other litmus paper and mastrip paper. Total no. of samples examined were 291 A laboratory manual is urgently required for elaborated details of examination under biochemical tests performed in the laboratory .As well; all the necessary reagents should be available with proper guidelines of preparation.

4.5.1 Biochemical Examinations:

Total No. of Samples	Urine	Milk	MetabolicProfiles (Biochemistry)
458	41	291	92

4.5.2 Urine Examinations: By using multistix and microscopic examinations of suspected urine sample, following defects were evident in some of the cases:

Result of Urine Examination:

Normal Urine	Abnormal urobilinogen	Abnormal Protein content	Abnormal Level of pH	Blood contents of urine	Abnormal Specific gravity	Content of ketone bodies	High Level of Bilirubin	High level of glucose	Microscopic defects
30	Nil	4	3	1	0	2	2	0	Pus cell-2

4.5.2 Milk Examinations:

Milk samples are brought to this laboratory either by farmer from the clinically evident case of mastitis or laboratory staff as well as JT/JTA submits the milk sample from sub clinical cases of cattle and buffalo. Usually commercial dairy farmers approach to do the sub clinical screening of mastitis in their herd. Positive cases of mastitis are always submitted to proceed for bacteriology and antibiotic sensitivity out of 291 milk samples examined in this laboratory, following are the result:

4.5.2.1 Result of Mastitis test:

No. of Negative sample	**No. of Positive sample of milk**				
	Milk from Fore Right Teat	**Milk from Fore left teat**	**Milk from Hind Right teat**	**Milk from Hind left teat**	**Total Positive sample**
198	17	12	34	30	93

4.5.2.2 Result of Subclinical Mastitis:

S.N.	Districts	Total No. of sample screening for Subclinincal Mastitis	No. Of subclinical Mastitis	no. of clinical Mastitis	Result of Antibiotic sensityvity
1	Kailali	179	27 (15.08%)	35	Nitrofurtoin=0% Gentamycin=75% Norfloxacin=50% Enrofloxacin=83.33% Ampicillin=22.2% Cotrimexazole=0% Ofloxacin=83.33% Tobramycin=40% Chloramphenicol=50% Amoxycillin=33.3% Cloxacillin=12.5%
2.	Dadeldhura	25	4(16%)	-	
3.	kanchanpur	34	5(14.7%)	22	

Remarks: The average percentage of subclinaical matitis is 15.26%, in which Dadeldhura is having the highest percentage that may be due to less no. Of sample size. Regarding the antibiotics sensityvity, Enrofloxacin & ofloxacin (each 83.33%) being the most sensitive antibiotics for mastitis cases. while Gentamycin (75%) is equally effective in many cases. Though Gentamycin is becoming resistant in many urban cases but effective in rurual areas where Antibiotics is not discriminately used to develop the resistant. There were the cases of fungal Mastitis, obviously, antibiotics is not of useful in such condition hence use of antiseptics and antifungal reagent is much useful

4.5.2.3 Leucocytes Count in Subclinical and clinical Mastitis Milk Under programme of NARC:

A technology has been introduced in far western region for the first time. This program of prevention of mastitis through teat dipping technology was initiated with the coordination of NARC, DLSO and RVL. This programme was conducted in the dairy farmers of Dhangadhi and Geta, in this progrramme Povidine and Glycerin (9:1) were mixed and distributed to the farmers. The farmer was advised to use the medicie as a teat dipping everyday after milking of the animal through out the milking period. The milk of all the animals were screened for subclinical mastitis by CMT reagent and the testing of the milk were repeated every month to find the reduction in leucocytes count. The increase in production was also recorded. Under this programme, 107 milk were tested and 36 samples were found to positive for subclinical mastitis. This programeme had a good impact among dairy farmers.

4.5.3 Results of Serum Biochemical Values:

S.N.	Biochemical Estimation for	Animal Species	Disease History	Standard Average	No.of Normal Animals	No. of Defective Animals	Total No. of Samples
1.	Calcium	Goat	Abortion	10.70mg/dl	11	11	22
2.	Phosphorus	Goat	Abortion	5.21 mg/dl	20	0	20
3.	Phosphorus	Bovine	Khari	5.56mg/dl	3	1	4
4.	Bilirubin	Goat	Abortion	1.0mg/dl	1	9	10
5.	Bilirubin	Bovine	Khari	1.0mg/dl	0	4	4
6.	Bilirubin	Poultry	Inclusion Body Hepatitis	1.0mg/dl	0	11	11

Clinical Significance: Low blood calcium level in livestock is clinically called milk fever, which is quite common in Nepalese context. The practice of feeding of the livestock only during production days usually causes the hypocalcaemia and will have loss in production during next milking cycle. The estimation of blood calcium level help to diagnose such condition. In addition to this, Total Protein, Albumin, Globulin,, Inorganic Phosphorus and Magnesium level in serum are also estimated by using spectrometer as per request made by farmers and on the basis of kits available in the laboratory. Since many commercial kits are available in the market, some of them are available in this laboratory, one of them was bilirubin kit, and the estimation of the same has shown the indication of jaundice

and liver dysfunction in many animals. The goat abortion due to insecticide poisoning, the disease of inclusion body hepatitis in poultry and khari disease as well show the case of high bilirubin level. This might be relevent to make the diagnosis.

4.6 . Microbiological Examinations

Totally 243 samples were cultured into the microbial growth media under microbiological examination in this laboratory. The media used were Mackonkey agar as well as Nutrient agar. Bacteria and Fungi were identified through nature of colonies and by gram staining. Due to the limitation of laboratory facility, biochemical tests couldn't be performed. However the microbes were identified as rod /cocci, gram negative / gram positive.

But on requirement, there were few plates, sent to CVL for biochemical examinations to identify the species of the bacteria. The details of the microbiological examination are described in table no.15:

4.6.1 Microbiological Examinations performed in RVL, Dhangadhi

Sample	No	Media	Identified organisms	Antibiotic sensityvity
Milk	93	Nutrient Agar	Staph., Strepto., E.coli., Bacillus Pseudomonas	Enrofloxacin Ofloxacin Gentamicin Cloxacillin Amoxycillin
Poultry (Swabs of Intestine, yolk, Joint, Lungs, Liver, Heart Blood	88	N. Agar Mcconkey Blood	Hemophilus Staph., E.coli, Strepto.	
Buff/ Cattle	18	Vaginal swab (Blood, Mcconkey, Nutrient)	Compylobacter	
		Skin swab in Saboraud	Microsporum	
		Khari (N. Agar, Saboraud)	E.coli, Staph., Cryptosporosis Blastomyces	
Goat (Liver, Vaginal, lungs, Bonemarrow, Teat, nasal, Joint swab)	42	Blood Maconkey N.Agar	Strepto., Staph.,E.coli. Corynebact., Campylo, Clostridium	
Swine (Swabs of intestines Lung, kidney, liver	6	Blood agar	Clostridium	
Dog/Cat/ Fish (Conjunctival swab Liver)	4	Blood agar	Clostridium E.coli Staph.	

DISEASE INVESTIGATION RELATED TECHNICAL ARTICLES

DISEASE INVESTIGATION

RELATED TECHNICAL

ARTICLES

Japanese Encephalitis Investigation Program at Central Veterinary Laboratory in Nepal

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Introduction

Japanese Encephalitis is an emerging disease of animals and humans. Most domestic animals are vulnerable to virus, including horse, cattle, sheep, goats and pigs. Other animals such as rabbit, rats, pigeons, dogs, ducks, chicken, wild birds and reptiles are also susceptible. Herons and egrets are able to maintain positive sera year-round. Snakes and bats are possible reservoirs. Pig is considered the most important natural amplifying animals for Japanese Encephalitis virus. Man, horses, donkey and monkey develop lesions in central nervous system. Pregnant women and pigs get abortion. Viremia does not develop in bovines, caprines and ovine and they are not amplifier or reservoir host. Pigeons and sparrow develop viremia. Pigs and ducks are main reservoir.

JE is caused by *Arbovirus,* which is a single-stranded ribonucleic acid virus. It is sensitive to ether, chloroform, sodium deoxycholate, and lipolytic or proteolytic enzymes. It is readily inactivated at 56 ^{0}C after 30 minute and has an optimal stability of pH 8.5. It is serologically similar to other viruses such as Murray Valley Encephalitis virus, West Nile virus and Kunjin virus.

The geographical distribution of this disease has been reported in Japan, Korea, Siberia, China, Philippines, Indonesia, Taiwan, Cambodia, Thailand, Vietnam, Laos, Myanmar, India, Bangladesh, Shri lanka, Nepal, Pakistan, Australia and Papua New Guinea in past.

A correlation between infection in pigs and infection in human is apparent with evidence indicating that swine play an important role in the building of the virus in the population. Mosquito are main vector for JE virus. They are bred in irrigated rice field, which brings the natural system into close contact with man and domestic animals. This is very favorable environment for JE infection. Consistence development of viremia in susceptible pigs ensures a continued supply of infected mosquitoes. Pigeons and sparrows can develop viremia and infect mosquitoes. Disease is transmitted by the bite of infected mosquitoes. There is ventral transmission from boar to sow or gilt and vice versa. Clinical symptoms manifested by JE infection in pigs are still borne, mummified foetuses, weak piglets. Boar develops oedematous, congested testicles, and hardening of epididymis as wells reduced libido (Barbara *et al*, 1999). Virus isolation, Competition Enzyme-linked immunosorbent assay (CELISA), Fluorescent antibody test (FAT), Plaque Reduction Test, and PCR techniques are used for the diagnose of JE in pigs and other animals (World Organization of Animal Health, 1996). There is no effective or specific treatment for JE, which has a case fatality of 20-40% and produce s residual neurological or psychiatric sequelae in 25-40% of survivor. It could be controlled by effective immunization program.

Status of Japanese encephalitis in Nepal

First epidemic of JE was introduced in Nepal in 1978. Since 1987 more than17, 000 cases and 4000 deaths due to acute encephalitis have been reported to Nepal health authorities. Epidemic occurs with the onset of monsoon rain in July and usually ends in October. Occurrence of JE has been reported 62% in the age group of 12 months to 15 years old children (Bista *et al*; 2001). Annually 2000-3000 cases 200 to 400 death occurs due to JE. Now it is prevalent in 24 districts of terai and inner tarai of country so 11.5 million people living in these districts are at risk. Three viral strains namely B-2524, B9548 and Nep-1/90 have been isolated from Nepal. Laboratory diagnosis facility for JE by using IgM capture ELISA is available for human beings (Epidemiology and Disease Control Division, 2001). The incidence of this disease in Kathmandu valley has been occurred which killed 7 persons recently in the third week of September 2003.

In Nepal existing population of pigs is 934462 and distribution of pig is about 0.22 per household (Central Epidemiological Unit, 2002). Population of pig in 24 endemic districts is 358805. Department of Livestock Services had vaccinated 45213 pigs with Live attenuated virus vaccine (SPF chicken embryo fibroblast)) in tarai zone in 2001 however sero-monitoring of vaccinated pigs could not be done due to lack of diagnostic facility for JE at Central Veterinary laboratory (CVL) in Kathmandu. (Summery of JE vaccination is given in Annex No.1). CVL under the Department of Livestock services, His Majesty's Government of Nepal has lunched a JE investigation program in the fiscal year 2002/2003.

Objective of project

The main objective of this investigation is to study the sero- status of JE virus infection in pigs and other susceptible animals in Nepal. This study also aims to provide scientific information useful to develop JE disease control strategy on the basis of epidemiological study and laboratory test result.

Activities performed

Japanese encephalitis investigation program was planned for three fiscal year and approved by His Majesty's Government of Nepal. In first fiscal year 2059/60 an annual budget of Rupees 250, 000 was allocated to start and lunch investigation activities. Co-ordination was made with heath authority and other related institutions before to implement work in the field. Meanwhile, Australian Animal Heath Laboratory, Australia was requested to support the diagnostic capability and facility of CVL and to do a collaborative research work on Japanese encephalin in pigs in Nepal. Field survey was done and a total number of 270 sera were collected randomly in fiscal year 2059/60 from pigs, ducks and horses by visiting various pig forms of 16 districts. These sera were labeled and stored at –20 ^{0}C until test. These sera were inactivated for 30 minutes at 56 ^{0}C before shipment to Australia. Then these sera were refilled into serum vials supplied by AAHL. Vials were disinfected by wiping with 0.01M Citric acid, packed well and sent to AAHL for test. Out of 270 sera, in fiscal year 2059/60, 70 were tested in Kathmandu after the establishment of JE diagnostic capability at CVL. All sera were tested for the detection of antibodies against JE virus by following the protocol of competition enzyme linked immunosorbent assay (C-ELISA)

developed by AAHL (2002). All necessary equipment and regents were made ready before to start the test.

Detection of antibodies

JE antigen was diluted at the ratio of 1:200 with coating buffer (ph 9.0). Plates were coated with 50 µl-diluted antigens per well, covered and incubated for 1 hour at 37 ^{0}C. Test sera were diluted at 1:10 dilution in ELISA diluent with ph 7.2 (1% skim in PBST). Control sera were diluted at 1:100, 1:800, 1:1000 and 1:2000 dilutions in ELISA diluent. Diluted test sera were kept in duplicate wells and then control sera were located in the last columns of the plate. Antigen coated plates were washed thoroughly with wash buffer (ph 7.2). 50 µl of serum dilution were added in duplicate to the plate wells according C-ELISA record sheet. Plates were covered and incubated for 1hour at 37 ^{0}C on the plate shaker. While serum incubation was in progress, monoclonal antibody was diluted in 1: 30000 dilutions in ELISA diluent. After the serum incubated ELISA plates were not washed. 50 µl of prepared monoclonal antibody was added in all well of the plate, except H (11&12). Plates were covered and again incubated at 37 ^{0}C for 30 minutes on the plate shaker. During incubation period, HRPO anti-mouse conjugate was prepared in 1:3000 dilutions in ELISA diluent. ELISA plates were washed at the end of the serum-Mab incubation and 50 µl of conjugated was added per well. Plates were again re-incubated for 30 minute at 37 ^{0}C. Plates were washed after incubation and 50 µl of TMB substrate prepared with hydrogen peroxide was added to each well. Reaction was stopped by adding 50 µl of 1M sulphuric acid to all wells after 10 minutes. Plates were read at 450 nm in ELISA reader to get OD values. Percentage of inhibition of each serum was received automatically by running ELISA program or calculated by using formula.

Result and discussion

The numbers of positive and negative sera were distinguished on the basis of percentage of inhibition on the development of color in each well. Sera resulting in level of less than 40% inhibition were considered negative where as sera resulting in level of greater than 40% inhibition were considered positive. Antibodies against JE virus were detected in 149 sera out of 270. (JE, C-ELISA results are summarized in Table No. 1&2.) In this way 55.18% of tested sera were found positive. Percentage of positive result in pigs, horses and ducks was revealed 57.25, 50 and 9.09 accordingly.

Percentage of positive samples according species of animals (Table No.1)

Species of animal	Total sera	Positive sera	Positive %
Pig	255	146	57.25
Horse	4	2	50
Duck	11	1	9.09

Antibodies against JE infection have been detected in Nepal in pigs in duck in the past (Joshi and Gaidamovich, 1981-1982) by performing heamagglutination test. However this is the first report for detecting antibodies against JE virus in horses

in Nepal. Only four horse sera collected from Parsa districts were tested. A total number of 11 duck sera was collected from Bara, Parsa and Banke districts but only one duck serum from Banke district was found positive. The evidence of this laboratory finding strongly indicates the persistence of JE virus infection in Nepalese pigs, ducks and horses. All these tested sera were collected from 16 districts of tarai regions, where high incidence rate of disease has been recorded and samples were collected only from unvaccinated pigs against JE disease. The highest incidence rate of JE in human being was recorded in Far western as well as Mid-western region and lowest in Central regions in the past (Epidemiology and Disease Control division, 2001). This study has also shown the highest percentage of sero–positive cases in Dang, Kanchanpur and Kailali districts of these regions (Annex No.2). Therefore result of this study is co-related with the incidence of human infection and epidemiology of JE.

Summary of JE, C-ELISA result according regions (Table No. 2)

Region	District	Positive/Tested	% of positive	Mean
Far-western				**93.18**
	Kailali	19/22	86.36	
	Kanchanpur	6/6	100	
Mid-western				**72.98**
	Banke	7/7	41.17	
	Bardiya	7/9	77.77	
	Dang	5/5	100	
Western				**33.48**
	Kapilbastu	4/15	26.66	
	Nabalparasi	8/14	57.14	
	Rupendihi	2/12	16.66	
Central				**28.71**
	Makwanpur	15/43	34.88	
	Bara	5/16	31.25	
	Parsa	2/10	20	
Eastern				**68.35**
	Jhapa	4/10	40	
	Morang	13/13	100	
	Sunsari	26/38	68.42	
	Saptari	14/24	58.33	
	Siraha	12/16	75.00	
Total		**149/270**		**55.18**

C-ELISA for the detection of specific antibodies (IgG) against JE virus infection in pigs and other susceptible animals has been first established at CVL in Nepal. It is considered very sensitive and specific diagnostic method which could be used for sero-surveillance and sero-monitoring of JE in all animals including human. Diagnosis of animal infection by detection of JE virus is little difficult due to short period of viraemia usually 2 to 4 days (Daniels Peter, 2001) and most JE infection of pig is asymptotic. In this situation C-ELISA is considered very

much suitable test to detect specific antibodies produced in response to JE infection. Some difficulties were found in the beginning in the establishment of this test at CVL. However these limitations were corrected after consultation with AAHL and test has been standardized. Reproducibility of this test was also confirmed by re-testing 10 sera, which were already tested at AAHL.

