ANNUAL TECHNICAL REPORT

2061/062 (2004-2005)

HMG/NEPAL
Ministry of Agriculture & Cooperatives
Department of Livestock Services
Directorate of Animal Health

Central Veterinary Laboratory (CVL)

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ANNUAL TECHNICAL REPORT

F.Y. 2061-2062
[2004-2005]

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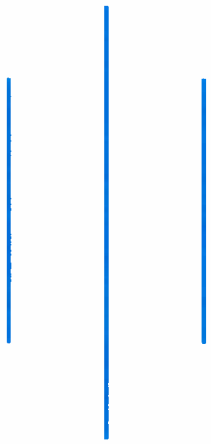

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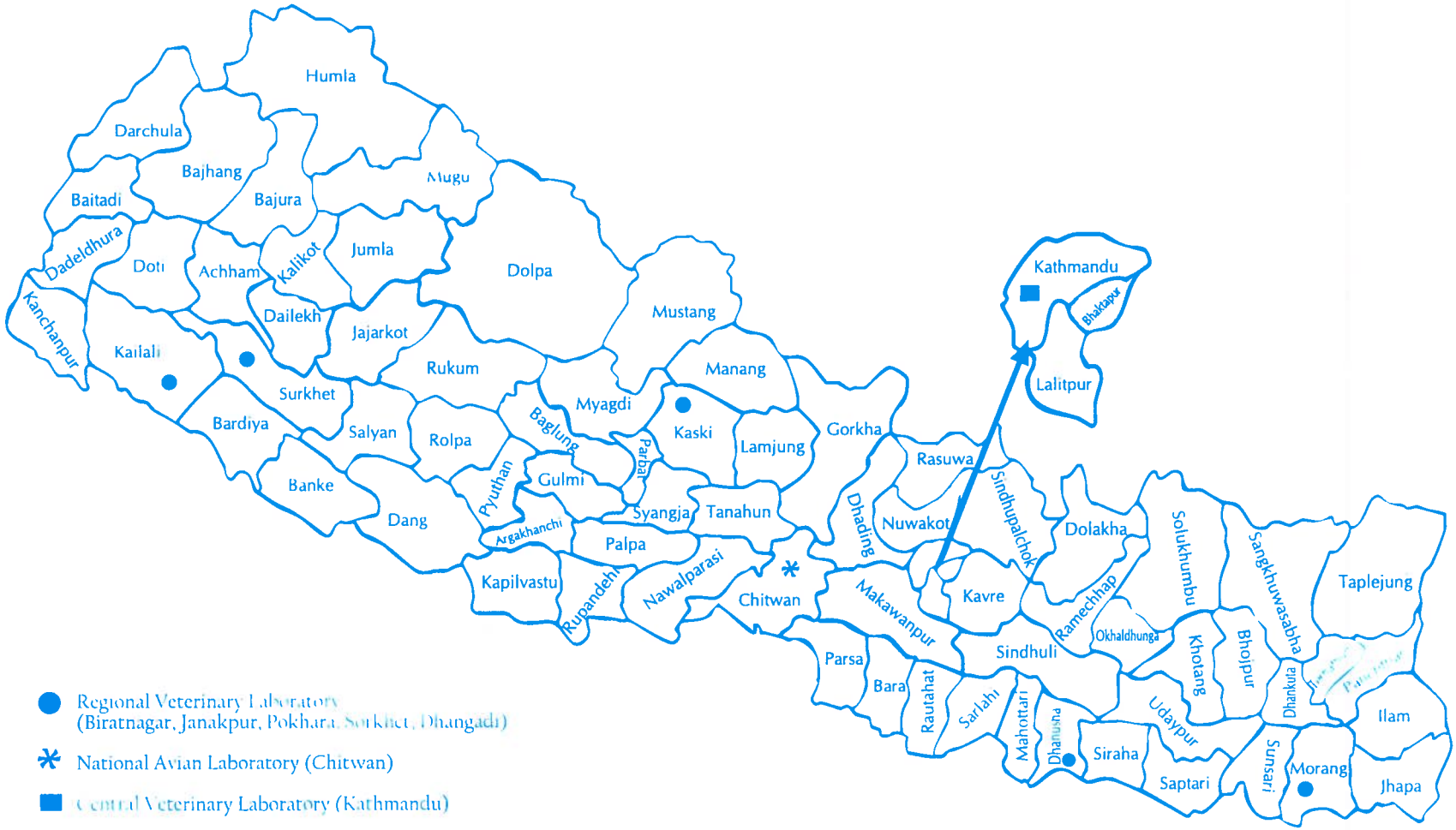
Location of CVL, RVLs & Avian Laboratory (Nepal).



CVL/VLC, Kathmandu

Foreword

Livestock sector is one of the important components of subsistence agriculture system in Nepal. Its contribution has been encouraging in national economy rating 31% to AGDP with the expected contribution up to 45% by the year 2015 as mentioned in Agriculture Perspective Plan (APP). Nepal is rich in animal resources but the output from them has been lagging than expectation. There are a number of obstacles among which animal health is an important issue. Animal health care has always been secured through symptomatic medication. Symptomic treatment has been comparatively costlier and has also led to injudicious use of drugs. This has resulted in higher cost of production of livestock commodities. However, treatment based on laboratory diagnosis has been practiced to some extent by some practitioners, which has been considered as a good initiation.

The role of veterinary profession has been modified tremendously in the course of development worldwide. Nepal has been the member country of World Trade Organization (WTO), and to meet with its requirements, we are compelled to follow the guidelines provided by Office International des Epizootics (OIE) for the provision of Sanitary and Phytosanitary (SPS) agreement. It secures the right of the member countries to apply measures necessary to protect human, animal, and plant life and health, which are based on scientific principles and evidences. In this way, the role of veterinary laboratories remains pivotal among others, which are ensured by the state veterinary services of a country.

Keeping in view the benefits of Nepalese farmers from the opportunity of trade globalization, HMG/Nepal has been paying due attention in strengthening the veterinary laboratories of the country and also we are committed in providing valid test results to our farmers for which we often compare our test results with OIE reference laboratories and other referral laboratories around the world.