Outcome of project

C-ELISA techniques were established at CVL for the diagnosis of JE disease in pigs and human beings in Nepal. The diagnostic capability CVL was also increased. The result of this study was disseminated and also provided to health authority for information to plan effective control strategy against JE infection in human beings. This test will be useful to monitor the level of protective antibody (IgG) produced in vaccinated human beings. International research link was established between CVL and AAHL for further collaborative research work to met the research need of the veterinary authority in Nepal.

Recommendation

It is essential to continue JE investigation program lunched by CVL, under the Department of Livestock Services to study different epidemiological aspect of this disease in depth for developing control strategy and to adopt control measures. In addition to C-ELISA, it is essential to establish JE virus isolation, virus neutralization test at CVL to differentiate JE virus from other related *Flavi viruses* that could be present in Nepal. Production of JE vaccination in Nepal for mass immunization of human beings would be the best option in future to control of this disease. Further collaborative work with national and international institution is needed to control JE.

Acknowledgement

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Department of Livestock Services had distributed 259377 doses of JE vaccine in different districts of Nepal on dated 2057/11/11 (B.S.) through Regional JE vaccine coordination committee formed in each region.

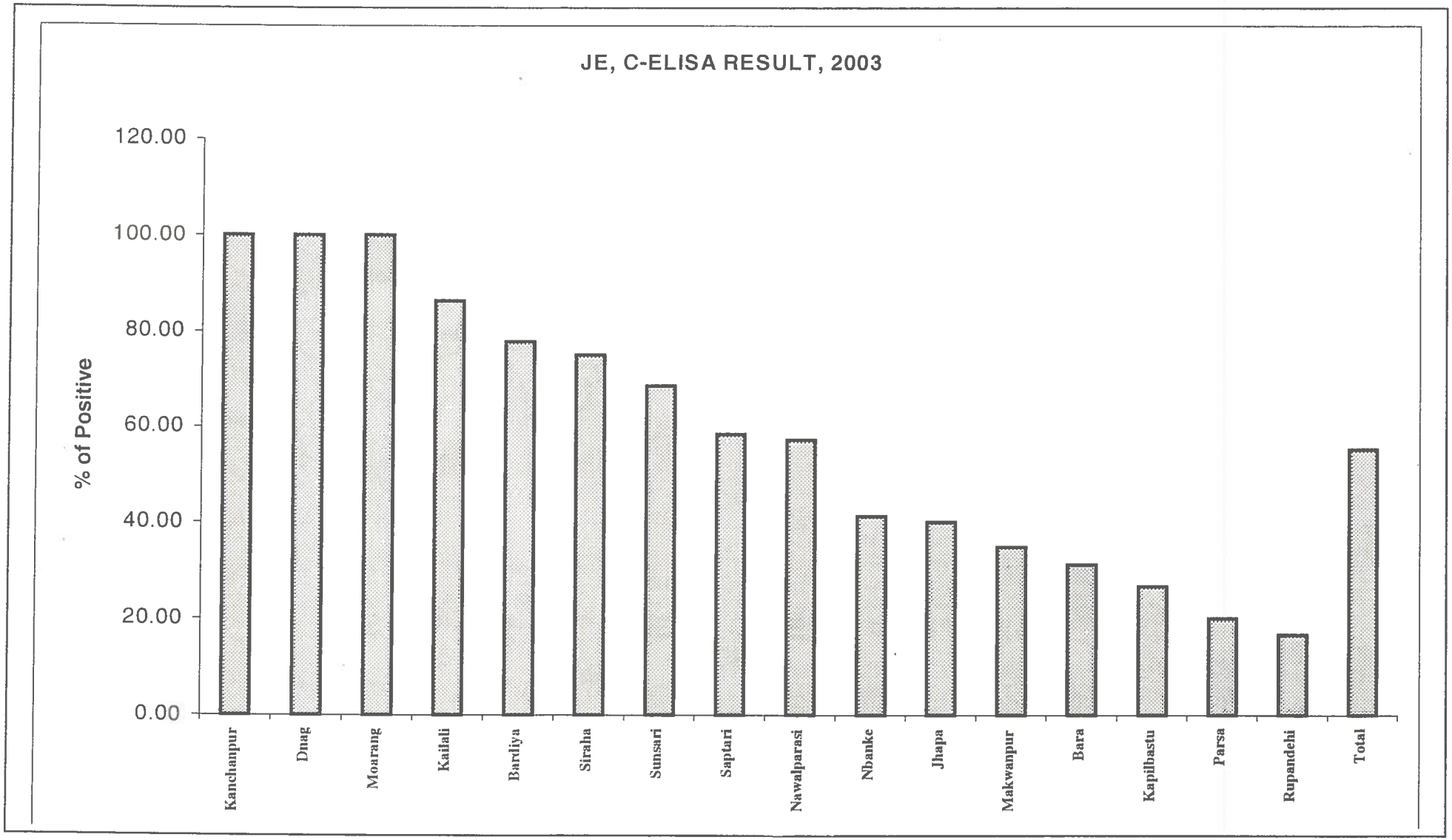
Record of JE vaccination in various districts (Annex No.1)

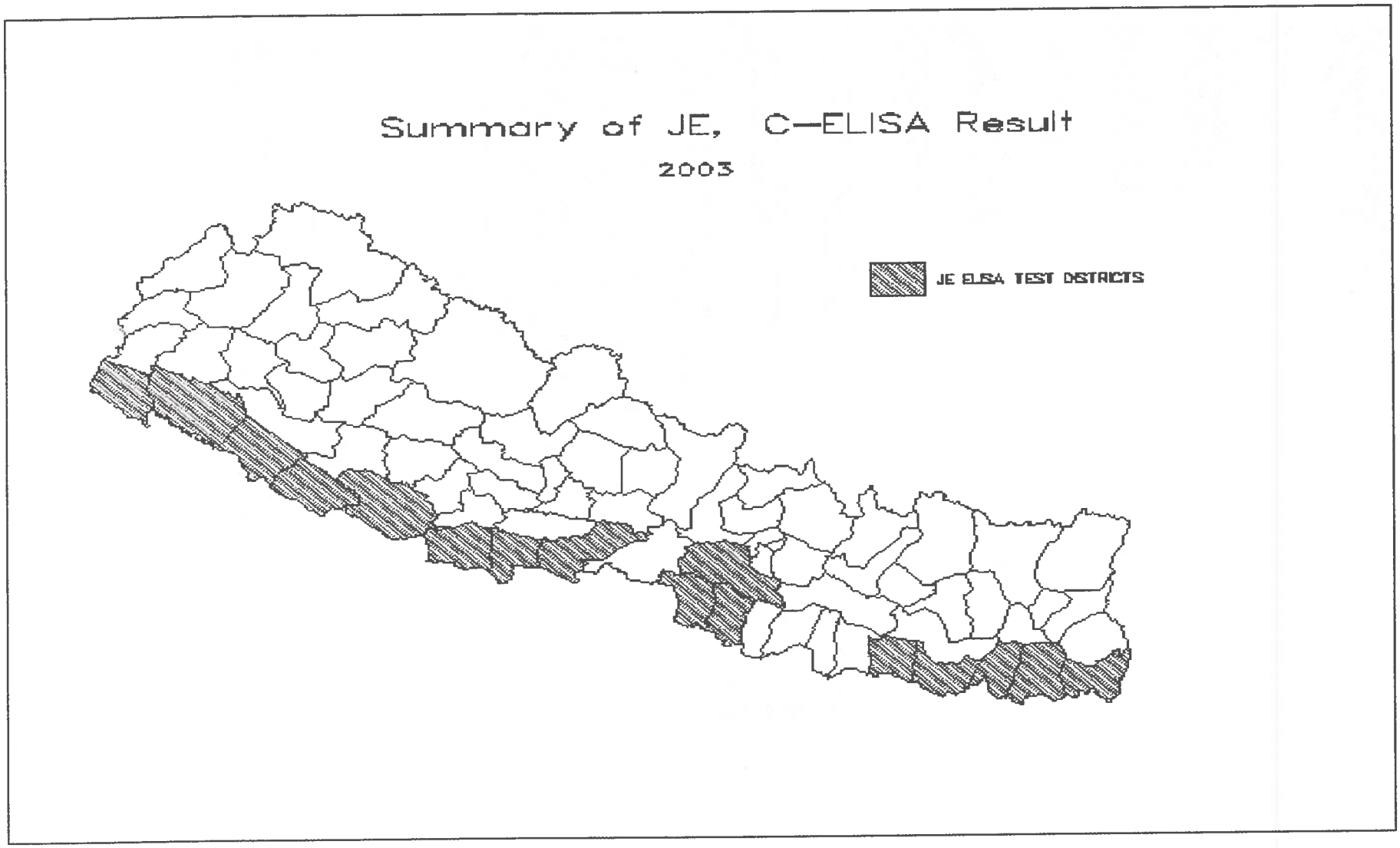
S. No	Region	District	Doses received	Doses given
1	**Far-western**		**42,800**	**17,386**
1.1		Kailali		12189
1.2		Kanchanpur		5197
2	**Mid-western**		**96,900**	**8,793**
2.1		Banke		824
2.2		Bardiya		1344
2.3		Dang		4000
2.4		Surkhet		625
3	**Western**		**24,000**	**5,028**
3.1		Lamjung		100
3.2		Nabalparashi		653
3.3		Palpa		150
3.4		Rupendehi		1034
3.5		Shyanja		791
3.6		Tanahu		2300
4	**Central**		**20,000**	**2,683**
4.1		Chitawn		600
4.2		Dhanusha		933
4.3		Makwanpur		550
4.4		Sharlahi		600
5	**Eastern**		**75,677**	**13,323**
5.1		Dhankuta		217
5.2		Jhapa		3706
5.3		Moranj		4650
5.4		Shiraha		280
5.5		Sunsari		4110
5.6		Udayapur		360
	Total		**259,377**	**45,213**

Above 45231 doses of JE vaccine were given to pigs until March 2001.This vaccination program was fully co-operated and actively participated by the staff of District Livestock Services Offices and members of Nepal Veterinary Association as well as Nepal Para Veterinary Association.

Summary of JE, C-ELISA result (Annex No.2)

District	Positive	Negative	Total	% of positive
Kanchanpur	6	-	6	100
Dang	5	-	5	100
Morang	13	-	13	100
Kailali	19	3	22	86.36
Bardiya	7	2	9	77.77
Siraha	12	4	16	75
Sunsari	26	12	38	68.42
Saptari	14	10	24	58.33
Nabalparasi	8	6	14	57.14
Banke	7	10	17	41.17
Jhapa	4	6	10	40
Makwanpur	15	28	43	34.88
Bara	5	11	16	31.25
Kapilbastu	4	11	15	26.66
Parsa	2	8	10	20
Rupendehi	2	10	12	16.66
Total	149	121	270	55.18


JE, C-ELISA RESULT, 2003
% of Positive
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Kanchanpur
Dnag
Moarang
Kailali
Bardiya
Siraha
Sunsari
Saptari
Nawalparasi
Nbanke
Jhapa
Makwanpur
Bara
Kapilbastu
Parsa
Rupandehi
Total


Summary of JE, C—ELISA Result
2003
JE ELISA TEST DISTRICTS

Investigation of Sarcosporidiosis in Buffalo Meat

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Abstract

An investigation of Sarcospidiosis in carabeef (buffalo meat) in Kathmandu, Lalitpur, Biratnagar, Pokhara, Bhaktpur, Hetauda, Nepalgunj, Dhangadhi and Mahendranagar metropolis, sub-metropolis and municipalities respectively was conducted in the year 2060/2061. The result revealed the occurrence of the parasite in buffalo meat in all the areas of study. The overall prevalence of Sarcosporidiosis in carabeef was found 80%..

Introduction

Sarcosporidiosis is a lesion encountered during meat inspection. It is caused by an intracellular protozoan parasite, the Sarcocystis. Later is a coccidian parasite. It occurs in both macroscopic as well as microscopic form. The occurrence of either of the forms depends on the animal species in which the infestation occurs. The parasite is cosmopolitan in distribution. It infects a wide range of creature including mammals, reptiles and birds. It can be rarely found in human skeletal and cardiac muscle when humans are the intermediate or accidental host. Humans can also serve as the definitive host for this parasite after ingesting the cysts through raw or undercooked beef or pork. They cause lesions in all the tissues or organs but especially in the skeletal muscle and nervous tissue. These parasites can resist freezing but they are susceptible to drying. The parasites are also destroyed on adequate cooking.

In all countries where there have been surveys the prevalence of infection in cattle, sheep and horses approaches with a lower but significant infection rate in swine. The major economic loss occurs with those Sarcocystis that produce macroscopic cyst and meat condemnation. However, clinical disease associated with Sarcocystosis has increasing recognition. It is also known that infection depresses the growth rate. The species from dogs are very pathogenic in experimental work but rarely cause disease naturally. However, Dalmeny disease is now thought to be due to Sarcocystis infection.

Frequency

In the US: *Sarcosporidiosis* has worldwide distribution. In the United States, more than 60 cases of muscle involvement by Sarcocystis species have been described, mostly in collections of case reports of 5-10 cases.

Since this finding is often incidental, many more undetected cases probably exist. The definitive form of Sarcosporidiosis causes self-limited nonspecific enteritis and is often not suspected clinically.

Internationally: Most cases of human Sarcosporidiosis occur in Southeast Asia. The incidence of intestinal Sarcocystosis infection in Thai laborers was measured at 23%. A study of autopsy specimens of patients in Southeast Asia showed a prevalence rate of 21% in 100 consecutive patients evaluated. The seroprevalence of Sarcosporidiosis in Malaysia was estimated at 19.8%.

Public Health Importance

Sarcocystosis is nowadays thought as a hidden zoonosis. The buffalo acts as the intermediate host for two species of the genus Sarcocystis- *S. levinei* for which dog acts as the definitive host and *S. fusiformis* for which cat acts as the definitive host. As dog transmitted cases are pathogenic, the humans may act as accidental host through the consumption of intected meat. Human Sarcocystosis may give rise to abdominal pain, diarrhoea, fever, tachycardia and an increased respiratory rate.

Economic importance

Sarcosporidiosis is a common pathological lesion encountered during meat inspection. The judgment is made on the basis of macroscopic appearance of the cysts. In light infection, the carcass is passed for consumption after trimming the infected portion of the carcass. In case of heavy infection, whole the carcass is condemned that leads to serious financial loss. Such type of meat is repulsive to the consumers.

Materials

The bodily skeletal muscles preferably from neck, thigh, shoulder, abdomen, tongue, esophagus and laryngeal muscles were collected from the slaughterhouses, slaughter sites and from the retailers in the concerned area.

Methodology

Questionnaire

A set of question was presented in the form of a format to the professional butchers as well as the retailers. The questionnaire format is presented in the annex.

Thirty-four butchers were inquired for the present study. Among them, 75% was found to be well aware and 25% percent was unaware about the occurrence of Sarcosporidiosis. According to butchers' information, 45% of the consumers are well aware about the infection and they do not use to consume such and infected meat. Fifty-five percent of them were found to be unaware. Of them, a few was

well known to the infection in meat but they used to consume such type of meat just saying that such type of meat is more nutritious than the healthy meat.

According to some of the butchers, Sarcosporidiosis is seen in carabeef, pork, chevon and even in poultry. The incidence and the severity of infection have been experienced in buffaloes imported through Jitpur, Nepalgunj and Tatopani quarantine checkpoints. It has also been learned that buffaloes brought in Kathmandu valley from Terai regions of the country also harbour the infection but the incidence has been found considerably lower than that present in the imported buffaloes.

Case history

The buffalo intended for slaughter may be quite healthy on ante mortem inspection. But the meat procured from such animals has been found to be heavily infected with Sarcosporidiosis. Similarly, most of the buffaloes are weak and emaciated and meat procured from them has also been found to be heavily infected. So it is very difficult to assure the infected animal prior to slaughter. The parasite has been found to be present frequently in the oesophageal, laryngeal and pharyngeal muscles. Besides, the important sites of predilection are tongue, buccinator muscle, neck muscle, shoulder muscle, thigh and pectoral muscles. Nevertheless, these findings ignore the generalized occurrence of the parasite in an animal.

Histopathology

The meat samples and the offal (tongue, esophagus, larynx) collected from different sites were preserved in 10% neutral buffered formalin. These samples, after fixation, were trimmed off for desirable size, and treated in ascending grades of alcohols following overnight treatment in 50%, two hours in 70%, one hour each in 90%, 95% and absolute alcohol and two treatments in the propanol. The tissues were then kept in the chloroform for clearing overnight and then impregnation in two wax baths having 60°C temperature each for 1.5 hrs carried out. After making the blocks through embedding them with the wax, they were cut into the section of 5μm thickness. Then the tissue sections were made into permanent slides through staining and mounting with DPX. The slides after cooling observed under microscope.

Results

The histopathological study of the tissues including skeletal muscles (Thigh, shoulder, abdomen, neck, larynx), tongue, and esophagus showed purple to blue coloured cysts located intracellularly in the muscle fibers. The cysts were found to be round, oval, ovoid and elongated shaped. The cysts were partitioned with incomplete connective tissue septa within which spherical spores were present in large number. The external morphology of the septa was found to be variable. In majority of the cases, the walls of the septa were thick and uniform while in some cases they were radially striated. Even in some cases, the walls were found to be provided externally with the spines. No inflammatory myositis was seen in the tissues presented for study. Rather, rupture of the myofibrils affected with

Sarcocystis, were seen. Probably it may be due to complete growth of the cysts when the later attain their optimum size.

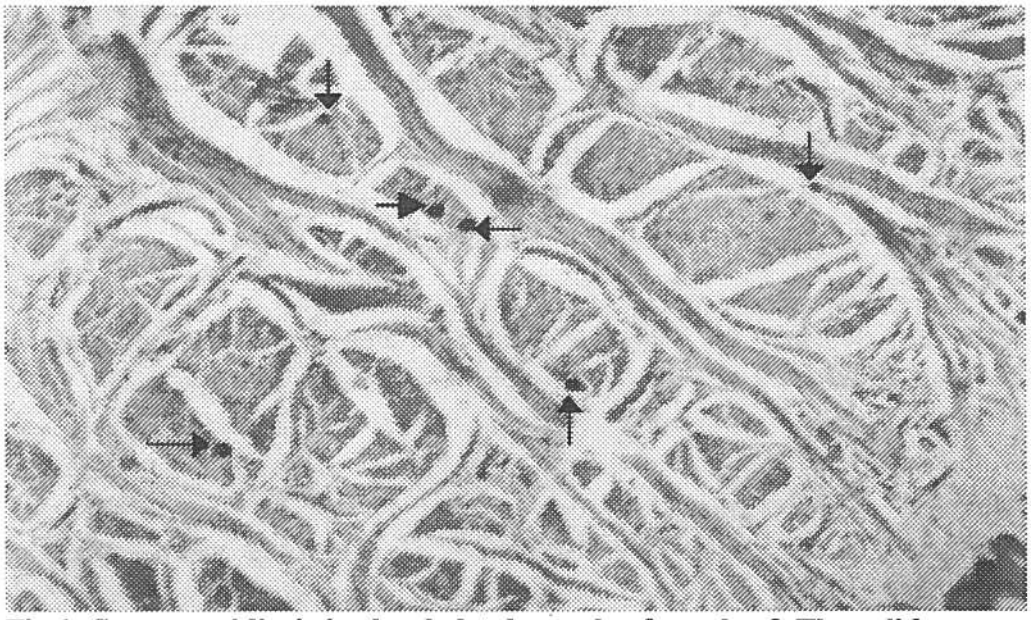

Fig 1: Sarcosporidiosis in the skeletal muscle of carabeef. The solid arrow shows the presence of *Sarcocystis* in the muscle.

The histopathological test result regarding occurrence and non-occurrence of Sarcosporidiosis has been tabulated below.

Table 1

Location	Total number of samples	Buffalo			
		Positive	% Positive	Negative	% Negative
Kathmandu	6	3	50	3	50
Lalitpur	8	7	87.5	1	12.5
Bhaktpur	4	4	100	-	-
Pokhara	3	3	100	-	-
Biratnagar	6	4	67	2	33
Hetauda	2	2	100	-	-
Nepalgunj	4	3	75	1	25
Dhangadhi	5	4	80	1	20
Mahendranagar	7	6	86	1	14
Total	45	36	80	9	20

Discussion

The investigation study was conducted in carabeef. The areas of study were Kathmandu, Lalitpur, Biratnagar, Pokhara, Bhaktpur, Hetauda, Nepalgunj, Dhangadhi and Mahendranagar metropolis, sub-metropolis and municipalities respectively. The areas selected for the study are all focal points for livestock trade in the respective zone.

In the present result, the sample size is not large enough. Nevertheless, these samples have shown the occurrence of Sarcosporidia in the buffalo meat. However, the small or large sample size certainly affects the percent occurrence. So, at least one hundred samples needs to be incorporated in the present tabular finding. As the aforementioned areas are central locations for livestock market

and trade in the respective zone and also internal quarantine are not so effective, it can be assumed that the infestation do occur in the adjacent areas or the adjacent districts of the study zone. With special reference to the common capital valley districts, the incidence, however, has been learned less in the buffaloes rose indigenous. So, it may be remarked here that buffaloes of either sex intended for slaughter imported from the Indian territories harbour marked infection of the parasite. These buffaloes may also be the source of introduction of infection in Nepal.

Recommendation

- The sample size is small. For the purpose of prevalence study, it is necessary to include a large number of samples.
- Species identification of the parasites in buffalo, pig and goat needs to be conducted to know the cause of non-infection of the parasites in case of humans.
- Seromonitoring in humans seems necessary to know the status of Sarcosporidiosis in humans. This is necessary because probably no case of human Sarcocystosis has been documented till date in Nepal.

Annex

Investigation of Sarcosporidiosis in food Animals

Name: Address:

1. How long have you been involved in this business?
2. What kind of meat do you sell?
3. Have you ever seen fried or boiled rice like thing in carabeef, chevon and pork?
4. Which of the organs are mostly involved?
5. What do you do with such meat?
6. Do the consumers purchase such kind of meat?

Survey by Date

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Polymerase Chain Reaction amplification and Molecular Characterization of *Pasteurella Multocida* species gene from cultural lysates of vaccine strain of Nepal

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Haemorrhagic Septicemia (HS) is an acute, peracute or chronic disease of livestock caused by *pasteurella multocida* organisms. HS has got different serotypes. Type B, known as type I in Carter's classification, is more commonly isolated in Asia, where as type E is the usual isolate in Africa. Outbreaks of acute pasteurellosis caused by other serotypes of P.multocida or by P. haemolytica should not be called haemorrhagic septicemia, even though septicemia may be a feature.

In Asia, the disease appears annually with the monsoon rains and is considered to be the most important infection of water buffalo and cattle.

The disease is prevalent in cattle, buffalo, pig, sheep and goat. The diseases may be three different forms.

1. Cutaneous form
2. Thoracic form
3. Enteric form

The disease can be transmitted by sick animal, saliva, blood, hide and skin and uncooked meat. It may be transmitted from respiration and there may be droplet infection too. The incubation period of the disease may be 2 to 5 days.

In Nepal, the disease is prevalent in Mid-hill, and Terai region with sporadic outbreak.

In the year 2003, there was 768 outbreaks of HS, in which 4321 animals affected and 557 dead. There was 273472 doses of vaccine used and 1805 animals were treated (annon, 2004).

Background:

Pasteurella spp. Are important primary and opportunistic pathogen as well as common commensals of the upper respiratory tract of various domestic and wild animals (Loubinoux *et al.*, 1999). Pasteurellosis is one of the most common disease of cattle sheep, and goats throughout the world where outbreaks usually lead to high mortality and great economic loss to the ruminant industry (Gilmour *et al.*, 1991; Links *et al.*, 1992). The symptoms of the disease are high fever, inflamation of tongue and throat. The disease may be peracute, acute and chronic form. There may be inflammation of brisket region and body. There may be dysentry.

Pathology:

The first lesion to be observed is usually oedema of the head, throat and brisket. The fluid is straw coloured and infiltration may extend from subcutaneous tissue into muscle. There are scattered petechiae. Pharyngeal cervical lymphnodes are swollen and may be haemorrhagic. Excess blood-tinged fluid is present in the thoracic abdominal and pericardial cavities. Pneumonia with oedema and thickening of interlobular septae may be seen in animals where the disease has lasted several days. Petechiae are seen under serous membranes throughtout the body. Apart from a few petechiae the spleen is unchanged.

Pasteurella multocida grows well on routine laboratory media and can be identified to species level by simple biochemical tests. Sero-typing, however is the work of a reference laboratory. Hem-agglutination tests detecting somatic antigens or mouse protection tests that detect capsular antigens are the most reliable.

Table No. I. Prevalence of HS in Nepal

Int year	Ecozone	outbreaks	Affected	Dead	Vaccinated
2000	Hill	579	5018	394	88315
2000	Mountain	292	2025	107	25646
2000	Tarai	799	7320	801	112356
2001	Hill	440	3775	537	106634
2001	Mountain	66	284	41	21912
2001	Tarai	535	5890	719	91375
2002	Hill	347	3263	321	85312
2002	Mountain	133	2343	50	21523
2002	Tarai	398	1617	486	71302
2003	Hill	316	912	202	70347
2003	Mountain	161	1987	28	31527
2003	Tarai	291	1422	327	171598

Objective:

1. Epidemiological study of HS in Nepal.
2. Course of disease, patholgical study and study of bacterial culture.
3. Isolation of DNA from *Pasteurella multocida* species.
4. Amplification of the gene fragment by PCR.
5. Molecular characterization of the gene fragment.

Material and Method:

Haemorrahagic septicemia seed bacteria were taken from Biological Product Division of Department of Livestock Services, Nepal. The bacteria were cultured in Microbiology laboratory of Central Veterinary Laboratory. The bacteria showed clear colony character of HS was taken for boiling and freezing method using liquid Nitrogen for extracting DNA. Water bath was also used for extracting DNA as an alternative method of extracting DNA.
There was set of primers namely KMT1SP6 (F) and KMT1T7(R).
Gene sequence of Forward primer is GCTGTAAACGAACTCGCCAC.
Gene sequence of Reverse primer is ATCCGCTATTTACCCAGTGG.
PCR reaction mixture was made up of 50 μ l. 1 μ l of genomic DNA or sample was taken. DNTP is of 200 μ M, 1.5 mM of $MgCl_2$, 1 U of Taq DNA polymerase and 1X PCR buffer.
PCR cycle is as follows.

1. 95° C for 5 minutes
2. 95° C for 1 minute
3. 55° C for 1 minute
6. 72° C for 1 minute
7. Repeat cycle 2 to 4 for 30 cycles.
8. 72° C for 6 minutes.

Then PCR product 5-μ l was mixed with 5-μ l of loading buffer (sucrose + methylene blue). The final product was loaded in 1.5% agarose gel and electrophoresis was done in 4V/Cm for 1 hour in 0.5X TBE buffer.
The gel was put in UV trans-illuminator and photographed.

Result:

In endemic areas, small numbers of healthy water buffalo or cattle carry organisms, in the naso-pharynx or tonsils. These animals act as the reservoir of infection between outbreaks of disease. Organisms are spread via the aerosol route. During an outbreak sick animals excrete large number of bacteria in nasal discharges, saliva and faeces.

The best tissues for smears and culture are blood, lung, liver and spleen. A long bone should be submitted if the animal has been dead for more than 8 hours. The organism is a small coccobacillus, which shows bipolar staining with Giemsa, methylene blue or equivalent stains.

The PCR product is 460 bp.

Discussion:

Outbreaks of H.S. in endemic areas are easily recognized. The rapid course of illness and the presence of throat swellings are suggestive of H.S. Anthrax, gas gangrene caused by clostridial species and certain snakes' bites occasionally give rise to similar lesions. Contagious bovine pleuro-pneumonia lesions are confined to the lungs but can closely resemble H.S.

Just before the monsoon, animals are in poor conditions, and are working hard preparing the land for the plating of crops. These stress factors coupled perhaps with viral or other infections, weaken host defences and predispose animals to pasteurella. In endemic areas, animals over 1 year old have often acquired immunity from previous outbreaks and disease is more common in the young stock. Outside endemic areas, clinical cases are seen in all age groups. Morbidity rates vary from low to high therefore, but in general case mortality rates are over 50% and may approach 100%.

The bacteria can survive for several hours in moist conditions but die rapidly if exposed to sunlight or desiccation. Indirect transmission of infection is possible and undoubtedly occurs. For several weeks ater an outbreak, between 20 – 50% of animals carry the organism and are capable of infecting susceptible in-contact-animals.

The same epidemiological pattern appears to exist in areas where the disease occurs sporadically.

It clearly shows that 460 bp of Pasteurella multocida gene in cultural lysates of vaccine strains. So that vaccine is potent and can give immunity up to the standard.

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INVESTIGATION ON INFERTILITY IN COWS OF EASTERN TARAI REGION OF NEPAL

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Abstract

A study was undertaken from the F/Y 2001/2002-2003/2004, to investigate the infertile cows reared in rural managemental system in the tarai region of Morang and Sunsari district of Nepal. Firstly, adequate information was collected, their breeding history recorded in the information sheet followed by gynaecological examination. Accordingly, 161 anoestrus cows were selected to assess Hb and serum levels of calcium, protein, phosphorus and copper. For this, blood was collected from the jugular vein under aseptic condition for the estimation of Hb and separation of serum. The serum was then stored at – 20 degree centigrade for biochemical studies. Estimation of Hb, total protein and phosphorus was done following the direction of the manufacturers provided with the specific kits. Values of Hb, calcium, total protein and phosphorus were found to be below normal while that of copper was in normal range. Medication was done aiming to deworm first followed by mineral supplementation. After mineral therapy, there was remarkable improvement in the condition of ovary and so, 56% anoestrus animals exhibited oestrus. As a whole, nutritional supplementation proved to be satisfactory for infertile cows, which must be provided for better reproduction.

Introduction:

Infertility problems account for more than half of all losses resulting from diseases of cattle. It has been considered as a serious problem in dairy cattle in Nepal with the resultant reduction in annual financial return over the managemental and feeding costs. In case of cow, it is logical to say that a cow should calve yearly for yielding the optimum production and economy in the life period, and this ideology, can only be achieved if the cow remains empty for not more than three months post calving. Every effort therefore should be applied to see, that a cow after calving should come into estrous after completing two months and should conceive by completing the three month post calving. Investigation on infertility requires a series of clinical examinations of affected animals and laboratory tests to determine the cause of the problem.

Infertility is a multifactorial syndrome. The causes of infertility may be broadly classified as infectious and non-infectious. In the non-infectious causes; it might be low energy intake, post-partum loss of body weight, deficiency of major trace minerals and vitamins, seasonal climatic factors etc. infertility due to nutritional causes is usually characterized by a failure of estrum or a cessation of the estrous cycle and only under certain conditions, it is characterized by a failure of conception or early embryonic death. Delayed maturity, anoestrus, prolonged post-partum anoestrus, silent or weak estrus and repeat breeding are the major problems of animal husbandry. (Kumar et al., 1988). There is a scope for improving the breeding efficiency of indigenous stock through better feeding, management and efficient health cover. In infectious causes; it might be bacterial, viral, fungal and parasitic disease condition.

Delayed puberty and post-partum anoestrus condition are common reproductive disorders encountered in rural cows leading to prolonged inter-calving periods, reduced milk production and thus affecting greatly the economy of farming community. Minerals as well as trace elements are closely involved in the maintenance of normal growth and development, reproduction and health of animals. They play an important role in animals by increasing the efficiency of livestock production and reproduction. Nutrients (Macro and microelements) are indispensable, either independently or collectively and are of great importance for the production, reproduction of cows and fertility management in dairy cattle. (Sharma et al. 2004). A low plane of nutrition due to lack of sufficient intake of protein and other elements necessary to maintain body weight may cause failure or delay in the onset of puberty or the onset of estrus cycle following parturition. This condition is seen most often in heifers maintained on poor hay or on poor pasture. As per Blood et al. (1983), the normal values of the Hb, total protein, and phosphorus in cattle are 11 gm%, 5.7-8.1 gm/dl, and 4-7 mg/dl respectively. All the above nutritional deficiency being recognized to have important roles in regulation of reproduction is blamed to affect fertility adversely.

In the rural areas of Nepal, dairy cattle are usually maintained on agricultural waste products with insufficient concentrates and fodder under the backyard system of management. As a result, marginal deficiency of macro and microelements occur affecting fertility adversely without manifesting specific deficiency symptoms. Moddie 1965, Prasad et al. 1984; have reported that certain biochemical constituents in blood serum affect the fertility status of cow and their reproductive behavior. Deficiency or excess of various biochemical constituents have been associated with fertility status of animals (Mc Clure, 1965; Prasad et al. loc. Cit.). The present study was undertaken to find out the levels of certain biochemical constituents during fertile and non-fertile period of rural cows.