This issue of annual technical report consists of various laboratory test procedures and disease investigation activities conducted at Central Veterinary Laboratory (CVL) Tripureshwor, its five Regional Veterinary Laboratories (RVLs) located one each in each development zone viz; eastern (Biratnagar), central (Janakpur), western (Pokhara), mid-western (Surkhet), and far-western (Dhangadhi) and National Avian Laboratory, Bharatpur, Chitwan in the fiscal year 2061/062 (2004-2005).

I would like to express my cordial thanks to all the regional veterinary laboratories as well as National Avian Laboratory for providing their annual progress report and technical articles in due time. I would also like to thank Dr. Ganesh Raj Pant, Dr. Poornima Manandhar, Dr. Kedar Bahadur Karki, Dr. Karuna Sharma Bhattarai, Dr. Salina Manandhar and Dr. Pragya Koirala Sharma for their support in publishing this report. My special thanks goes to Dr. Vinay Kumar Karna, CVL, Dr. Rajesh Yadav of Directorate of Livestock Marketing & Promotion, Mr. Prakash Devkota, and Mr. Asal Tamang (Technicians, CVL) for their sincere contribution in compiling and editing the report in this form.

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

Dr. Rebati Man Shrestha
Chief Veterinary Officer
Central Veterinary Laboratory

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INTRODUCTION

Central Veterinary Laboratory (CVL) was established in 1995 under Department of Livestock Services, HMG/Nepal with the aim of conforming healthy national herd/flock of livestock and poultry based on scientific evidence of animal diseases diagnosis. From its inception till to date, CVL has been involving in providing diagnostic services of animal diseases, investigational activities and caring public health issues indirectly which is limited but of great importance. The direct benefit has been experienced in the field of veterinary medical care based on valid laboratory test results. To achieve these multidimensional activities, CVL works through a variety of disease diagnostic procedures. The major diagnostic applications are achieved through its different disciplinary set up as Pathology, Parasitology, Microbiology, Serology, Haematology and Biochemistry units.

With the advancement in disease diagnostic technique worldwide, modern disease diagnostic technologies comes continuously. CVL is always aware in adopting modern disease diagnostic technologies regularly. We have now Molecular Diagnostic Unit to achieve more precise diagnosis of animal and poultry diseases. At present the molecular based diagnosis of Avian Influenza is in the course of advancement. Similarly, we have also worked with Haemorrahgic Septicaemia in the past with encouraging result.

The multidisciplinary approach in disease diagnosis is followed with the principle of verifying a disease through tentative diagnosis, presumptive diagnosis and finally confirmative diagnosis. For this, OIE standard test protocols for disease diagnosis are followed in many instances. Sometimes, we are unable to adopt the precise test methodologies required for a particular disease and we refer relevant samples, in such cases, to OIE reference laboratories as well as other relevant laboratories of the country and abroad. The test results are subjected for standardization in this way.

To provide diagnostic facilities throughout the country, CVL works through its five Regional Veterinary Laboratories (RVLs) located one each in each of the development region of the nation viz; eastern (Biratnagar), central (Janakpur), western (Pokhara), mid-western (Surkhet) and far-western (Dhangadhi) as well as through National Avian Laboratory established in 2004 A.D. at Bharatpur, Chitwan. To make the laboratory services easier in the forthcoming days, CVL has established 15 basic laboratories in 15 potential districts of the country. These basic laboratories are able to perform microbial cultures. Samples that could not be processed in the aforementioned laboratories by means of insufficient facilities are referred to CVL.