Keeping these facts in consideration, the project had been designed with the following objectives: -

Objectives:

- To find the incidence of infertility in the region under investigation.
- To assess the nutritional status of infertile animals.
- To evaluate the response of vitamin – mineral supplementation.

Sites:

Year	District	VDCs
2001/2002	Morang	Jhorahat, Sorbhag Tankisinbari
2002/2003	Morang Sunsari	Jhorahat Sidhraha Pu. Kusaha Duhabi
2003/2004	Sunsari	Chhitaha Chimari

Materials and Method:

A study was conducted from F/Y 2001/2002 to 2003/2004 at Jhorahat, Sorbhag, Tankisinbari, Purba Kusaha, Duhabi, Chhitaha and Chimari VDCs of Morang and Sunsari districts to investigate infertile cows. Formats were designed and primary records related to reproductive performance were collected by direct interviewing with farmers adopting door-to-door survey system of selected sites. Under the process, the activities were accomplished as follows: -

Phase 1

- A total no. of 161 anoestrus cows (Heifers with delayed puberty and Post-partum anoestrus) were identified. Breeding history, body score, milk yield and other necessary information were recorded in the questionnaire sheet. Apart from this, each anoestrus animal was examined to determine the condition of ovaries, uterus, cervix and vagina.

Year	No. of selected anoestrus animals	Heifers with delayed Puberty	Post-partum Anoestrus
2001/2002	51	30	21
2002/2003	50	32	18
2003/2004	60	39	21
Total	161	101	60

- Every year, 10 apparently healthy cows having normal estrus cycle and free from reproductive disorders were also selected.

Phase 2

- Nutritional assessment of all anoestrus and control animals were done. For this, blood was collected from the jugular vein under aseptic condition for the estimation of Hb and separation of serum. The serum was then stored at – 20 degree centigrade for biochemical studies.

- Estimation of Hb, total protein and phosphorus was done following the direction of the manufacturers provided with the specific kits. For estimation of copper, serum samples were sent to the Central Veterinary Laboratory, Kathmandu.

Phase 3

- All anoestrus cows were dewormed with broad spectrum anthelmintics named Oxyzan-L (Oxyclozanide and Levamisole).

- All the anoestrus cows were supplemented with mineral mixture named Agrimin forte at the dose rate of 30 gm/day for 40 days.

- Again on 21st and 42nd days, gynaeco-clinical examination along with blood collection for nutritional assessment was done.

Phase 4

- Re-evaluation and follow up and follow up was done. Apart from this, those animals that came into estrus during the investigation period were inseminated.

Result and Discussion:

The average value of Hb in anoestrus cows ranged between 5.9-8.5 gm%, which is in accordance with the statement of Hansel who mentioned that low concentration of Hb below 9 gm% was noted in all anoestrus cows. However, Awasthi and Kharche (1987) did not find any difference between haemoglobin levels in normal and infertile group of cows.

The values for Hb and other biochemical constituents in the blood/serum of anoestrus rural cows are presented in the table below-

	Period	Hb gm%			Total protein gm%			Phosphorus mg/dl			Copper Mg/dl			Calcium Mg/dl		
		Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Ma	Min	Avg	Max
Normal values		8.0	**11**	15	5.7	**6.9**	8.1	4.0	**5.5**	7.0	0.10	**0.15**	0.20	8.0	**9.2**	10.5
Control Animals		8.4	**9.2**	10.3	6.0	**8.0**	9.7	5.0	**6.0**	8.1	0.09	**0.16**	0.20	7.0	**8.0**	9.0
Anoestrus cows	**O day**	5.9	**7.3**	8.5	4.3	**5.4**	7.5	3.5	**4.4**	6.3	0.08	**0.12**	0.18	5.8	**6.9**	8.0

In the present study, the average value of serum calcium in anoestrous cows was found to be 6.9 mg/dl. The finding of present study is less than the normal value of serum calcium (8.0 – 10.5 mg/dl) as reported by Blood et al. (1983)

Deficiency of serum calcium doesnot have direct bearing on reproduction as reported by Roberts (1971) and Marrow (1986). Calcium deficiency might be associated with low nutritional plane, stage of lactation, environmental condition, parasitism etc.

The average value of total serum protein in anoestrus cows ranged between 4.3-7.5 gm/100ml. These values of total protein are nearer to the value reported by Samad et al. (1980), Sharma et al. (1984), Kavani (1987) and Baruah et al. (1988). Patil and Deshpande (1979) reported that in anoestrus cows, the total serum protein was low as compared to those exhibiting oestrus. In control animals, the average value of total serum protein was 8.0 gm%. However, high levels of protein in the diet of dairy cows have been shown to increase the incidence of anoestrus (Gould, 1969). The effect of low level intake of protein on reproduction may be to reduce the total intake of feed, resulting in delay in estrus (Wiltbank et al. 1965).

Protein is very much important in supplying the energy to the animals for body requirement is well known. Deficiency of protein under usual condition of management of cattle is not common. Such deficiency is frequently observed in normal animals with malnutrition and high parasitic infestation. Low intake of protein therefore will put the animals in negative energy balance and continued status of such energy obviously will affect the body system including the reproductive phenomenon.

In this study, the average value of serum phosphorus in anoestrus cows was from 3.5-6.3 mg/dl. This value is in consistent with the Morris (1976), who suggested serum phosphorus level less than 4 mg/dl. in affected animals. Likewise Bansal et al.; 1978, commented that anoestrus animals possessed low level of phosphorus in the blood serum. The average value in control animals was ranged between 5.0-8.1 mg/dl. Whereas, normal values of inorganic phosphorus reported was 4.0-7.0 mg/dl. (Blood et al.; 1983)

Above finding shows the importance of inorganic phosphorus in reproduction leading to delay in puberty and occurrence of postpartum anoestrus. Phosphorus

deficiency is the most widespread and economically important of all the mineral deficiencies affecting grazing livestock. Significantly lower level of phosphorus in delayed pubertal heifers might be a contributing factor in inhibiting the function of anterior pituitary resulting in the suppression of both ovarian and genital activity. Diagnosis of phosphorus is only possible by analyzing the blood serum because in most of the cases, phosphorus deficiency occurred with no clinical sign of aphosphoresis such as lameness, rough coat, hoof abnormalities as described by Blood and Handerson (1968). Deficiency of phosphorus usually tends to occur due to poor or low protein intake and soil deficient in phosphorus.

The average values of serum copper in anoestrus animals ranged between 0.08-0.18 mg/dl. This value is nearly similar to the values recorded by Arthur. According to him, values of 0.1 mg/dl or above are normal, with deficient animals showing lower levels of 0.07 mg/dl or less.

Effect of mineral mixture therapy-

Out of 161 anoestrus cows supplemented with mineral mixture for 40 days, 90 cows exhibited oestrus.

Effect of mineral mixture therapy

Year	Total no. of anoestrus animals	No. of animal exhibited oestrus	Percent
2001/2002	51	31	61 %
2002/2003	50	27	54%
2003/2004	60	32	53.33%
Total	161	90	55.90%

In the present study, no. of animals exhibited oestrus was 55.90% whereas Dabas et al. (1987) treated anoestrus animals with 60 gm of Nuvimine forte for 15 days and observed 75% cows in normal heat. Singh and Vednere (1987), observed 72.22% of induced oestrus in cows with sodium phosphate treatment. The difference of finding in present study might be due to feeding, age, climatic and managemental conditions as well as quality of mineral mixture.

Effect of mineral mixture therapy on blood biochemical profile

During the study period, nutritional assessment was done on 0^{th}, 21^{st}, and 42^{nd} days. There was increasing trend in blood biochemical profile of the animals. The values for Hb, total protein, phosphorus and copper in anoestrus animals increased from 0^{th} to 42^{nd} day.

The average serum levels of Hb, calcium, total protein, phosphorus and copper in anoestrus animals before treatment were 7.3 gm%, 6.9 mg/dl, 5.4 gm%, 4.4 mg/dl and 0.12 mg/dl respectively which increased to 8.1 gm%, 7.4 mg/dl, 6.3 gm%, 4.8 mg/dl and 0.14 mg/dl on 21^{st} day. On 42^{nd} day, the values for Hb, calcium, total protein, phosphorus and copper were 9.3 gm%, 8.6 mg/dl, 8.4 gm%, 6.5 mg/dl and 0.15 mg/dl respectively.

Effect of mineral mixture therapy on blood bio-chemical profile from 0 day to 42 day:-

Biochemical constituents	Period	0 day	21 day	42 day
Hb gm %	Min.	5.9	6.0	7.2
	Avg.	7.3	8.1	9.3
	Max	8.5	9.1	9.8
Calcium mg /dl	Min.	5.0	5.6	6.2
	Avg.	6.9	7.4	8.6
	Max	8.9	9.2	11.0
Total protein gm %	Min.	4.3	4.8	6.2
	Avg.	5.4	6.3	8.4
	Max	7.5	8.0	10.6
Phosphorus mg/dl	Min.	3.5	3.8	4.8
	Avg.	4.4	4.8	6.5
	Max	6.3	6.6	8.0
Copper mg/dl	Min.	0.08	0.09	0.09
	Avg.	0.12	0.14	0.15
	Max	0.18	0.19	0.20

Effect of mineral mixture therapy on ovary

During the study period, all the anoestrus animals were examined per-rectally to know the condition of ovaries as well as other genital organs on the day 0, 21, and 42. Per-rectum gynaecological examination on 0 day revealed infancy state of genital organs, small and smooth inactive ovaries in most of the heifers and smooth, flat non-functional ovary with or without palpable corpus luteum in lactating animals. The results are in accordance to the findings of several workers. Low level of serum protein has been reported to be associated with inactive ovaries (Roberts, loc.cit.). Likewise, low level of serum protein has been reported in anoestrus animals having inactive ovaries (Aminuddin et al.; 1984). Occurrence of ovarian dysfunction / ovarian inactivity at lower phosphorus levels were recorded by Hignett (1952). On 42nd day, there was remarkable improvement in the condition of ovaries and 90 animals showed signs of oestrus out of 161 animals.

Effect of mineral mixture therapy on the ovary from 0 day to 42 day

Category of animal	No. of anoestrus animals	Condition of ovary			No. of animals exhibited oestrus
		0 day	21 day	42 day	
Heifers with delayed puberty	107	Small / round smooth – 87 Palpable C.L. – 10 Growing follicle – 8 Hypoplasia of ovary - 2	Inactive – 52 Active -55	Inactive – 26 Active - 81	72
Post partum anoestrus	54	Flat / round smooth – 26 Palpable C.L. – 21 Growing follicle – 7	Inactive – 18 Active -36	Inactive – 12 Active - 42	18
Total	161				90

Feeding of anoestrus cows with mineral mixture evinced oestrus in 56% of the cows. This encouraging result is an indication of the stimulatory effect of the trace elements contained in it in improving the reproductive efficiency in anoestrus animals.

Conclusion & Recommendation:

Role of minerals in reproductive performance of cows is shown by the present study, which indicates that Hb, protein and phosphorus has definite role in reproduction. Altogether 56% animals exhibited oestrus after mineral supplementation for 40 days. Hence, nutritional supplementation is proved to be satisfactory for infertile cows, which must be provided for better reproduction. Based on above findings, it is recommended to improve the nutritional status of animals by offering mineral supplements in addition to grazing will definitely increase the number of fertile animals in rural areas. In addition to this, effective extension services on this aspect will surely help to overcome this problem.

Acknowledgement:

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Investigation of Epidemics

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Various disease outbreaks of animal and poultry were investigated during fiscal year 2060/61. Whenever request for investigation of an outbreak is received from the district to the laboratory, a veterinarian or technician or a team of technicians with necessary sampling kit visited to the site of epidemic, collected possible epidemiological information and appropriate pathological samples. In the laboratory, pathological samples collected from the field were processed to find out the etiology of the outbreak. Epidemiological information gathered from the site of an outbreak was used to decide the test to be performed in the laboratory and to assist in the confirmation of disease diagnosis. Samples, not possible to process in this laboratory were referred to CVL, Kathmandu.

A total of 15 epidemics were investigated during 2060/61. Of 15 outbreaks investigated, some were confirmed by laboratory while others confirmations were based on clinical signs and postmortem findings. The outbreaks epidemiological findings are presented in Table 9.

Table 9: Epidemiological findings of animal disease outbreaks investigated during 2060/61

S. N.	Outbreak Month /Year	District	Species of animal affected	Disease diagnosed	Animal population at risk	No. of animal affected	No. of animal died
1	Bhadra 2060	Kaski	Poultry	Ranikhet	1600	1250	490
2	Kartik 2060	Nawalparasi	Goat	Mange	80	15	2
3	Kartik 2060	Arghakhanchi	Goat	Mange	100	12	-
4	Mangshir 2060	Baglung	Goat	Pneumonia	200	15	4
5	Magshir 2060	Parbat	Cattle /Buffalo	F.M.D.	120	15	2

6	Paush 2060	Lamjung	Buffalo	F.M.D.	60	40	7
7	Paush 2060	Nawalparasi	Goat	Mange	120	24	-
8	Paush 2060	Kaski	Poultry	Ranikhet	3000	950	865
9	Paush 2060	Kaski	Sheep	Acute fasciolosis	26	14	7
10	Magh 2060	kaski	Poultry	Mycotoxicosis	1400	465	160
11	Baishakh 2060	Gorkha	Goat	Abortion	70	10	-
12	Baishakh 2061	Shyanja 2060	Buffalo/ Cattle	Plant Poisioning	12	4	2
13	Jesth 2061	Kaski	Poultry	Gumboro	2500	590	350
14	Ashad 2061	Kaski	Poultry	Egg Drop Syndrome	4500	2600	3
15	Ashad 2060	Tanahu	Goat	Mange	122	16	1

Single Intradermal Tuberculin tests of milking cows and buffaloes in Kaski and Kathmandu districts

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Introduction:

Bovine tuberculosis is an infectious disease caused by *Mycobacterium bovis*, and is usually characterised by formation of nodular granulomas known as tubercles. *M. bovis* is seen mainly in ruminants, and is a zoonotic agent with a wide range of mammalian hosts including humans. Infection in humans occurs largely through consumption of infected milk and spread can also occur by inhalation. Nowadays, clinical evidence of tuberculosis in cattle is seldom encountered in most countries because intradermal tuberculin test enables presumptive diagnosis and elimination of infected animals before signs appear.

Materials and Methods:

An investigation on prevalence of tuberculosis in milking cows and buffaloes in Kaski and Kathmandu districts was carried out using Single Intradermal Cervical Tuberculin Tests (SICTs). In 2003 September, 654 lactating buffaloes and 800 cows in milk pockets areas of Kaski and Kathmandu districts were examined for tuberculosis, using SICTs with purified protein derivative of bovine tuberculin obtained from Indian Veterinary Research Institute. The SICTs was carried out following the methods as described by OIE (1996).

Results and Discussion:

The result of SICTs is presented in Table 1.

Table 1: Results of tuberculin tests in lactating buffaloes and cows of Kaski and Kathmandu districts

District	Buffaloes		Cows	
	Tested	Positive (%)	Tested	Positive (%)
Kaski	558	25 (4.48)	396	19 (4.79)
Kathmandu	96	10 (10.41)	414	13(3.14)
Total	**654**	**35 (5.35)**	**800**	**32 (4.0)**

Figures in parenthesis indicates percentage

It can be seen in table 1 that 5.35% lactating buffaloes and 4% lactating cows from Kaski and Kathmandu districts were positive for SITCs. The results of SICTs positivity in milking cows and buffaloes appear to be a clear indication for the presence and extent of bovine tuberculosis in Nepal. Further studies on bovine tuberculosis are necessary covering a large animal sample size from different eco-zones of Nepal.

Reference

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Investigation of Johne's disease in Cattle and Buffaloes in Western Region of Nepal

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Introduction:

Paratuberculosis (Johne's disease) is a chronic infectious disease of ruminants characterized by chronic diarrhoea; progressive weight loss and decrease in milk production. The disease persists in breeding stocks after the introduction of infected animals. A potential source of infection in calves is milk from infected cows or milk that is contaminated with the faeces of diseased cattle or buffaloes. Johne's disease (JD) is caused by *Mycobacterium paratuberculosis*. Although death losses are not high, the consequence of long period of ill health and reduced productivity causes severe economic loss.

In Nepal Dhakal and Tiwari (1993) reported that 34% of buffaloes were positive to intra-dermal Johnin test while examining 146 buffaloes in Chitwan district. Joshi (1999) reported 34% buffaloes and goats, 27% cattle and 24% sheep positive for specific antibodies to *Mycobacterium paratuberculosis* in the western hills of Nepal.

With an objective to study on isolation, identification and characterization of *M. paratuberculosis* from cattle and buffaloes RVL, Pokhara carried out this investigation work during 2060/61.