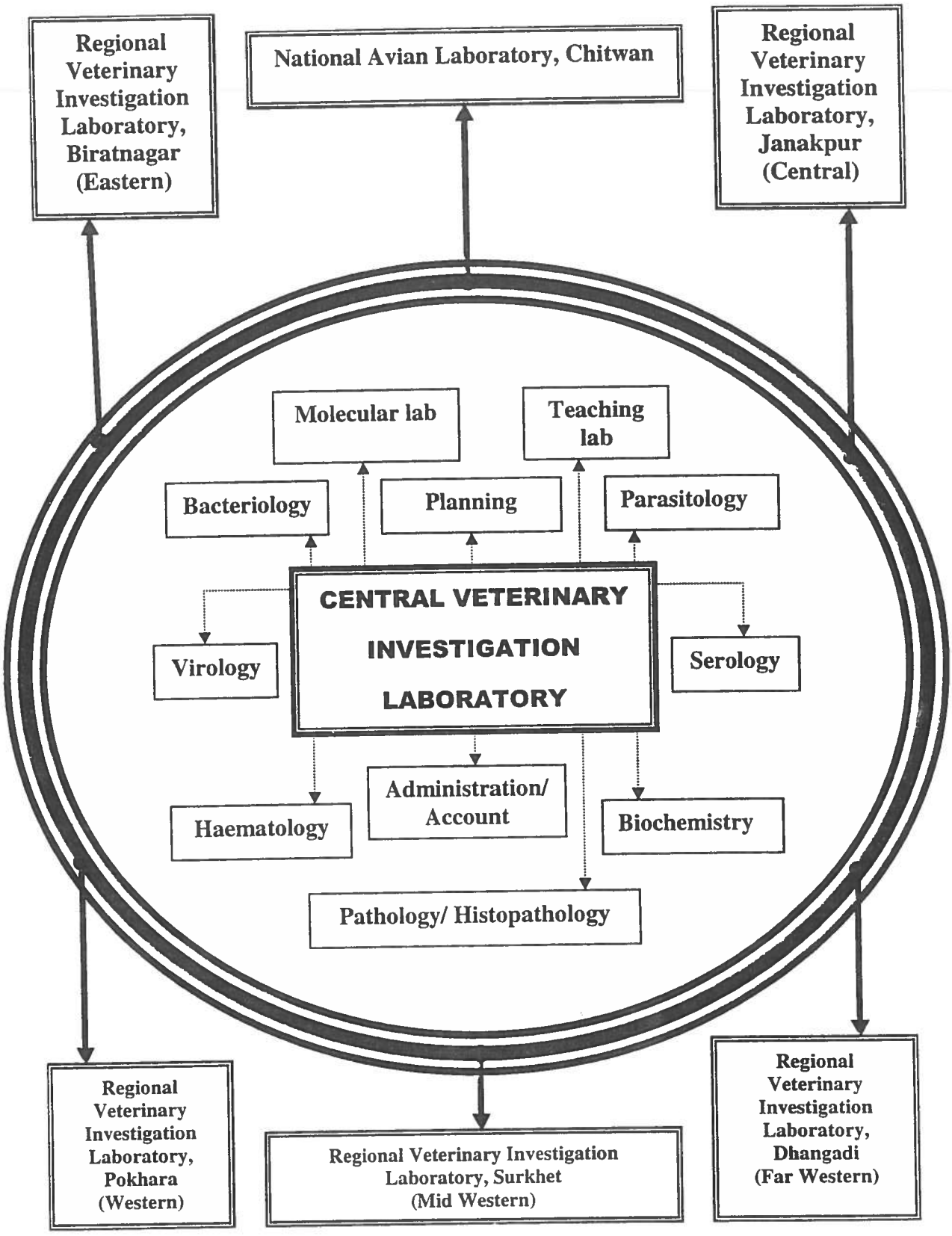
Objectives

The role of veterinary profession has been modified tremendously in the course of civilization worldwide. Its effect has also sensitized Nepalese societal life as well as Nepalese economic activities related to livestock sector. An immediate outcome of such a situation is evidenced with the fact that Nepal is now the member country of World Trade Organization (WTO). To

meet with its requirements, Nepal is compelled to follow the guidelines provided by Office International des Epizootics (OIE) for the provision of Sanitary and Phytosanitary (SPS) agreement that seeks scientific procedures and evidences in the course of disease diagnosis as well as production chain. In such situation, the challenges and opportunities ahead a veterinary laboratory are great. To maintain pace between the present demand of the situation and advancement in diagnostic technologies, CVL works with the following objectives.

- Support the national disease control and surveillance programme.
- Act as national referral laboratory
- Conduct disease investigation and research.
- Acquire, adopt, upgrade and disseminate new as well as different diagnostic test methodologies for animal and poultry diseases.
- Assist in formulating epidemic control strategies
- Capacity building of veterinarians and paraveterinarians by organizing training on laboratory technology
- Strengthen and coordinate regional and district laboratories
- Collate, analyze and maintain national livestock disease database and strengthen animal health information system
- Disseminate information concerning animal and avian diseases to national and international organization.

Organization chart



Annual Work Programme & Annual Progress of CVL (2061-062)

S.N.	Activities	Unit	Target	Budget allocated	Achievement	Progress %
1.	**Diagnostic Services**					
1.1	Parasitology	Number	1600	80000.00	1633	100
1.2	Microbiology	Number	2000	270000.00	2627	100
1.3	Pathology	Number	1200	237000.00	1340	100
1.4	Serology	Number	4500	735000.00	6403	100
1.5	Haematology	Number	500	75000.00	506	100
1.6	Biochemistry	Number	800	130000.00	832	100
1.7	Molecular Diagnosis	Number	30	118000.00	24	80
1.8	Rabies Diagnosis	Number	30	60000.00	30	100
1.9	Dispatch of samples to other laboratories	Number	500	110000.00	500	100
2.	**Disease Investigation and Surveillance Program.**					
2.1	Investigation & Surveillance on Infertility in Cattle & Buffalo	Times	12	160000.00	12	100
2.2	Outbreak Investigation	Times	12	220000.00	14	100
2.3	Investigation and surveillance of Japanese Encephalitis	Times	6	140000.00	5	83
2.4	Serum Bank Management	Times	12	165000.00	12	100
2.5	Investigation and surveillance of Sarcosistosis in Buffalo Meat	Times	3	75000.00	3	100
2.6	Investigation and surveillance of Khari	Times	6	240000.00	7	100

	Disease					
2.7	Surveillance of Avian Influenza	Times	6	45000.00	7	100
2.8	Surveillance of RP	Times	6	50000.00	7	100
2.9	PPR Surveillance	Times	6	43000.00	7	100
2.10	Pen Side test of PPR	Times	3	0.00	3	100
2.11	Sero Epidemiological Surveillance of PPR	Times	6	0.00	9	100
3.	**Teaching Lab Program**					
3.1	Teaching Lab Management	Times	12	175000.00	12	100
3.2	Training on Laboratory Technology (3 months/ JT, JTA/10 Persons)	Time	1	460000.00	1	100
4.	**Supervision and Monitoring Program**					
4.1	Follow-up & Reporting of Laboratories	Times	12	85000.00	12	100
5.	**Workshop Program**					
5.1	Technical Workshop on disease investigation	Times	1	70000.00	1	100
5.2	Participation in regional workshops (5 regions)	Times	5	40000.00	5	100
5.3	Workshop on Prog. & Budget of the next F/Y	Times	1	35000.00	1	100
6.	**Publication**					
6.1	Annual Technical Report	Times	1	150000.00	1	100
7.	**Contract Service**					
7.1	Sweeper & Gardener	Times	3	25000.00	3	100
8.	**Purchase**					

9.1	Technical Books & Journals	Times	3	40000.00	3	**100**
9.3	Elisa Reader	Set	1	800000.00	1	**100**
9.4	Automatic Tissue Processor	Set	1	200000.00	0	
Total				**5033000.00**		**95**
Administrative Expense				**3359000.00**		
Grand Total				**8392000.00**		

Man Power Situation of Central Veterinary Laboratory

(F/Y 061-62)

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	2	2	-	
3.	Veterinary Officer	G III	5	5	-	
4.	Vet. Technician	NG I	7	7	-	
5.	Stock Man	NG III	8	7	1	
Total Technical			**23**	**22**	**1**	
B.	***Administration/Account***					
1.	Typist	As per efficiency	1	1	-	
2.	Accountant	NG I	1	1	-	
3.	Kharidar	NG II	1	1	-	
4.	Driver	Light Vehicle	1	1	-	
5.	Peon	-	6	6	-	
Total Administration			**10**	**10**	**-**	
Grand Total			**33**	**32**	**1**	

Staff of Central Veterinary Laboratory
(At the end of F/Y 2061/062)

S.N.	Name of Staff	Post	Class	Starting from	Remarks
1.	Dr. Rebati Man Shrestha	CVO	G.I	058.11.02	
2.	Dr. Ganesh Raj Pant	SVO	G.II	055.09.23	
3.	Dr. Poornima Manandhar	SVO	G.II	057.12.22	
4.	Dr. Kedar Bahadur Karki	VO	G.III	060.10.21	
5.	Dr. Karuna Sharma Bhattarai	VO	G.III	062	
6.	Dr. Vinay Kumar Karna	VO	G.III	059.01.22	
7.	Dr. Pragya Koirala Sharma	VO	G.III	062	
8.	Dr. Binu Shrestha	VO	G.III	059.09.29	
9.	Mr. Asal Bahadur Tamang	VT	NG.I	052.04.01	
10.	Mr. Ashok Pd. Shrestha	VT	NG.I	052.04.01	
11.	Mr. Prakash Devkota	VT	NG.I	060.08.01	
12.	Mr. Bal Bdr. Kunwar	VT	NG.I	053.02.24	
13.	Mr. Tek Bahadur Air	VT	NG.I	058.09.04	On Leave
14.	Mr. Gyan Bahadur Bogati	VT	NG.I		
15.	Mr. Braj Kishor Thakur	VT	NG.I	060.07.24	

S.N.	Name of Staff	Post	Class	Starting from	Remarks
16.	Mr. Laxman Sijapati	S.Man	NG.III		
17.	Mr. Mukunda Acharya	S.Man	NG.III		
18.	Mr. Purna Maharjan	S.Man	NG.III	053.12.20	
19.	Mr. Hari Pd. Pyakurel	S.Man	NG.III	054.12.02	
20.	Mr. Prahlad Basnet	S.Man	NG.III	057.12.01	
21.	Mr. Hari Bhakta Karki	S.Man	NG.III	059.01.01	
22.	Mr. Bhimsen Adhikari	S.Man	NG.III	057.08.01	
23.		S.Man	NG.III		
Administration/Account					
24.	Mrs. Kamala Shrestha	Typist	NG.I	055.07.11	
25.	Mr. Nim Bahadur Woli	Account.	NG.I		
26.	Mr. Laxman Kumar Khanal	Kharidar	NG.II		
27.	Mr. Macha Kaji Maharjan	Driver	Lo. lev.	055.07.01	
28.	Mrs. Chiri Maya Maharjan	Peon	Lo. lev.	055.10.01	
29.	Mr. Santa Raj Budathoki	Peon	Lo. lev.	059.11.01	
30.	Mrs. Bhima Acharya	Peon	Lo. lev.	055.04.01	
31.	Mr. Hari Gobinda Shrestha	Peon	Lo. lev.	059.11.06	
32.	Mr. Chandra Bdr. Rana	Peon	Lo. lev.	056.08.23	
33.	Mr. Anoj Bajracharya	Peon	Lo. lev.	058.11.01	

Pathology unit

Introduction

Pathology discipline in a biomedical laboratory acts as the welcome door wherefrom disease diagnosis process begins. In the Central Veterinary Laboratory (CVL), this discipline covers two different kinds of disease diagnostic approach: Post-mortem examination (Post-mortem unit) and Histopathology technique (Histopathology unit). Being a referral veterinary laboratory of the nation, CVL receives a wide variety of samples from all over the country either directly or through respective Regional Veterinary Laboratories (RVLs). Besides, District Livestock Service Offices (DLSOs), veterinary practitioners, hatcheries as well as farmers also deliver samples for disease diagnosis.

While post-mortem examination provides a tentative diagnosis of a disease, the histopathology technique always lays a presumptive diagnosis. In this way, it is essential to circulate necropsy and sometimes biopsy samples through various laboratory disciplines. It is the post-mortem discipline that decides over collection of appropriate sample and possible pathologic cause of the disease. Thereafter it delivers the samples to appropriate discipline of the concerned laboratory.

Histopathology unit processes the samples and provides the result over 7-10 days period. Though it has been regarded as an obsolete laboratory procedure nowadays, it is of prime importance in developing countries likes Nepal and also its diagnostic value can not be underestimated in the field of disease diagnosis. On one hand, we cannot go through virology technique for all of the viral diseases due to some relevant causes. On the other hand, we cannot deny the indispensable role of Histopathology in the diagnosis of chronic and obscure diseases as well as neoplastic diseases.

Post-mortem examination

The total post-mortem samples received during the fiscal year 2061/062 were seven hundred and sixty-two. Of them, Six hundred and ninety-two samples (91%) were received from poultry and rest 70 (9%) 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 farming. The annual disease occurrence pattern in different animals and birds has been presented in tabular form as shown in Table 1.

Histopathology

Histopathology unit received a total of 115 samples from different animal species during the fiscal year 2061/062. Poultry contributes a total of 46 (40%) samples and the samples received from different species of animals and birds other than poultry are 69 (60%). Later includes 26 cases from buffalo, 12 cases from dog, nine cases from pig, eight cases from goat, four cases from fish fingerling, two cases from duck and

one case each from elephant, leopard, peacock, quail, rabbit, sheep, tiger and wild bird.

It has been essentially realized that the histopathology unit receives the samples without adequate information about a disease/process and species/strains of animals/birds. Also this unit receives irrelevant and incomplete set of samples. Example, the cases of non-specific bacterial infections, Salmonellosis, Colibacillosis, Gumboro disease, Ranikhet disease etc. need not necessarily be assigned for Histopathological tests. Among them, bacterial diseases can be confirmed through bacteriology while diseases like Gumboro, Ranikhet etc. can be tentatively diagnosed on the basis of distinct post-mortem lesions and confirmed through Virological techniques. In the present report analysis, some of the diseases have been simply interpreted as the general pathological conditions like hemorrhage, inflammation and necrosis due to obvious reasons.