Materials and Methods:

In a close collaboration with the District Livestock Services Offices of Kaski, Lamjung, Gulmi, Tanahu and Syanja; a questionnaire was used to identify the cattle and buffaloes with a history of chronic diarrhoea, debilitated condition and progressive weight loss. Details of the identified animals were recorded. Rectal scrapping and rectal feacal samples were collected from identified cattle and buffaloes and the samples were brought to the laboratory on ice.

Smears were prepared from the rectal scrapping and were subjected to Ziehl-Neelsen (ZN) staining technique.

Fecal samples were subjected for culture examinations. Fecal samples were cultured on ready-made culture media for Mycobacterium paratuberculosis manufactured by Kyouritsu Seiyaku Pharmaceutical Company, Japan. The culture procedures were followed as per the culture media manufacturers instructions. The inoculated culture tubes were incubated for 3 months at 37 C.

During the incubation weekly observation of inoculated tubes were made for growth of any colonies resembling *Mycobacterium paratuberculosis* and records were made. After 3 months of incubation of the inoculated samples, the colonies of the culture tubes were stained by Ziehl-Neelsen staining technique.

Results and Discussion:

Twenty-eight buffaloes and 26 cows were identified for rectal scrapping and fecal sample collection. Rectal scrapping could be collected from only 12 animals. However fecal samples of all 54 animals were collected.

The ZN staining of 12 rectal scrapping smears revealed that 4 scrapping smears were positive for acid-fast organisms.

The results of fecal sample cultures revealed that 5 cultures yielded colonies resembling *Mycobacterium paratuberculosis*. The ZN staining of *Mycobacterium paratuberculosis* suspected colonies revealed 3 cultures positive for acid fast organisms. All 3 acid fast positive cultures are from buffalo's fecal samples. For further confirmation and characterizations, the isolates will be sent to Gunma Prefactural Institute of Public Health and Environmental Sciences, Japan.

It is evident from this study that *Mycobacterium paratuberculosis* infection is prevalent in the bovine population of western region. This is the first laboratory report of isolation of *Mycobacterium paratuberculosis* form bovines in Nepal. Further studies on the disease epidemiology, isolation and characterization from a larger population would be necessary to formulate effective control measures against Johne's disease in farm animals of Nepal.

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An Outbreak of Egg Drop Syndrome in a Layer Poultry Farm of Kaski district

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Introduction:

Egg Drop Syndrome 1976 (EDS 76) is a major cause of egg production loss through out the world. Although the laying birds look healthy but the egg produced are thin shelled or shell -less eggs with reduced egg production. EDS 76 is caused by an adenovirus and the birds become infected through direct or indirect contact with the infected wild or domestic birds.

Materials and Methods:

In June 2004, sudden reduction in egg production in a laying poultry flock for last 2 weeks was reported to the Regional Veterinary Laboratory, Pokhara. Subsequently the farm was visited and the clinical signs in the affected flock were observed and epidemiological data including egg production performance of the flock was recorded. The eggs produced by the flock was checked for shape, size, color and if any abnormality present. Ten serum samples were collected from the birds of the affected flock. The serum samples were sent to the Central Veterinary Laboratory for the detection of antibody to Infectious bronchitis (IB) and EDS 76.

Results and Discussion:

The poultry farm had two different flocks in separate housing in a distance of 50 meters. Total laying birds population at the farm was 10600. The affected flock poultry population was 5247 and the birds were 37 weeks old. At the age of 35th week the egg production in the flock was 83%. At 36th week the egg production came down to 74%. Similarly at 37th week 63%. At 38th week egg production was 61%.

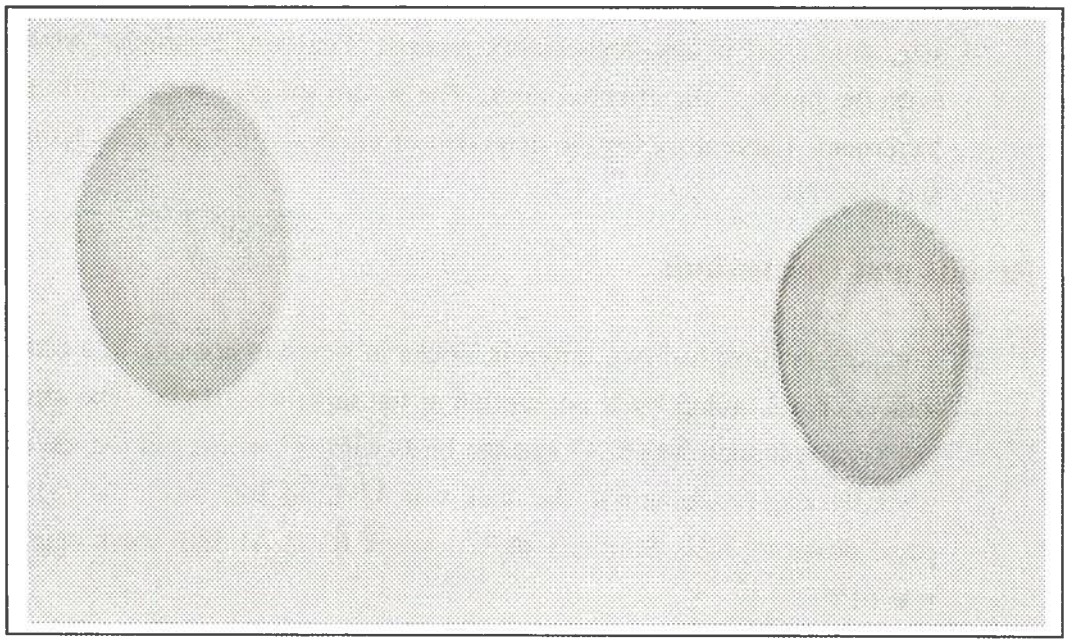
No mortality was observed in the affected flock. During the visit no any dead bird could be found. No any evident clinical symptoms could be seen in the affected flock. However the farmer noticed that there was reduced feed intake by the birds and some birds had diarrhoea.

Visual examination of the laid eggs revealed that about 15 % eggs had loss of colour; thin shelled or soft-shelled eggs and small sized eggs. The thin-shelled eggs had rough texture.

The abnormal eggs layed by the flock are presented in figure1.
The serological examination results obtained from Central Veterinary Laboratory, Katmandu revealed that out of 10 serum samples subjected to ELISA for the detection of antibody to IB and EDS 76, 9 serum samples were positive for IB antibodies and 8 serum samples were positive for antibodies against EDS 76. It is worth to mention here that 6 weeks before the outbreak the flock was already vaccinated against IB, therefore the presence of antibodies against IB was due to vaccination. The affected flock had no any vaccination against EDS 76 therefore the antibodies found against EDS 76 was due to infection of the flock with the EDS 76 virus. Hence this outbreak was due to the introduction of EDS 76 disease in the flock.

After treatment of the birds with chlortetracycline powder, vitamin AD3EC and Calcium and Phosphorus liquid the egg production increased gradually from 39 weeks onwards.

In Nepal, the incidence of EDS 76 in laying poultry flocks is in increasing trend and vaccination of laying flocks against EDS 76 is not practiced. For prevention of EDS 76 in the laying flocks routine vaccination of layer flocks/parent flocks against EDS 76 at the age of 15 weeks is recommended.



Abnormal size and shape of eggs due to EDS 76 disease in laying flock

Poverty Alleviation through Semi commercial Goat farming Program during 2060/61

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Animal Production Directorate had given the RVL, Pokhara the following responsibilities for supporting the Poverty Alleviation through Semi commercial Goat farming Program.

Seromonitoring and serosurveillance of the goats distributed under this programme at Arghakhanchi, Baglung and Nawalparasi districts.

Faecal sample collection from the goats and examination of samples for internal parasitic identification.

Outbreak investigation and support the concerned DLSOs in controlling the diseases in the goats.

Drug trial and recommendation of the effective drugs.

Participation in programme related workshop and training.

Reporting of the progress on monthly, trimestral and annual basis.

The progress report of the activities under the programme conducted by RVL, Pokhara during 2060/61 is presented as follows.

Activities	Unit	Annual target	Achievement
Seromonitoring	Times	3	3
Serosurveillance	Times	3	3
Disease outbreak investigation and control	Times	3	3
Faecal sample examination	Times	3	3
Participation in meeting/ Seminars	Times	7	7
Drug trial and recommendation	Times	3	3
Monthly, Quarterly and Annual progress reporting	Times	16	1

Table 1: Goat population in the farming committee

District	Committee	Members	Goat population
Arghakanchi	Khidim Bakhra palan samiti	63	447
Baglung	Binamare Bakhra palan Samiti	88	440
Nawalparasi	Narayani bakhra Palan Samiti	186	964

Table 2: Disease investigation in goats

District	Disease problem
Arghakanchi	Plant Poisoning, Internal and External parasites
Baglung	Pneumonia, Abortion, Internal parasites
Nawalparasi	Conjuntivitis, Pneumonia, Paralysis, Abortion, Poisioning Internal parasites

Serum collection and Examination:

One hundred and fifty one serum samples from goats were collected from Arghakhanchi, Nawalparasi and Baglung districts. All the serum samples were sent to CVL, Kathmandu for the detection of antibody to PPR. The result obtained is presented in table 3.

Table 3:Results of serum samples tested for PPR antibody

District	No. of serum tested	No. Positive	Positive percentage
Arghakhanchi	25	12	48
Nawalparasi	68	44	64.7
Baglung	58	1	1.7

According to the DLSOs of Arghakhanchi, Baglung and Nawalparasi all the distributed goats were vaccinated against PPR. However the results of the randomly collected serum samples for the detection of antibody against PPR revealed that only 48%, 64.7% and 1.7% serum were positive for PPR antibody in Arghakhanchi, Nawalparasi and Baglung districts respectively. Principally, of the total goat population in a flock, 80% goats must have PPR antibody so as to prevent the introduction of PPR in the flock. Therefore all these three districts were requested to revaccinate their flocks as early as possible to prevent introduction and mortality of goats from PPR.

Regarding Baglung district it seemed that the vaccine cold chain was not maintained as a consequence 98.3 % sampled goats had no antibody against PPR.

800 doses of PPR vaccine was provided to DLSO Baglung for revaccination of the flock.

Among the collected serum samples 30 samples belonging to aborted goats were subjected for brucellosis test ((RBPT), All samples were found negative for brucella antibody.

Twenty-five serum samples of aborted goats were also subjected for the biochemical estimation of Calcium, Phosphorus and magnesium.

Table 4: Serum biochemical values in goats

Estimation	Result Value range	Normal value range	Remarks
Calcium	2.7 - 7.74 mg%	8 -11 mg%	Deficiency
Phosphorus	3.58 - 8.36 mg%	4 -7 mg%	Normal
Magnesium	2.24 - 2.93 mg%	1.9 - 2.5 mg%	Normal

Fecal sample collection and Examination:

A total of 156 fecal samples were collected from three districts and brought to the laboratory in closed plastic bag with cotton mixed with 10% formalin. The collected samples were subjected for fecal examination for the detection of internal parasitic eggs.

Table 5: Results of Fecal examination

Detail	Arghakhanchi	Nawalparasi	Baglung
	Total samples	Tested	
	50	46	60
Parasites			
Strongyloides	11	13	13
Strongyle	17	10	10
Trichuris	5	3	5
Fasciola	1	0	2
Paramphistome	3	2	0
Monezia	3	0	11
Coccidia	9	14	19
Negative	1	4	0

It can be seen in table 5 that almost majority of the sampled goats were positive for internal parasites. The results of fecal examination were dispatched to all three districts and suggested for routine drenching of all the goats.

RVL, Pokhara also distributed Panacur (150 mg) 850 tablets and Albendazole (200 mg) 100 tablets for drenching the goats found positive for internal parasites at Nawalparasi district.

Results of Drug trial:

Fenbendazole was found effective against G I nematodes in goats.
Both Ivermectin and Ectomin were found effective against ectoparasites in the goats.

Problems faced by farmers:

Agalactiae problem in majority of lactating does.

Internal and External parasitic major problems in the goats.

Due to predator difficulty in grazing the goats in Jungles.

Mortality in kids.

Suggestions:

RVL can play an active role for the protection of health status of goats through early disease diagnosis and control. DLSOs are requested to inform us immediately if any disease problem arises in the flocks.

DLSOs concerned staff should also collect appropriate samples and send to RVL for the disease diagnosis.

As majority of the goats are continuously infested with internal parasites therefore RVL recommends at least 4 times drenching of goats in a year (Baishakha, Shrawan, Kartik and Magh).

RVL recommends to provide training on goat health and disease diagnosis to the concerned field staff as soon as possible.

Investigation of diseases of goat under commercial rearing system

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Introduction

Goat rearing is one of the traditional occupation particularly popular is the rural poor community of Nepal. The total 5.4 millions of goats of Nepal (DFMAS 1991) is mostly reared under conventional rearing system .The efforts made in the past to commercialize is showing it's positive impact in some parts of the country. With the commercialization of goat rearing, newer disease is being notice, which needs to be diagnosed properly to adopter preventive and control measures. Keeping in view, the regional veterinary laboratory (RVL) surkhet and dhangadi has been assigned to investigate the diseases of goat under commercial rearing system in this fiscal year 2055/2056 .The project was started with the objective to find out the virus disease problem faced by the goat raisers under commercial rearing system: to confirm the virus diseases of goat based on the laboratory confirmation and to establish the virus normal physiological parameters of Nepalese breed of goat.

The methodology envisaged at the beginning comprises retrospective study, site selection, participatory rural appraisal (PRA) and finally prospective study. The retrospective study involves recording of previous case record 2-3 districts of this region to list out the problems / diseases of goats faced by the farmers in the past. Based on the criteria like commercial goat rearing parasite manage by farmers group. Easy accessibility and near by service centre / sub centre. DLSO, or RVL the first site selected is sivashakti, dangapari goat breeding society at satabariya - 4 dang. The site is located near to lamahi (dang) beside the east west highway. The society is recognized for their on breed of goat dangapari as they claim. The society has the strength of its 22 members 317 goats they possesses on tenth magh -2055 (Annex-1) further to site is aimed to extend in letter years on the basis of the working experience of first site. Participatory rural appraisal (PRA) was made in the farmers group of the selected site to find out the main problems/ diseases of the goats faced by them. Prospective study has been started to record day to day problems / diseases of the goats at the site in the format (Annex-II): to retrospective study /PRA /day to day case register recording of the monthly cases of goat on day to day basis is being under taken by the group leader J.T./J.T.A. of the selected site. Herd inventory of every individual goat raisers recorded every month during regular visit by the veterinarian from RVL and DLSO in the first week of every month. Extra visit scheduled whenever needed or in emergency of out break.

The work is in progress at the site and samples are being collected to process them at RVL surkhet. In this paper the previous records of district livestock service office DLSO collected as part of PRA study is analyzed travel the main diseases /problem of the goat.

Material and method

Available case record previous of surkhet. Bankey and dang district was planned to record to list out the case of goats. Unfortunately the case records of previous year of dang district couldn't be found. Different types of cases were recorded on monthly as well as yearly basis. To see the more frequent problems percentage calculated. Weight age of different disease problem were shown in different pie chart .The record of three fiscal year I.E., 2052/053-2054/2055 of bankey and of only one fiscal year i.e., 2053/2054 of surkhet district could only found and analyzed here in this paper .The main problem found in the study are as follows.

Round worm

It is one of the most frequent problem found in the both of the districts and the percentage ranges from 34-42% .It is not clear whether this cases are clinically diagnosed of lab confirmed.

Diarrhea

It was found another main problem in goats and parents ranges from 11-15% pyrexia. It appears another important problem in goats and the percentage from 10-16% surgical problem. It includes wound and castration cases and the percentage range from 9-18%.

Digestive disorders

It includes indigestion, timpani, stomatitis, dysentery etc.

Skin problems

It includes mange, dermatitis, abscess etc.

Other problems

Besides the above-mentioned problems other problems notice in the retention of placenta, posterior paralysis (kumri) the bite, abortion, respiratory problems etc. The different problems in countered in the records of the previous years was monthly a symptomatic guessing. Show, to investigate the diseases of goat under commercials rearing system needs a well thought planning and quick diagnostic measures when ever necessary. The work is under progress on the above-discussed line and it is expected the some of the symptom of diseases could be diagnosed in the laboratory in the near future.

Acknowledgement

The regional veterinary laboratory express its sincere thank to the DLSO Surkhet, dang and Bankey for their support. Likewise, the direction given by the CVL, AHD and RDLS Surkhet is also appreciated.