Among poultry, twenty-four samples of broilers, 16 samples of layers and six samples of parent stocks were received. Among the samples received from broilers, eight cases were interpreted as the case of Fatty changes and Inflammation of liver, four cases of Inclusion body hepatitis-hydropericardium syndrome (IBH-HPS), two cases of Avian Encephalomyelitis (AE), one case each of Inflammation and cystic degeneration of Bursa and neoplasia of unknown origin. Six cases tentatively diagnosed for avian encephalomyelitis and one case for Sarcosporidiosis was found negative for their occurrence and a case of broiler was received without any information. Therefore, interpretation could not be established. Among layer birds, six cases were interpreted as visceral form of Marek's disease (MD) followed by three cases of classical form and two cases for its non-occurrence. Rest five samples interpreted were, one each as Lymphoid Leukosis (LL), IBH-HPS, AE, Inflammation and cystic degeneration of Bursa and Inflammatory changes. The three cases of parent stock were interpreted as Fatty changes and inflammation of Liver, and one each for visceral form of MD, AE and pneumonic lesions.

The samples from the buffalo were all intended to investigate for the occurrence of microscopic form of Sarcosporidia in the muscle and histological interpretation of these samples revealed 16 samples containing the parasite with 10 samples showing its non-occurrence among a total of 26 cases. Similarly, of the twelve samples of dog, four cases were interpreted as the cases of canine transmissible venereal tumor (CTVT), three cases of Leomyoma of vulva, and one each case of Adenoma of vulva, Squamous cell carcinoma, and Rhabdomyomma and chronic inflammation. Samples from case of ventral hernia were not processed as no remarkable pathological lesions were appreciated.

In case of pig, eight cases were interpreted as Haemorrhagic inflammation (septicaemic condition but could not be appreciated as either the case of Haemorrhagic septicaemia or Classical swine fever, while one case was interpreted as Gastroenteritis, which was tentatively diagnosed as Transmissible gastroenteritis.

Among eight cases of goat, two cases were interpreted for Sarcosporidiosis and one case of poisoning. The two tentatively diagnosed cases of Peste des Petits Ruminant (PPR) were interpreted as the case of Pneumonia and Gastroenteritis and three cases

were not concluded. Later was intended to diagnose Brucellosis provided placenta as pathological specimen.

The four cases of fingerlings were received from Master's scholar from Tribhuwan University to appreciate the occurrence of Trematodes, of which one case was interpreted as suspected and the same in the rest three samples could not be appreciated. Both the cases of ducks were diagnosed as hepatitis. One of the each case of rabbit was diagnosed as parasitic gastroenteritis, peacock as Pasteurellosis, quail as hepatitis and elephant as Z-N fast bacteria. Each one sample from leopard, sheep and tiger could not be interpreted either for the sample putrefied or no relevant organs/tissues or information was available.

Table 1: Disease occurrence pattern in commercial poultry in Kathmandu Valley

S.N.	Disease/Condition	1	2	3	4	5	6	7	8	9	10	11	12	Sub-Total			Total
														B	L	P	
1	Colibacillosis	21	18	11	5	3	8	11	23	13	39	31	16	199	1	-	248
1.1	Omphalitis	1	-	2	2	1	-	-	2	2	1	1	1	13	-	-	
1.2	Colisepticaemia	11	1	-	-	-	2	-	1	-	-	7	1	23	-	-	
1.3	Coligranuloma (Hjarre's Disease)	-	-	-	1	-	-	-	-	-	-	3	-	4	-	-	
1.4	Early Chick Mortality	1	-	-	-	-	-	1	-	2	2	2	-	8	-	-	
2	Infectious Bursal Disease (IBD)	11	7	7	6	2	4	1	5	8	11	5	3	70	4	1	124
2.1	IBD+ Colibacillosis	7	3	1	1	1	-	3	2	1	1	2	2	24	-	-	
2.2	IBD+CRD	1	1	3	1	-	-	1	1	1	-	-	-	9	-	-	
2.3	IBD+IBH-HPS	3	-	1	-	-	-	-	1	-	1	-	-	6	-	-	
2.4	IBD+ Fowl Cholera	-	-	-	1	-	-	-	1	-	-	-	-	2	-	-	
2.5	IBD+RD	-	-	-	1	-	1	-	1	1	-	-	-	4	-	-	
2.6	IBD+Salmonellosis	-	-	-	-	-	-	1	-	-	-	1	-	-	2	-	
2.7	IBD+Coccidiosis	1	-	-	-	-	-	-	-	-	1	-	-	1	-	-	
2.8	IBD+RD+Colibacillosis	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	
3.	Undiagnosed	4	3	8	-	4	5	6	2	3	6	6	5	52	9	2	63
4.	Salmonellosis	2	2	1	-	2	2	4	10	7	4	4	4	42	9	3	54
5	IBH-HPS	9	2	4	-	2	2	-	2	1	-	-	1	23	2	-	44
5.1	IBH+ Colibacillosis	6	-	-	-	1	-	-	1	4	1	-	-	13	2	-	
5.2	IBH+ Coccidiosis	1	-	-	-	-	-	-	-	1	-	-	-	2	-	-	
5.3	IBH+ Salmonellosis	1	-	-	-	-	-	-	-	-	-	-	1	2	-	-	
6	Caecal coccidiosis	2	1	-	1	1	-	1	-	2	4	1	1	14	1	1	30
6.1	Intestinal coccidiosis	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	
6.2	Coccidiosis+ Colibacillosis	1	1	2	1	3	-	-	1	-	1	-	-	10	-	-	
6.3	Caecal coccidiosis+ Necrotic enteritis	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	
6.4	Intestinal coccidiosis+ Necrotic enteritis	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	
6.5	Caecal + Intestinal Coccidiosis	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	
7.	Chronic Respiratory Disease (CRD)	2	2	-	1	-	-	1	-	2	2	-	1	11	1	-	28
7.1	Complicated CRD	-	1	2	1	2	-	1	1	1	1	4	2	16	-	-	
8	Aflatoxicosis	-	-	-	-	-	1	-	2	2	-	1	1	7	3	1	20
8.1	Aflatoxicosis+ Colibacillosis	1	-	-	-	-	-	-	-	5	3	-	-	9	-	-	
9	Stress condition	2	3	-	-	4	2	3	2	-	-	-	-	16	0	0	16
10.	Marek's (Classical)	1	-	3	-	1	-	-	1	1	-	-	1	0	8	0	14
10.1	Marek's (Visceral)	-	-	1	3	-	-	-	-	-	-	-	-	0	4	0	
10.2	Marek's Disease+ Ascarid Infestation	-	-	-	-	-	-	-	1	-	-	-	-	1	1	0	
11	Ranikhet Disease	2	-	1	2	2	-	-	-	1	-	-	1	9	1	0	12
11.1	RD+Colibacillosis	1	-	-	-	-	-	-	-	-	-	-	-	1	0	0	
11.2	RD+Pasteurellosis	-	-	-	-	-	-	-	-	1				1	0	0	
12	Fatty Liver Syndrome	-	-	1	3	-	-	-	-	-	-	2	-	6	0	0	6
13	Avian Encephalomyelitis	1	-	-	-	1	-	-	-	-	1	-	-	3	2	1	6
14	Avian Leukosis Complex	1	1	1	-	-	1	-	-	-	-	-	-	1	4	0	5
15	Fowl Cholera	-	-	-	-	-	-	-	3	-	-	-	1	4	1	0	5
16	Infectious bronchitis	-	-	1	-	1	-	-	-	-	-	-	-	2	2	1	5
17	Ascaridia Infestations	-	-	2	-	-	-	-	-	-	-	-	1	1	3	0	4
18	Poisoning+ Toxicity	1	-	-	-	-	-	-	-	-	2	-	-	3	-	-	3
19	Vitamin+Mineral Deficiency	1	1	-	-	-	-	-	-	-	-	-	-	2	1	0	3
20	Cestode Infestations	1	-	-	-	-	-	-	-	1	-	-	-	0	2	0	2
21	Necrotic enteritis	-	-	1	-	-	-	-	-	-	-	-	-	1	1	0	2
22	Fowl Pox	1	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
23	Pasty Vent Disease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Total		98	47	55	30	33	28	34	63	61	81	70	43	616	65	11	692