Investigation of respiratory problems of goat

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Background

Investigation of diseases of goat under commercial rearing system was undertaken as project from F/Y 2055/056 for 3 years. The project was started with objective to find out the various diseases/problems faced by the goat raisers under commercial rearing system: their laboratory confirmation: and to establish various normal normal physiological parameters. The activity of the project was designed as retrospective study, PRA and prospective study. The end of F/Y 2057/058 concluded the project. The project successfully accomplished the retrospective study and PRA. The prospective study part could not be fully completed as expected at the beginning. Even This study has veiled many use full information about the feeding, housing, economic and health status of commercial goat rearing of the study site, i.e. shivashakti dangapari goat breeding society satwaria, Dang.The retrospective study conducted at Surkhet and bankey revealed the major problems as worm infestation, diarrhea, digestive disorders, skin problems etc.when the farmers were asked to rank the problem/diseases, they realized the problems in order of nasal discharge, worm infection, hydropneumonis, timpani posterior paralysis, white dysentery, circling plus sky grazing, poor weight gain and death due to arthritis. The problem of nasal discharge, hydropneumonis, swelling of head, salivation etc. was also realized during prospective study. The problems, which are related to the respiratory system, are very important because there is no certain known diagnosis and treatment for these problems unlike the problems of worm infection, timpani, posterior paralysis et. This respiratory problem causes much death loss than others. Definite treatments for these are also not recommended many field veterinarians and technicians also experience such respiratory problems. It needs to be investigated in detail. The outcome of this study will certainly help to reduce the death loss and production loss caused by respiratory problems like pneumonia. Simultaneously, it will improve the diagnostic capabilities of this regional veterinary laboratory. Also it will guide the programs like poverty alleviation through goat raring. Hence this second phase of investigation program for goat respiratory problems is designed.

Objective

1. General objectives:

i) To investigate the various types of respiratory problems in goat for the benefit of goat raisers, field veterinarians, extension workers and planners,

ii) To build the diagnostic capabilities of this regional veterinary laboratory.

1. **Specific objective:**

   i) To find out the prevalence of respiratory problems in the selected locality (1 mid-hil, 1 terai)
   ii) To investigate the associated bacterial, parasitic, protozoan, fungal, viral or environmental causes or respiratory problems.
   iii) To site the correction between different hematological parameters and different respiratory problems.

Duration of the project

3 fiscal years (2058/059-2060/061) ie. 2001-2004 AD

Working approach

1. **Receiving of samples and records from veterinary hospital surkhet and Bardiya:**

   An arrangement will be made with the veterinary hospital of surkhet and bardiya to record the cases of goat suffering from respiratory problems and to collect the pre-decided samples from each case. The staff from RVL, surkhet will collect these records and samples once a month. The recording will be made on the supplied format. Only the decided samples will be obtained from the monthly epidemiological report.

2. **Direct investigation at 3 sites:**

   i) Chhinchu service center of surkhet district-monthly clinic
   ii) Kohalpur service center of Bankey district-monthly clinic.
   iii) Lamahi service center/satwariya of dang district-monthly clinic.

   These monthly clinics will be run on a fixed date. Goat raisers of the area will be pre -informed about it. In these clinics, if some goat showing severe signs of respiratory problems can be purchased for post mortem and detail laboratory investigation. Besides these, The technicians of the site will be provided with a separate case register for goat, formats for individual case records and samples will be brought back to RVL at the time of monthly clinic operation.

3. **Goat health camp 2 per year (one in terai and one in mid-hill)**

   These camps will be organized to get enough number of samples from the goat suffering from respiratory problems. Other types of problems will be treated in general and different types of samples will also be collected. The same type of case register, individual case records for respiratory problems and sample collection kit will be used.

Methodology

1) Recording of cases of goat showing respiratory problems in a pre- designed format (Annex-ii)

Anex II

Regional Veterinary, Surkhet
Investigation of respiratory problems in goat
Case Report format

Date:

Site: -
Case Reg. No.: -
Name of the owner: -
Address: -
Breed of goat: - Local / Jamunapari / Barberi / Ajmeri / Cross / any other .....
Sex: - Male / Female / Castrated male
Age: -
Housing system: - Free range / Goat pen / Mixed
Ventillation in goat pen: - well ventilated / poorly ventilated / unventilated
Feeding system: - Stall feed / Grazing / Stall feed +Grazing
What was feed before becoming ill .....
Anything special: -
Temp. ..... Respiration rate ...../min Pulse rate ...../min
Mucous membrane: - Normal / Anemic / jaundiced / others .....
Nasal discharge: - serous / Mucoid / Mucopurulent / Rusty / Foetid
Type of respiration: - Normal / dyspnoea / Abdominal
Cough: - Absent / Dry / Moist / Coupious / Frequent
Respiratory sound: - Vesicular / Bronchial / Frictional / Moist rales / cripitation sound
NO. of goat in your village suffering from same type of disease :-
Any new goat purchased and brought to your flock recently? Yes/No, If yes, how many days ago .....and from where .....

Sample collected:

1. Nasopharyngeal swab.
2. Blood smear fixed in Methanol.
3. Blood in EDTA (2ml)
4. Serum.
5. Faucal sample.
6. PM sample...
   1.
   2.
   3.
   4.
   5.
   6. Any other sample.....

Tentative Diagnosis.....

Result of lab test .....

N.S. Result -
B.S. Result -
Faecal Result -
Hb in gms/100ml-

PCV -
RBC Count in million / cmm -
N - M -
E -
B -
L -

2) **Collection of following sample from goat showing respiratory problems in each clinical case:**

i) Fecal sample (without any additive)
ii) Nasopharyngeal swab
iii) Blood smear made and fixed in methanol at site
iv) Blood in EDTA (2ml)
v) Serum

Preservation and transportation of samples to RVL as per the standard condition for various types of samples will be made.

3) **Purchase of diseased goat to sacrifice for detail investigation (maximum 10goats per year)**

Following diseases causing respiratory problems in goat will be investigated detail in laboratory:

i. Paste des petites ruminants (PPR)-serology, histopathology, and hematology
ii. Contagious caprine pleuro-pneumonia-bacterial culture, isolation, sensitivity (procedures attached)
iii. Pasteurella pneumonia -bacterial culture from the sample collected from postmortem, isolation and sensitivity.
iv. and Lung worm- By simplified Bayerman's method: identification of larvae its preservation.
v. Babesiosis- Blood smear examination and preservation of positive slides.
vi. Caseous lymphadenitis- smear from pus and Germ staining bacteriology

General guidelines for sample processing in different laboratories:

i) Bacteriology:

Inoculate each bacteriological sample into nutrient agar, blood agar and mccone key agar, Record the observation after 24 and 48 hours. Subculture to get pure colony and record the colony characteristics. Study the bacterial morphology and perform biochemical test to identify the bacteria. Alternately use API kit if available. Preserve the bacteria in appropriate media using appropriate technique. Perform antibiotic sensitivity test. Record all the results. Also inoculate SDA plate with same bacteriological samples and proceed as per need to see the fungal infection.

ii. Virology:

Samples (serum and pm samples) will be cent to CVL for the available test.

iii. Histopathology:

Collected samples will be brought and processed to CVL. Fixation and trimming of samples will be performed at RVL. Post mortem will be done as and when required. The recording will be made in the format attached. All types of possible samples will be collected.

Data analysis and reporting

The available data for the different types of causative agent of respiratory problems or

*Disease to the total number of case presented

*Prevalence rate of a disease at particular site.

*Season of outbreak for different diseases problems.

*Management practices vs. respiratory problems/disease

*Details of the diseases confirmed (History, inspection, clinical exam, result of lab, tests. etc

*In vitro sensitivity and a therapeutic response.

Action plan

In first quarter of 2058/059

+ Agreement with different conceded DLSOs'

+ Workshop on sample collection, preservation and dispatch from the diseased goat.

  The workshop will be including.
  i. Pm technique in goat
  ii. Sample collection technique
  iii. Sample preservation technique
  iv. Sample dispatch technique
  v. Filling of different formats

+ Supplying the site technicians with sample collection kit, apron and other accessories
  2058 marga (1st dec -2001) on wards

+ Recording and collection of samples at veterinary hospital Surkhet and bardiry.

+ Monthly visit at direct investigation sites- on the 1st, 2nd and 3rd of every months according to English calendar.

+ Goat health camp-

  Ist-2nd quarter of 2058/059
  2nd-3rd quarter of 2058/059
  3rd-1st quarter of 2059/060

4th-2nd quarter of 2059/060
5th-3rd quarter of 2059/060
6th-1st quarter of 2060/061 + sample testing in lab: as and when required

+ Technical reporting: once a year, at the end of every year according to English calendar.

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Respiratory Diseases in Goat in Mid-western region in relation to age, Season and Housing system

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Introduction

- One of the important diseases in goat in mid western region.
- From the earlier investigation, it is found that the disease in prevalent in Mid-western region.
- In this disease:
  -Increased respiratory rate,
  -Labored breathing,
  -Rapid exertion,
  -Abnormal sounds associated with breathing,
  -Nasal discharge,
  -Coughing or fever.
  -PPR test is negative for all bacteriological positive cases.
  -All These bacteria were isolated only in rainy and winter season.
  -In rainy season the prevalent of E. coli were maximum.

Possible problems associated with respiratory diseases

- Chronic progressing pneumonia
- Lung worm
- Pasteurellosis
- Tuberculosis
- My co plasma infection
- Anemia
- Dusty of mould hay
- Pregnancy toxemia
- Ammonia or other fumes
- Rumen acidosis
- Bloat
- Heat stroke
- Irritant fumes
- Nasal obstruction
- Respiratory sync teal virus
- Inhalation pneumonia
- Pulmonary adenomatosis
- Melioidosis
- Cryptococcosis

Objectives:

- To identify the causative agent of respiratory diseases.
- To strengthen the capability of RVL for diagnosis of different discipline i.e. bacteriology, hematology, serology and parasitology.
- To prepare RVL for the diagnosis of any emergency outbreaks.

Materials and methods:

- Site selection (3 site)
  - Chinchu of Surkhet,
  - Dhakeri of Bankey,
  - Satbariya of dang district.
- Total goat population -1860
- Selection criteria:
  - Commercial goat farming system
  - Conscious about drenching, vaccination and also they are innovators.

Activities:

- From jan, 2002-bi-monthly visit to these sites,
- Clinical examination (temp., respiration, nasal discharge) of these goat .
- Identifying the diseased one.
- Collection of the sample
  - Blood smear fixed in methanol
  - Blood in EDTA
  - Fecal
  - Nasopharyngeal swab
  - Any sample which felt necessary
- 242 cases of respiratory problems were recorded in 12 visits during 24 months of program
- Examination conducted:
  - Bacteriology.
  - Hematology.
  - Parasitic identification
  - Blood parasite examination
  - Intra dermal examination for tuberculin test
  - Brucellosis test
  - Serum examination for PPR.

-The goat between ages 1-3 years was easily infected with respiratory problems.

Result and discussion:

Out of 242 goat, we mainly categorized the age group i.e. 0-1yrs, 1-3yrs, 3-6yrs.

Age	0-1yrs	1-3yrs	3-6yrs	total
No.of goat	48	132	72	242
%	20	50	30	100

The table shows younger goats are mostly affected with respiratory problems.

Parasitological identification

No.	Parasite	Total Number	% Parasites
1.	Trichuris	13	14
2.	Strongyloides	16	17
3.	Liverfluke	10	10.75
4.	Trichostrongyles	29	31.18
5.	Liverfluke &Trichuris	3	3.22
6.	Liverfluke & Trichostrongyles	9	9.67
7.	Ostegia	1	1.07
8.	Cocci	2	2.15
9.	Dictyocalus	8	8.60
10.	Haemonchus	2	2.15

◊ Totaly, 8.6% of cases were only were found to be affected with lungworm.

Bacteria isolated from different age groups and diferent season

Age 0-1yrs		*1-3yrs*		*3-6yrs*	
E.coli	2	E.coli	28	E.coli	14
Staphylococci	2	Strptopyogens	4	Staphylococci	2
	-	Staphyloepidermis	6	Staphyloaureus	4
		Staphyloaureuss	8	Streptviridans	2
		Pasturella	4	Pasturella	4
	4		50		26

Bacteria isolated

Total no of bacteria isolated		**% of bacteria isolated**	**% of positive cases**	**% of negative cases**
E.coli	44	55	33	67
Staphylococci	4	5	-	-
Streptopyogenes	4	5	-	-
Staphyloaurens	12	15	-	-
Streptoviridons	2	2.5	-	-
Staphyloepidermis	6	7.5	-	-
Pasturella	8	10	-	-

Hematological value

✓ The average value of lymphocyte, monocyte, basophil, eosinophil, neutrophil, PCV and Hb. are a follows

Lymphocyte%	Monocyte%	Basophil%	Eosinophil%	Neutrophil%	PCV%	Hb gm%
50-60(70)	2-3(207)	0	2-3(2020	40-41(205)	35	8
>50(55)	3-5(30)	0	3-5(30)	42-43(30)	40	10-12
<60(117)	6-10(5)	0	6-8(80	45(7)	40	<12
<60(117)	2-3	0	2-3	40-41	35	10-12

✓ Lymphocytosis is present in more than 100 cases. That may incicate there is chronic infection of some agent may be (viral , allergic)

Discussion:

- As the problems of respiratory diseases, which were analyzed above, suggests that no involvement of protozoan parasites on blood smear by giemsa stain and lungworm were also very low up to 4% in positive cases.
- The incidence of E. coli is mainly in rainy season mainly due to water contamination.
- The lymphocytosis (more than 100 cases) indicates chronic infection in those farm or shed. That chronic infection may be due to some respiratory syncytial. Virus along with different parasites & bacteria.
- In those periods the death of 18 goats were reported.
- Only 2 goats were post-mort med but didn't find any special indication.
- The plate agglutination test for brucellosis was also performed and found negative.
- Also tuberculin test was conducted and found negative.
- The poor ventilation & mixed type of housing system, the production of ammonia gas in goat pen were also pre-disposing factor for the causative of respiratory diseases.

Suggestion:

❖ So, we concluded that the serum sample should be tested for my co plasma and other viral antigen. Further investigation to be needed in the direction of my co plasma and viral infection.

The status of EPG counts in different district of mid-western region in relation to poverty alleviation: Goat keeping program

- Project Districts - 8

Total sample collected - 1143 Status of parasitic load (EPG) in mid-western region

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Objective:

- ✓ To identify the different parasites found in mid-western region.
- ✓ To count the parasitic load in goat in different location .
- ✓ To repair our laboratory for routine work.

Material and methods: -

- Visit of the districts where the poverty alleviation program is going on Clinically examination of the Goats.
- Collection of fecal sample for proper

EPG count: During the period of 2 years all together 1143 sample were collected

S.N.	Particles	Positive sample	% in gestation	Negative
1.	Trichuris	86	17.13	56.68 sample were negative
2.	Strongyles	252	50.19	
3.	Ascaris	9	1.79	
4.	Fasciola	67	13.34	
5.	Cocci	11	2.19	
6.	Ostertegia	21	4.18	
7.	Cotylophoron	16	3.18	
8.	Monetize	22	4.38	
9.	Lung worm	18	3.58	
	Total	502	100%	

EPG count in different

A.N.		0-5	5-10	10-15	Positive	Remark
1	Trichuris	59	13	0		
2	Strongyles	143	109	0		
3	Ascaris	9	0	0		
4	Liver fluke	67	0	0		
5	Ostergia	21	0	0		
6	Cotylophoron	16	0	0		
7	Monezia	22	0	0		
8	Lungworm	18	0	0		

- Result: It shows results of various parasitic loads in goats across the districts in two years time.
- Results: It shows results of various parasitic loads in goats across the months in two years time.

Distribution of positive cases of EPG counts on Feacal examination

Parasites	Banke y	Dan g	Salya n	Pyutha n	Surkhe t	Jajarko t	Ruku m	Rollp a
Trichuris	25	27	13	5	10	-	-	6
Strongyles	43	92	40	25	37	-	-	15
Ascaris	2	2	1	2	2	-	-	-
Liverfluke	17	38	1	4	6	-	-	1
Ostertagia	1	-	-	8	5	-	-	7
Cotylophoro m	3	8	1	-	2	-	-	2
Moneizia	3	6	5	2	3	-	-	3
Dictyocalus	-	3	1	1	2	-	-	8

Distribution of positive cases of EPG counts on Feacal examination across months

Month	Trichuris								Total
Baisakha	10	24	2	3	-	-	5	7	51
Jestha	11	60	1	1	15	2	3	2	95
Ashad	15	20	-	3	-	3	1	1	43
Shrawan	-	-	-	-	-	-	-	-	-
Bhadra	-	4	-	-	-	-	8	5	17
Ashoj	7	58	-	4	-	4	4	3	80
Kartik	8	10	-	6	-	-	1	-	25
Mangshir	10	8	4	11	-	1	-	-	34
Push	13	25	-	19	2	3	-	-	62
Magha	12	43	2	20	4	3	-	-	84
Falgun	-	-	-	-	-	-	-	-	-
Chitra	-	-	-	-	-	-	-	-	-

Results &Discussion:

- Total sample collected -1143
- Positive for parasitic infestation - 502
- Negative - 641

Although the different species of parasites may infect the population in different way but incase of gastro-intestinal parasites specially some worm egg per gram count is necessary in case of strangles 43% of the feces count in more than 600 egg per gram. The efficacy of drug as de wormers vary in different situation out the use of benzimidazole is very frequently. Is usually the dose recommended for nematode group B albendazole of @ 10mg/kg body wt. levamisol.