Table 1 needs to be carefully interpreted. The numbering from 1-12 stands for the months of Nepalese calendar year in which 1 stands for Shrawan (first month of the running fiscal year) and so on. Similarly, monthly distribution of disease/condition has been provided according as the familiar host for a particular disease. For example,

colibacillosis is commonly seen in broilers but not frequent in layers and parents due to comparatively good hygienic measures adopted at layers and parent farms. The monthly total indicates the highest number of cases for a particular month. The number of undiagnosed cases is comparatively large. An undiagnosed category includes poultry birds either received alive or putrefied so that no conclusion could be derived. Similarly, stress is not itself a disease rather it is an alteration in environment (in the form of low brooding temperature, high brooding temperature, ill ventilation, therapeutic stress etc) that can create heavy mortality among the flock.

Table 2: Disease profile of animals and birds other than commercial poultry

S.N.	Disease/Conditi on	Duck	Local Fowl	Pig	Goat	Pigeon	Parrot	Quail	Dog	Game Bird	Jungle fowl	Peacock	Rabbit	Total
1.	Bacterial Infestation	4	-	-	-	1	1	1	-	1	-	-	-	8
2.	Colibacillosis	5	2	-	-	-	-	-	-	-	-	-		7
3.	NAD	3	1	-	1	1	-	-	-	-	-	-	-	6
4.	Pasteurellosis	4	-	1	-	-	-	-	-	-	-	-		5
5.	PPR	-	-	-	4	-	-	-	-	-	-	-	-	4
6.	Ranikhet Disease	2	2	-	-	-	-	-	-	-	-	-	-	4
7.	Swine Fever	-	-	4	-	-	-	-	-	-	-	-	-	4
8.	Viral Hepatitis	4	-	-	-	-	-	-	-	-	-	-	-	4
9.	Mycoses	2	1	-	-	-	-	-	-	-	-	-	-	3
10.	Salmonellosis	3	-	-	-	-	-	-	-	-	-	-	-	3
11.	Ascarid Infestation	-	-	-	-	1	-	-	-	-	1	-	-	2
12.	Aspergillosis	1	-	-	-	-	-	1	-	-	-	-	-	2
13.	Cestode Infestation	-	2	-	-	-	-	-	-	-	-	-	-	2
14.	Fowl Pox	-	2	-	-	-	-	-	-	-	-	-	-	2
15.	Transmissible gastroenteritis	-	-	2	-	-	-	-	-	-	-	-	-	2
16.	Avian Encephalomye litis	-	-	-	-	-	-	-	-	-	-	1	-	1
17.	Coccidiosis	-	1	-	-	-	-	-	-	-	-	-	-	1
18.	Food Poisoning	-	-	1	-	-	-	-	-	-	-	-	-	1
19.	Gumboro Disease	1	-	-	-	-	-	-	-	-	-	-	-	1
20.	HS+ Swine fever	-	-	1	-	-	-	-	-	-	-	-	-	1
21.	Parasitic Gastroenteritis	-	-	-	-	-	-	-	-	-	-	-	1	1
22.	Parvo Virus Infection	-	-	-	-	-	-	-	1	-	-	-	-	1
23.	Pasteurellosis + Salmonellosis	1	-	-	-	-	-	-	-	-	-	-	-	1
24.	Poisoning	-	-	-	1	-	-	-	-	-	-	-	-	1
25.	Spirochaetosis	-	-	-	-	1	-	-	-	-	-	-	-	1
26.	Ventriculitis	-	-	-	-	-	1	-	-	-	-	-	-	1
27.	Visceral Gout	-	-	-	-	1	-	-	-	-	-	-	-	1
Total		30	11	9	6	5	2	2	1	1	1	1	1	70

Table 2 shows total cases of animals and birds other than commercial poultry are merely seventy. Despite of less inflow of post mortem cases among animals and birds other than commercial poultry in central veterinary laboratory as shown in the present table, the variety of them is not discouraging. It indicates the increasing importance of laboratory diagnostic services in the valley. Hopefully a good variety of diseases among the animal species as well as birds would be appreciated for the obvious reason in future.