- The gastro intestinal tract, in our stances does substantial losses to goat owners effective control of parasites will make a significant contribution to goat's health & well being.
- The sample presence of these parasites will make only presence hot only disease case but the parasites loads become excessive than only disease persist. On that case EPG count should be necessary to identify whether drenching is necessary hot. In our investigation 43.9% of case were positive for parasitic infestation but only in case of tricuspids & strangles the egg per gram count is greater than 600. As already illustrated in different literature the ie. (www.goatworld.com/articles/wormsncsu.shtm.) Disease only occurs when the egg count is greater than 600/gram of feces.
- Although the different species of parasites may infect the population in different way but incase of gastro-intestinal parasites specially some worm egg per gram count is necessary in case of strangles 43% of the feces count in more than 600 egg per gram. The efficacy of drug as de wormers vary in different situation out the use of benzimidazole is very frequently. Is usually the dose recommended for nematode group B albendazole of @ 10mg/kg body wt. levamisol.

Conclusion

- The present review on gastrointestinal helminthes infection of small ruminant in Nepal has attempted to collate available information in this field and following conclusion could be drawn from it.
- Goat and sheep rearing is and important aspect of farming system and these animals are important source for cash income for most of the household in Nepal.
- Animals are reared under the traditional management without much external inputs.
- Gastrointestinal nematodes are the important constraints for increasing the productivity of sheep and goats reared under the sedentary and migratory managements.
- The epidemiological information on infection of animals is well understood and the infection is confined to the wet summer months only.
- The response to treatment is very encouraging in terms off increased productivity of the animals.
- The mechanism and system of disease control have not been developed well and needs to be investigated.
- The availability of chemical anthelmentic is a problem in the remote areas.
- No information on anathematic resistances available.
- Some of the plants have been shown to possess anathematic properties but their efficacy is not well evaluated.
- The native cage breed was found to have greater resistance to haemonchus infection than the other two breeds.
- Goat breeds have not been evaluated for their genetic resistance against helminthes infection.
- There is a considerable potentiality to improve the productivity of the animals with improvement in the health and nutrition management.

A study on Meat Borne zoonoses in far western region of Nepal

Regional Animal Disease Investigation Laboratory
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Introduction:

All communicable diseases of man, transmitted by vertebrate animals are usually called Zoonoses. There are certain diseases which are either communicable through meat animals or through meat and meat products to human being are usually meat borne zoonotic diseases. The total meat production of the nation is 0.203 million metric ton in which buffaloalone contributes 64.14% while sheep, goat, pig, poultry and duck contribute 1.35%, 19.45%, 7.66%, 7.23% and 0.13% respectively. (CBS 2003/2004)

A study, conducted in 41 slaughter stalls and butchers in six municipalities of Far western region by Regional Veterinary laboratory, dhangadhi during F/Y 2001/2002 has indicated that Far western region was having 10.1kg / per year / paecapita meat consumption (the study was not conducted in any village of this region) in which buffalo meat, goat meat, mutton, pork and chicken contributed 32%, 18%, 3.6%, 11% and 26% respectively. (Annual Technical Book, RVL, Dhangadhi 2001/002)

Materials and Methods:

The surveillance programme was conducted through through cross sectional and longitudinal study. The study was of three years started during 2001/2002. Initially the study was conducted in all municipalities of far western region Viz. Dhangadhi, Mahendra nagar, Tikapur, Amar gadhi, Dipayal silgadhi And Dasharth chand. Later, next year onward, longitudinal study was mainly focused at Mahendra nagar, Dhangadhi, Tikapur and Amar basti of kanchanpur. Visual inspection of the carcass during slaughtering and processing was done to find the unhealthy meat and organs. The suspected sample was collected and submitted to RVL, Dhangadhi for parasitological, Pathological and bacteriological examinations. Serum of live animals especially before the slaughter or at the quarentine checkpost while importing, were collected and submitted to Laboratory to perform RBPT, Salmonellosis and mycoplasmosis.

Result and Discussion:

Defects found during Ante mortem Examination of live animals/ Birds and Post mortem Examination after slaughter:

S.N.	Species	No.of animals /birds	A.M. defects observed	P.M. defects observed
1.	Buffalo	36	Usually the animals were smaller, younger age group and have less than one quintal. There were animals found to be affecting by diarrhea, Jaundice, debility coughing. Their faces were foul smelling and conjunctivitis with purulent discharge was also noticed. The sick animals, so far were found around 30%.	Liver fluke, loose faecal contents. Lungs having white patches, hemorrhages, icterus. Rumen having paraamphistomes, ascaris. Liver haemorrhage and congestion. Intestine having haemorrhage and ulceration.
2.	Goat	124	Debility, diarrhoea, emaciation, pyrexia, icterus, red urine tick infestation, old female goats were commonly observed in many cases. Usually imported goats were found healthier than the local. Unhealthy goats to be slaughtered were 10%.	Liver- haemorrhage, congestion, lungs- severe haemorrhages white patches, pneumonic. Rumen- having paramphistomes cyst in mesentry. Spleen- enlarged, haemorrhages
3.	swine	17	Emaciated, nasal discharge, conjunctivis otitis, pyrexia found in less than 10%	Lungs- congested, whitish putrefied regions, hyperemic. Liver- blackish coloration, intestine-hemorrhage, whitish discoloration, ulcers, reddish bloody discoloration.
4.	Poultry	40	white diarrhea, Bloody dropping, fall of feathers dehydrated birds were common A.M. signs found during surveillance.	Liver- enlarged, hemorrhages and nodules present. Lungs- severe congestion and discoloration. Heart- leechi heart and ascitis. Kidneys- severe haemorrhages, pale. Intestines- haemorrhages, button ulceration. Bursa – enlarged, hemorrhages. Proventrticulus- haemorrhoges (probable diseases- Ranikhet/ IBD/ IBH/ Coccidiosis/ ascitis/CRD)

Laboratory Investigation:

Findings of Parasitic infestation in meat animals:

	Species	Sample collected	Result of Lab. Examination	Zoonotic Importance
1.	Buffalo	Faeces from live animals	Liver fluke	Fluke in man
		Worm collected from stomach and intestines	Paramhistomes	Not important
			Strongylus	Not important
			Taeniasis	Contaminated food and water causes autoinfection in man
			Hydatidosis	Contaminated food and water causes autoinfection in man
2.	Swine		Ascaris	Not important
		Cyst	Taeniosis	Contaminated food and water causes autoinfection in man
		Meat on histopathology	Cysticercosis	Contaminated food and water causes autoinfection in man
3.	Goat	Faeces	Strongylus Strongyloides Ostetragia Cooperia Trichuris Monenzia Liver fluke	Not important
		Cyst- intestine and mesentry	Hydatidosis	Contaminated food and water causes autoinfection in man
		Blood slides	Babesiosis	Not important
4.	Poultry	Faeces	Tapeworm Ascaris	Not important
		Defective organs	Ranikhet	Not important
			Ascitis	Not important
			Infectious bursal disease	Not important
			Leechi heart disease	Not important
			CRD	Not important

- **Haematological examination** of 5 goats to be slaughtered revealed, there was eosinophilia in two goats (which may be due to heavy parasitic infestation), another two goats were found to be having neutrophilia which could be due to

bacterial infection in those animals as there were pneumonic lungs, noticed during post mortem examination.

- Though **serological examination** was performed but nothing revealed of zoonotic importance, 33-goat serum was tested against Brucellosis and salmonellosis by using plate agglutination test, all were found to negative. 14 poultry sera were found to be negative for salmonellosis. And also, 21 goat sera samples, tested through ELISA for PPR, all were found to negative. There were huge evidence of sera being positive for mycoplasma (the antigen was of Poultry M.gallinarum)

Microbiological Findings:

Under the study of microbiological contamination of meat, totally 48 samples were collected from the suspected live animals, meat, visceral organs and other suspected lesion. Mostly nutrient, Blood and Mackonkey agar were used in totally aerobic condition .The results are as follows:

S.N.	Species	Suspected samples	Media used	Organism isolated	Zoonotic importance
1.	Buffalo	Buffalo meat=15 Intestinal swab=2	Nutrient Mackonkey agar	Streptococcus Staphylococcus Pasteurella	Suspected water contaminated meat as well as presence of environmental may cause food poisoning.
2.	Goat	Nasal swab=6 Lungs=4 Liver=3	Blood Mackonkey Nutrient	Streptococcus Bacillus	Food poisoning in case of heavy infection.
3.	Swine	Liver=3 Lung=1	Nutrient Blood	Pasteurella	Not so important
4.	Poultry	Intestine=6 Lungs=1 Trachea=1 Liver=2 Eggs=4	Blood Mackonkey Nutrient	Streptococcus Clostridium	Food poisoning

The major findings of bacteriological examination under aerobic condition and nonselective media are streptococcus, staphylococcus, Bacillus, Pasteurella and clostridium in all kinds of meat and their viscera. Though there environmental microbes or they may be due to water contaminated or unhygienic dressing as well as manual handling of meat by customers. These are zoonotically important bacteria, their load and hours of storage at room temperature is important. So,

further investigation of microbial load with particular span of time is very important.

CONCLUSION:

- The surveillance of butcher and meat cutting places reveals the unhygienic handling of meat in far western region of Nepal. Though it is a good source of income, butchers do not maintain their health status, neither they treat the wound in their hands nor they are aware of meat borne zoonoses. There is the habit of selling defective meat. Training for meat slaughtering, meat borne zoonoses and healthy meat production is very important to butchers.
- Ante Mortem and Post Mortem Examinations of live animals and their meat and viscera revealed there is the habit of slaughter sick animals. Butchers slaughter sick animals having diarrhoea, emaciation, icterus, pyrexia, conjunctivitis, red urine, nasal discharge. They also slaughter the birds, which are ready to die due to diseases like Ranikhet/ IBD/ leechi Heart disease/ Coccidiosis and white diarrhoiea.
- Liver fluke, paramphistomes, ascaris, cooperia, Monenzia, trichuris etc are common worm infestation in ruminants but these are not zoonotically important but the investigation of Taeniasis, Hydatidosis, cysticercosis are the cocern of public health and further investigation is required.
- Microbiological Load in meat is threatening due to unhygienic handling, unhealthy meat production and use of contaminated water and utensils as well as knives, wooden block etc. The growth of streptococcus, staphylococcus, pasteurella, bacillus, clostridium are zoonotically important. Further investigation is necessary to estimate their load in different meat with particular span of time.

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A study on Caprine abortion in far western region of Nepal

Regional Animal Disease Investigation Laboratory
Dhangadi (Far-western Region)

Background:

Goats are reared even by small farmers and are multipurpose animals, which provide meat, milk, manure, fibers and power for transportation. Total no. of goats in Nepal is 6.4 million and goat meat contributes 19.4% of total meat production in Nepal (MOAC, 2000/2001) . It is the second largest source of meat after buffalo and goat alone shares 10.2% of national livestock GDP in term of meat (NCAP, 1991/92). There is abundant scope of exporting goat to other countries. Epidemics of caprine abortion was observed during 2001/2002 in goats, distributed under poverty alleviation programme of department of livestock services. The incidence was observed in far and mid western region of Nepal, later on reported from other regions too.

Methodology:

Though this study was performed by regional veterinary laboratory, Dhangadhi for the span of two years and this has covered only the districts of far western region. However the same etiology has been observed in mid western region as well as other regions of Nepal, (Sharma, Banshi CVL 2002). Both retrospective and perspective studies were performed in this study. Epidemiological records of three years have been studied and the record of epidemic abortion in goats from several districts has been analyzed. a clinical and management of goat rearing surveillance was conducted.. Also, laboratory investigation has been performed in several caprine abortions as well as during epidemics of the same.

Clinical Symptoms of Caprine abortion:

Clinical observation shows there would be the causes of both infectious and non-infectious but mostly the abortion occurred with second to third kidding. The major clinical observation were rise in temperature, weakness, posterior lumbar paralysis (setariasis), coughing diarrhoea straining during evening and night usually after grazing, abortion during last months of gestation, there would be single or twin fetus, retention of placenta is quite usual.

In some cases, animal becomes anorectic, having joint swelling, icteric eyes, conjunctivitis and mucous watery discharge from the nose.

Abortion observed mostly during July/ August/ September, which are usually the months of goat distribution in Nepal through different schemes. Not only the abortion but the death of newborn too notices during these months.

Laboratory Findings:

A total of 600 faecal examinations have revealed the highest infestation of strongyles (54.7% of total cases) with high Epg count of 2000, followed by Coccidiosis (26.2%), Strongloides (13.5%) and Fascilosis (12.2%). The mixed infestation was as high as 36.5%. The parasitic load during monsoon makes the animals prone to other infectious diseases. Multiple hemorrhages in visceral organs, foul smelling of reproductive tract, accumulation of fluid in abdominal cavity, congestion of lungs, found during post mortem examination also reveals the causes may be infectious which later on, confirmed by histopathological examination which has indicated the etiology was the acute toxic reaction of bacterial origin. The haematological examinations showed the cases of lymphocytosis, neutrophlia and eosinophilia cases in different cases. 29 sera out of 34 of the aborted goat revealed the positive cases of mycoplasma through plate agglutination test (Mycoplasma Capri). The microbiological culture showed the presence of important bacteria like compylobacter. The level of bilirubin was high in many aborted goats but those were having low level in serum calcium and phosphorus level.

Conclusion:

It should be considered that many abortions are spontaneous (idiopathic) with no actual or detectable causes (capricorn publication, web page 2312). However this study indicated both infectious and non-infectious causes.

1. Abortion due to Stress:

It is suggestive that the epidemic of caprine abortion after distribution of goats is mainly due to stress factors. This factor may differ from place to place. In some districts, it is mainly due to inadaptable climatic condition as many goats are supplied from terai to high and mid hill districts and vice versa. At the same time, long duration of transportation from teari to hills either by road or foot causes the abortion. The lack of grazing land or any other source of nutrition to those animals of comparatively high-density population has caused stress and abortion of pregnant animals.

There is general consideration that if 50-100 animals assembled at a place, there is always some 5-10% abortion when they go to their shed at evening. A PPR mass vaccination or seromonitoring if done in its shed, there is no problem while if animals of different sheds are assembled together to perform the same has caused the abortion as well. So, there is behavioral stress of goat, which may cause the abortion.

2. Abortion due to poisoning:

An investigation of the epidemic abortion in a commercial pocket area of goats revealed that the cause as poisoning due to chemicals (insecticides) which were used in a canal for the purpose of fishing but since that small running drinking water canal was only the source of drinking water to small ruminants. Another remarkable poisoning was Lantana camara in the periphery of east west highway

where there was deprived of brewers in the forest and goats were compelled to browse those poisonous plants. In both cases, there were remarkable signs of icteruis and high level of bilirubin in the serum of live does.

3. **Parasitic causes of Abortion:**

What ever may be the cause of abortion; there has been the epidemic mostly during monsoon. One of the immediate causes is parasitic load during these months. The infestation of Strongyles and coccidiosis is very high during these months, caused not only severe diarhhoea but also debility of animals, which may cause the expulsion of fetus. One of the parasitic causes is due to Posterior lumbar paralysis, which has been established to be due to satyriasis. The ineffectiveness of many of commercial Ivermectin products also causes the persistent parasitic stress to the animals.

4. **INFECTIOUS CAUSES OF ABORTION:**

4.1 Abortion due to viral infectious diseases:

There have been some PPR outbreaks, which usually causes the high mortality but also causes abortion in many pregnant animals.

4.2 Bacterial Causes of Abortion:

The laboratory investigation did not reveal the epidemic abortion due to Brucellosis except three cases of Brucella melitensis in Dadeldhura. However, Placental impression smear and culture showed the presence Compylobacter, is significant. Though laboratory test did not support in this study, but the clinical findings of sick animals ha given some indication of the causes of abortion due to Chlamydia, Toxoplasma and coxiella. Certainly there is need of further investigation of these causative agents.

4.3 Abortion due to Mycoplasma:

Out of 19 goat sera, 16 sera found to be positive for M.Capri agglutination test (IVRI, India 2001/2002). Also, out of 34 sera, 29 found to be positive for Mycoplasma (RVL, Dhangadhi 2002/2003). M.Capri in sheep and goat affects respiratory tract and joint and causing the moderate pathogen city of diseases like pleuropneumonia, arthritis, conjunctivits and ultimately abortion.

RECOMMENDATION:

Many abortions are spontaneous with no detectable cause. Because causes are various from shed to shed and differs from individual animas within a shed. However, This study indicates there is several manage mental aspects, which may cause abortion and can be prevented by applying proper care in management of the goat rearing. The infectious causes of abortion can be eliminated through proper medication. Certain tips are necessary to reduce the causes of the fatality and mortality due to abortion in and has been discussed a follows:

- The goats should not be given long transportation either by vehicle or by foot.
- The goats of hills should only be supplied to hills. These goats may not be adaptable to terai region. The plain region goats have very much cold stress when they are supplied to high hills.
- The no. of goats to be distributed in certain area should be decided by the availability of grazing land and other nutritional sources.
- Mixing and assembling of flocks together may give stress to the goats; such incidents should be minimized while drenching, vaccination or doing any other medication to the animals.
- The chances of poisoning through plant and water should be minimized through proper care and management of goats; especially care should be taken in the case of pregnant and new born goats.
- All the goats should be dewormed prior and during monsoon to reduce the parasitic load of the animals. Selection of most effective drug can be done through the advice of veterinarians.
- Though there is less incidence of PPR now a days but it may arise any time due to its transboundry nature and other viral diseases like goat pox, orf etc should be eliminated through proper disinfecting and caring of Animal's health.
- There is a chance of Buck to be infected by some contagious diseases and that may spread to the breeding does and hence such bucks should be removed from the flock. A periodical screening of such diseases is very important.
- A balanced diet must be required for all the animals. The practice of rearing goats only on grazing should be discouraged in stead 300-500 g concentrate with 4-5 hours grazing will be optimum.
- Mold contaminated feed is obviously dangerous to the animals This may lead to mass abortion in goats, so, such practice should immediately be eliminated.

Epidemic Disease Investigation: Epidemiological Findings of animal's disease outbreaks investigated during 2060/2061.

Regional Animal Disease Investigation Laboratory, Dhangadi (Far-western Region)

S.N.	Month	Place/ District	Species of animals	Disease diagnosed	Total animal population	No. of animal affected	No. of animals died	Morbidity (%)	Mortality (%)	Case Fatality
1.	Bhadra	Rajeepur	Goats	Babesiosis	2200	150	18	6.8	0.8	12
2.	Kartik	Kailali Kanchanpur	Poutry	Inclusion body hepatitis/ Leechi heart disease	50,000	5000	500	10	1	10
3.	Poush	Chipur Dadeldhura	Goats	Epidemic abortion (Stress and Mycotic)	1200	150	22	12.5	1.8	14.6
4.	Jestha	Amargadhi Dadeldhura	Cattle and Buffalo	Red Urine (Babesiosis, Theleria Anaplasma	250	10	2	4	0.8	20
5.	Jestha	Suda Daijee Kanchanpur	Goat	Lungworm Epidemic	2000	250	15	12.5	0.75	6
6.	Ashadh	Kanchanpur	Bovine	Foot and Mouth Disease ('o' type)	2000	50	8	2.5	0.4	16

Highlights of other major disease investigation and their case Report:

Regional Animal Disease Investigation Laboratory, Dhangadi (Far-western Region)

1. Investigation of Lung worm Epidemic in Goat: A Case Report-

Incidence:

There were several incidence and case reports of of goats having respiratory complications, coughung and sneezing during April and May, which is not supposed to be season of cold effect to the livestock. Farmers from Kailai and Kanchanpur reported several death among kids and goats as these cases were later, complicated with diarrhoea and Pneumonia (DIsease Investigation Of Goat programme, conducted by RVL, Dhangadhi 1998/1999). The complication couldnot be eliminated through drenching of Albendazole like other anthelmintics, neither symptomatic injectable treatment wiith antibiotics, antihistaminicas and cough syrup / powder could reduce the incidence.

In suda and Daijee V.D.Cs. of Kanchanpur district, several commercial farmers rearing around 200-300 goats complained such incidence to DLSO and RVL. A visit was made to the affected site and shed. The grazing lands and forest were inspected. The animals, which were clinically sick, were kept under supervision. The faecal, blood, Bacteriologicals swabs were collected and submitted for examination.

Epidemilogical Study:

The suda and daijee villages are situated near East West highway and of 5 km distance from the foot hills .The natural and community forests are still available for grazing by the livestocks. The small ruminants always graze at the humid places of canal and small rivers. The snail (Intermediate host) is found through out these places .The temperature remains as cold as 15 degree centrigate during winter while it goes more than 40 degree centrigrade during April /May. The infection has been reported in all ages groups of goats but the non carrier which has been newly introduced i.e. age group of six months are having high level of clinical symptoms. The population under theatening was up to 2000 goats while the morbidity is 12% and tme mortality is around 1%, the case fatality is 6%.

Pathogenesis and Clinical Findings:

Lungworm infection is otherwise called as verminous Bronchitis or Verminous pneumonia. Dictyocaulus filaria, protostrongylus rufescens and Muellerius capillaris are found to be affecting sheep and goats. Though D.filaria is recognised as pathogen but also muellerius capillaris is prrevalent worldwide. Dictyocaulus having direct life cycle in which adult females lin the bronchi lay larvated eggs that may hatch in the bronchi or in he host's faeces after having

coughed up and swallowed. The first stage larva in faeces develops to infective third stage larvae in minimum of 7 days but many take longer time depending ambient temperature and humidity. Grazing animal ingest infective larvae that than undergo 2 further molts while migrating to the lungs from intestine via. pulmonay arterial blood supply. Larvae emerge into the alveoli and migrate to the bronchioles and bronchi where they mature. The prepatent period is 5 weeks. Pastures become a source of infection early in the year as a result of larvae surviviving on the herbage or inthe soil during prervious winter, which they can do even at low temperature, or being grazed by apprently normal animals that carry light infection from previous years.

Muellerius sp. require slugs or snails as intermediate hosts, sheep and goats become infected by eating the intermediaste host. In this 10 weeks may be required. The pathogenic effect of lungworms depends on their location within the respiatory tract, the no. of infective larvae ingested and the animal's immune system. During prepatent phase, the main lesion is blockage of small bronchioles and Bronchi by infiltrate of eosinophilic in response to the developing larvae, these results in obstruction of airways and collaspe of alveoli distal to the block. In the patent phase, the adult in the segmental and lobaar bronchi cause a bronchitis with eosinophils, plasma cells, lymphocytes in the bronchial wall and a cellular exudate, frothy mucus and adult nematodes in the lumen. The bronchial irritation causes marked coughing, a notable feature of lung worms. Later development of chronic, nonuppurative eosinophilic, granulomatous pneumonia in response to eggs and first stage larvae aspirated into alveoli and bronchioles.

Clinical findigs: The clinical signs of lungworm infection range from moderate coughing with slightly increased respiratory rates to severe persistent coughing, respiratory distress and respiratory failure, reduced weight gain. The most consisitent signs are tachypanea and coughing. The reinfection in adult is usually seen in autumn. Pulmonary signs usually are not associated with M. Capillaris.

Control:

- Grazing at the place of nonhumid grasslands may be reliable. Combinig a grazing routine with strategic anthelmintics with prolonged period of activity (e.g. Ivermectin effective up to 3 weeks) may now make this a more reliable approach.
- In the case of epidemic in Suda and daijee, administration of tetramisole orally at the dose rate of 10 mg/kg of body weight and Ivermectin injection at the rate of 1 ml/25 kg of body weight has given a good approach in many flocks.

- Also, Levamisole with Ivermectin injection was proved to be effective in many cases.

- The use of diethylcarbazine orally 2-4-tabs/ day has been found to be supportive in controlling the disease.

2. Investigation of Blood protozoans in livestock of Dadeldhura:

Incidence:

Red Urine cases are quite commonly reported from all the mid and high hills of far western region especially: Dadeldhura, Baitadi, Doti and Bajhang. The etiology is yet to confirmed as several etiological factors are involved from place to place. Such disease has been reported throughout the year from all above-mentioned districts.

The crossbred of Jersey and Holestein in amargadhi municipality showed the clinical symptoms of high fever (104-105 c), coffee colored urine (Haemoglobinuria), lacrimation, recurrent pyrexia, drop in milk yield and heavily infested ticks. The antibiotics and antipyretics therapy didnot show any cure to the sick animals. DLSO, Daldeldhura and RVL combined team visited the site and examined the clinically sick animals. Blood slides, EDTA blood, whole blood, ticks from body surface of tweleve animals were. These samples were fixed at DLSO laboratory and brought to submit in RVL, Dhangadhi.

Epidemiology:

The red urine casewas reported by several farmers of the municipality to DLSO, Dadeldhura. Amargadhi municipality is 120 km away from the East west highway, connected through feeder road. The altitude is around 1600 meteres and natural and community forests are just around the market and the shed of the animals. Animals are usually fed with the fodder brought from the forest. The temperature ranges 7-30 degree centigrade. Winter is very cold with snowy winds. The affected animals were all cross bred..There was no death. Clinical signs comprised of anoexia, red urine (hemoglobinuria), high fever, dry muzzle, constipation with blood-tinged faeces, drop in milk yield and reccurence of fever.

Diagnosis:

The blood slides were fixed with methanol at the same place of dadeldhura, later on in RVL, Dhangadhi, all 24 slides (2 from each animals) were stained with Giemsa and examined under oil of emersion. The same slides were sent to CVL, Kathmandu for reconfirmation.

Result:

Parasitological Examination of Blood protozoans:

S.N.	Name of the owner	Address	No. of the animals	Species of animal	Sex	Breed	Result
1.	Bhoj Bahadur Tamrakar	Dadeldhura	2	Cattle	Female	Cross	Anaplasma marginale
2.	Man Bahadur sahu	Amargadhi	3	Buffalo	Female	Murrah	Theleria Anaplasma marginale A. Centrale
3.	Narendra Bahadur Bohara	Amargadhi	4	Cattle	Male Female	Cross	T. Parva A. marginale B. A.centrale
4.	Khagendra Kapad	Amargadhi	3	Cattle	Male and Female	Cross	Babesia bovis T. parva A.marginale B.centrale

Recommendation and control:

- Since the forest is generally nearby the village and livestocks are brought to those areas, there is always a chance of infestation of ticks to the animals. Though these ticks are carrier of protozoa, animals' donot immediately show the symptoms, but it becomes sick during lean perion when there is stress and lean period, usually during summer or monsoon. The same situation of tick infestation during 2003 in goats of Chaumala showed the epidemic. Hence, the major control measure can be the control of ticks.

- This epidemic of blood protozoans was controlled by Berenil 3-5g deep intramuscular and was repeated in few cases after the alternative day. The supportive therapy with B-complex and liver tonics was also administered. Almost all animals were cured but there were severe drop in milk yield in milch animals, which could hardly be reassumed during the milking period.

3. *Drug trials for anthelmintics in Goats:*

Objectives:

There are several anthelmintics of varoius brands and commeercial preparations availabe at Nepalese market. Farmers used to report the inefficacy of the drug, administered in their animals though the price is competetive. However there are several anthelmintics, whose price has been lowered dramatically. The repeated faecal examination in RVL also suggests the inefficiency of many anthelmintics. Hence this trial was carried to find the efficacy of the drugs available in mthe local market.

Limitation:

The trials were done on the field basis; no animal has so far been kept under laboratory superisison. The goats were selected at randomly irrespective to age; sex and breed but most of the animals were clinically suffering from the parasitism. The trials were conducted duiring different seasons.

Methodology:

After the selection of the sick and helminth suspected animals in the farmer's house, faecal samples were brought to Laboratory for parasitological examination .The sites were selected from plain to mid hills viz. suda, Tribhuvan basti, Beladandi and krishnapur of kanchanpur district and Bhasu, malakheti, Chaumala and Matiyari of kailali districts. Totally, the no. of the flock were around 2000. All the farms were commercial, keeping more than 50 goats. The goats were reared under semi intensive system in which 8-10 hours of grazing is preferable. Also, very small amount of concentrtae feed is generally provided to pregnant and breeding stock.

After 10-15 days of drenching (in case of helminths positive cases), the condition of the animals was clinically inspected and faecal samples were collected and examined in Laboratory to confirm the efficacy of particular drug. There were some cases, which were readministered in the case of nil efficacies even after 15 days.

The anthelmintics drugs used were Ivermectin, Fenbendazole, Oxyclozanide with Levamisole and Tetramisole with the dosage rate of 1ml/25 kh for Ivermectin, 10 mg/kg for all other drugs used. These were commonly used medicines either purchased by farmers themselves or dispensed on the prescription of clinician in far western region.

The owner of the flock too confessed the use of same medicine in their farms, usually twice a year or sometimes once in a year.

The faecal examination was done by sedimentation, floatation, larva culture, Lungworm examination by Baerman's method.

Result and Discussion:

Totally 42 farm houses (or flocks) were used for drenching of anthelmintics to about 2000 goats under the programme of poverty alleviation. In most of the cases, strongyles, strongyloides, Trichuris, Coccidia, Monenzia were tne common mixed infestation in goats while there were also the cases of FD|ascioliasis, Paramphistomiasis and Lung worm infection in many goats (Annual Technical Book, RVL2003-004).

While administering the anthelmintics, Albendazole and Oxyclozanide were used inthe case where the sample was positive for Fascioliasis and paramphistomiasis but other products were given on the basis of history of drug administration. There were few cases of failure of first drug administered (mostly in the case of Albendazole), repeated with the dosage of Ivermectin injection along with oral administration of Albendazole and Fenbendazole or Tetra misole as well e.g. in the case of lungworm infestation.

Scoring:

SN 1	Name of medicines 2	No. of trials 3	No. of positive cases before Medication 4	No. of positive cases after medication 5	Score 6=4-5	% of efficacy	dosage	Prioritization
1.	Ivermectin	7	79	13	66	83.5%	1ml/25kg	I
2.	Fenbedazole	3	32	8	24	75%	10mg/kg	III
3.	Albendazole	4	44	22	22	50%	10mg/kg	V
4.	Oxyclozanide with Levamisole	5	47	13	34	72.4%	10mg/kg	IV
5.	Tetramisole	4	49	10	39	79.5%	10mg?kg	II

Recommendation:

- The highest scorer was Ivermectin (83.5%) and the lowest effective drug was Albendazole (50% eeficacy).
- Tetramisole was too effective followed by Fenbendazole and Oxyclozanide. Though there are drugs havinfg the combination of Oxyclozanide and Levamisole to counter the mixed infection of roundworm and liverfluke in the goat, which could be a better choice instead of Ivermectin, but since the drug available in the market having the fewer efficacies was not up to the useful.
- In the case of mixed infestation of Liverfluke and multiple roundworm and tapeworm, oral tetamisole along with Ivermectin s/c is more useful than any other drugs.
- However, we can't blame directly to the composition made from generic compounds, it is always advisable to administer the drug which is having qualitative product in the market because this trial intends to make aware to clinician and farmers who use the drug without knowing their efficacy and ultimately the failure suffers farmers through loss in productivity and mortality of the animals.

4. Khari Disease Investigation:

Introduction:

Khari disease has been affecting the buffalo rearing in Baitadi and Darchula districts for more than 30 years. In this disease, the milk production drops to nil, animals get deblitated, and there will be protrusion of bones and skin. There is a postule and dusty materials come out from body surface as well as from hooves. Finally animals become downer and die after a long sickness .If animals become pregnant or being treated with mineral mixture along with Ivermectin or with supplementation of green fodder during monsoon, the animals get cured automatically but may get infected during subsequent year. Finally animals die after 4-5 years.

Use of tonophosphan, Ivermectin or any other mineral mixture has the effect over sick animals but there is reccurance in same animal during following year (Dr. Ratala, U.M. singh et al). The biochemistry of seurum of healthy animals and unhealthy animals has given the evidence of deficiency of some macro and micronutrients, this has not pointed whether the disease itself is infectious or noninfectious, need to have elaborative study on that. (Dr.S.N.Mahato)

Objective and Methodology:

- RVL, Dhangadhi is always trying its best to find the etiology ofKhari disease but since it needs an elaborative study, this laboratory can't make a good result alone. A collaborative effort is necessary in this regard. This needs not only the efficient manpower but also a handsome amount of budget. With the constraint of budget of investigation in RVL,
- We have made attempt to visit both districts and digital photographs were taken. This study has focused on laboratory examination of soil so that the deficiency could be indicated.
- The bacteriological and fungal cultures of the hooves dust were perfomed to identify the existing microorganism.

Result & Discussion:

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

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

The above table shows the situation of the soil Phosphorus content . Though The sample is very less in no, however it gives the indication of low phosphorus content of the soil , especilly the soil where the fodder " Ganjyo" grows and other plants in the garden which are used as feed materials of the livestock.

Bacterilogical Examinations:

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

The above Bacteriological Examination indicates that the hoof scrap contains the presence of Fungus. There was the evidence of systemic fungal infection too, which might generalise the situation of the disease. Of course, before arriving into any conclusion, a thorougho investigation is required.

Recommendation:

The test of soil shows the deficient of Phosphorus in soil and Bacteriology of the hoof scrape shows the presence of systemic fungus. This could be a step to continue further investigation of Khari disease.