Microbiology unit

This unit is responsible for routine diagnosis and outbreak investigation works especially concerned with bacterial, fungal, and viral diseases. In addition to this, the unit is doing certain development work such as antigen production as well as development of certain test methodology like Penside test for PPR diagnosis. The Salmonella antigen produced in Microbiology unit is supplied to different regional laboratories, National Avian Laboraotry and private practitioners as per demand. This unit is also involved in research activities for graduate as well as post-graduate students received from various academic institutions. Recently the role and responsibility of this unit is added for its full-fledged involvement in the isolation and diagnosis of Avian Influenza virus from samples suspected for bird flu collected from different species of birds such as pigeon, wild bird like Eunice and commercial poultry.

Being reference veterinary laboratory of the country, it receives a wide variety of samples from field condition, veterinary hospitals, farms, DLSOs, Animal Quarantine Check-Post (ACQPs) and post mortem unit of CVL itself. It also receives samples/primary isolates from regional laboratories as well as National Avian Laboratory for confirmation/reconfirmation through further identification and verification.

Microbiology unit comprises four sub-units which are as follows

Bacteriology and Mycology laboratory

Virology laboratory

Rabies Diagnosis laboratory

Washing and Sterilization unit. This sub-unit functions for the shake of whole the laboratory.

Bacteriology and Mycology Unit

This unit is responsible for the isolation and identification of bacteria and fungi from respective diseases. It also performs drug sensitivity test to isolate organisms that helps in formulating proper line of treatment. The major samples include milk, various tissues, pus and swabs followed by egg, blood and urine from various diseases/disease processes from different species of animals. Similarly water samples are also received from different hatcheries for the appreciation of microbes present therein. Besides these routine works, this unit also carries out research works for postgraduate students on *Salmonella spp* in poultry meat.

Progress

During the fiscal year 2061/062, a total of 827 samples were received from different species of animals. Among these, only 549 samples were found positive from which

different organisms were isolated through different types of culture techniques. Out of these positive samples, different species of fungus were isolated from 49 samples.

A total post mortem sample of different species received from PM unit of CVL was two hundred and eighty-five. Out of these, 257 samples were received from poultry and rest from different species of animals comprising 11 samples from pigs, 10 samples from ducks and seven from dog. Various organisms like *E coli, Salmonella, Staphylococcus, Streptococcus, Pasteurella, Klebseilla* etc. were isolated from 206 positive samples accounting for a total of 235 isolates as shown in **Table 1.** It may be mentioned here that the number of isolates has been more than the number of positive cases. This is due to the fact that more than one bacterial isolates have been confirmed in some of the samples.

Table.1: Result of Bacteriological analysis of post mortem samples

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Poultry	257	191	66	*E.coli* *Salmonella* *Staphylococcus* *Streptococcus* Others (*pseudomonas, proteus, sheigella)*	107 45 43 21 5
2.	Swine	11	6	5	*E.coli* *Pasteurella* *Staphylococcus* *Streptococcus*	2 1
3.	Duck	10	6	4	*E.coli* *Staphylococcus* *Salmonella*	4 1 1
4.	Canine	7	3	4	*Stoph* *Klebseilla*	2 3
	Total	285	206	79		235

A total of 274 milk samples were received from field. Out of these, 220 samples were found positive for California Mastitis Test (CMT). The major organisms isolated from milk were *Staphylococcus, E.coli, and Streptococcus. Pseudomonas, Proteus, Micrococcus, Klebsiella* was also isolated from few milk samples, the result of which has been presented inTable 2

Table 2: Result of Bacteriological analysis of milk samples received from field

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	274	220	54	*E.coli* *Staphylococcus* *Streptococcus* *Others (pseudomonas, proteus, Micrococcus,*	84 140 40 12
	Total	274	220	54		

Similarly, total sample as vaginal swabs received from field were sixty-one. Out of these, 51 samples were received from Bovine and rest from Swine (9) and Canine (1). Among them, only forty-three samples from bovine were found positive with 20 negative samples from all species given. The major isolates from positive cases were *Enterobacter and Staphylococcus* with the presence of *Aerobactor, Klebseilla, Proteus, Pseudomonas and Streptococcus.* The result of culture and isolation of vaginal swabs are presented in
Table.3

Table 3: Result of Bacterial isolates of vaginal swab

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	51	43	8	*Enterobacter* *Staphylococcus* *Klebsiella* *E.coli* *Aerobacter* *Others (Streptococcus, Proteus and Pseudomonas)*	15 10 10 3 2 3
2.	Swine	9	-	9	-	-
3.	Canine	1	-	1	-	-
	Total	61	43	18		43

Total blood samples received from field were forty-nine. Out of these, 24 samples received from Bovine and rest samples from various species of animals and birds that constitutes 18 samples from poultry, six from canine and only one sample from caprine. Detail of the test result has been given in table 4

Table.4: Result of blood culture

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	24	2	22	*Pasteurella*	2
2.	Poultry	18	1	17	*Staph* *Strepto*	1 1
3.	Canine	6	-	6	-	-
4.	Caprine	1	1	-	*Haemophilus*	1
	Total	49	4	45		

Total ear swab samples received for bacteriological technique were eighteen in which 17 samples constituted of canine and only one sample of caprine. The description of test result has been provided in table 5.

Table 5: Result of ear swab culture

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Canine	17	14	3	*Staphylococcus* *Streptococcus*	11 3

					Pseudomonas *Proteus*	2 1
2.	Caprine	1	1	-	*Strepto* *E.coli*	1 1
	Total	18	15	3		

Total urine samples inclusive of bovine and canine received from field condition were eleven. Out of these, only seven samples were found positive. Details of the result has been provided in table 6

Table 6: Description of test result of urine sample

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Bovine	9	6	3	*Staphylococcus* *Strepto*	6 2
2.	Canine	2	1	1	*Staphylococcus*	1
	Total	11	7	4		

Pus samples received from canine was three and from poultry did one, constituting a total of four samples. Detail of the culture and isolation are presented in table 8.

Table 7: Description of test result of pus sample

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Canine	3	3	-	*Staphylococcus* *Strepto*	3 1
2.	Poultry	1	1	-	*Staphylococcus*	1
	Total	4	4	-		

The Lab also received eggs from different AQCPs for isolation of *Salmonella spp.* During the fiscal year 2061/062, a total of 55 eggs samples were received. Similarly the total water samples received from different hatcheries were fifteen. Details of the test results of egg and water are presented in table 8.

Table.8: Result of bacteriological culture with egg and water samples

S.N.	Species	Total	Positive	Negative	Isolate Organism	No.
1.	Egg	55	1	54	*Salmonella*	1
2.	Water	12	6	6	*Staphylococcus* *E.coli*	3 6
	Total	67	7	60	-	

The total samples received for fungal culture were sixty-eight. Out of these, 37 samples were received from canine and 21 samples each from poultry and bovine (21) and only one from zoo animal. Description of the test results are given in table 9

Table 9: Result of fungal culture and isolation from different species of animals

S.N.	Species	Total	Positive	Negative	Isolate Fungus	No.
1.	Canine	37	22	15	*Penicillium*	21

					Candida	1
2.	Poultry	21	18	3	*Penicillium*	18
3.	Bovine	9	8	1	***Penicillium***	6
					Candida	2
4.	Zoo animal	1	1	-	***Candida***	1
	Total	68	49	19		49

Virology Unit

This unit is responsible for diagnosis of viral diseases through isolation and identification of different viruses. It is achieved through inoculation of Virological samples in embryonated chicken eggs through different approaches and performs HA/HI test and Agar gel Immunodiffusion Test. It receives samples mainly from post-mortem section of CVL and a few from field cases.

PPR diagnosis is done through Penside test which can be performed on the spot in field condition. Ocular and nasal swabs of goat and 1% piglets RBC are required for this test.

Progress

During the fiscal year 2061/62, a total of 555 samples from chicken, duck and goat were tested for different viral diseases. Out of these, only 270 samples were found positive.

A total of 24 samples comprising five from duck and 19 from chicken suspected for New Castle Disease were received from Kathmandu district. Only six samples were found positive, result of which haas shown in table 10

Table 10: Examination of New Castle disease through egg inoculation method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kathmandu	Duck	5	3	
2.	Kathmandu	Chicks	19	3	
	Total		24	6	

A total of 32 samples from chicken suspected for Avian Encephalomyelitis received included 11 samples each from Kathmandu valley and Kaski district and 10 samples from Chitwan district. Out of these, four samples from Chitwan were tested through egg inoculation were found positive. However, three samples from Kaski district tested positive for AE with AGID. The result of both tests performed for the diagnosis of AE is shown in Table 11 and table 12.

Table 11: Examination of Avian Encephalomyelitis through egg inoculation method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Chitwan	Chicks	4	-	
	Total		4	-	

Table 12: Examination of AE through AGID method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kathmandu	Chicks	11	-	
2.	Kaski	Chicks	11	3	
3.	Chitwan	Chicks	6	-	
	Total		28	3	

Pox samples received from goat were four and from chick were four constituted a total of eight samples. These samples were received from Kaski and Bhaktapur district, tested through egg inoculation method result of which is given in table 13.

Table 13: Examination of Pox disease through egg inoculation method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kaski	Goat	4	-	
2.	Bhaktapur	Chicks	4	4	
	Total		8	4	

A total of four samples from Kathmandu district suspected for Inclusion body hepatitis (IBH) were examined through egg inoculation method. Only two samples were found positive as shown in Table 14

Table 14: Examination of IBH disease by egg inoculation method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1	Kathmandu	Chicks	4	2	
	Total		4	2	

A total of 178 Avian Influenza suspected samples was received from Kathmandu, Chitwan, Lalitpur, Taplejung and Kaski district. Out of these, 120 samples were examined through AI virus antigen test and rest was examined through egg inoculation method and HI method respectively. None of them were found positive. The result of these tests are shown in table 15, 16 and 17

Table 15: Examination of Avian Influenza disease by AI virus antigen test

S.N.	District	Species	Sample tested	sample positive	Remarks
1.	Kathmandu	Duck	11	-	
2.	Kathmandu	Chicks	58	-	
3.	Chitwan	Chicks	23	-	
4.	Lalitpur	Chicks	20	-	
5.	Taplejung	Chicks	8	-	
	Total		120	-	

Table 16: Examination of Avian Influenza disease by egg inoculation method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kathmandu	Chicks	6		
2.	Chitwan	Chicks	4	-	
3.	Pokhara	Chicks	4	-	
	Total		14	-	

Table 17: Examination of Avian Influenza disease by HI method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kathmandu	Chicks	23	-	
2.	Chitwan	Chicks	18	-	
3.	Dhankuta	Chicks	3	-	
	Total		44	-	

Besides, this unit received 201 samples for the diagnosis of PPR from various districts of the country viz; Kathmandu, Dhading, Krishnanagar, Lahan, Dang Valley, Sunuali, Achham and Bajhang. Theses samples were tested through Penside test. A total of 151 samples were found positive within which 108 samples were verified through HA/HI test and 104 samples were found positive. Details about the test result ig presented in table 18 and 19

Table 18: Examination of PPR disease by Penside test method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kathmandu	Goat	117	97	
2.	Dhading	Goat	18	-	
3.	Kapilbastu	Goat	8	4	
4.	Siraha	Goat	3	2	
5.	Dang	Goat	4	4	
6.	Rupandehi	Goat	1	1	
7.	Achham	Goat	11	11	
8.	Bajhang	Goat	15	15	
9.	Lalitpur	Goat	16	16	
10.	Kailali	Goat	8	1	
	Total		201	151	

Table 19: Examination of PPR disease by HA/HI method

S.N.	District	Species	Sample tested	Sample positive	Remarks
1.	Kathmandu	Goat	108	104	
	Total		108	104	

Rabies Diagnosis Section

One of the most important works done in Microbiology unit is diagnosis of Rabies. Although it is the part of virology, various tests related to diagnosis of this disease are performed separate than others because of public health importance. Generally this section performs three different types of diagnostic tests, namely Negri body test, Fluorescence antibody test and biological test.

During the fiscal year 2061/62, the total rabid specimen received were 22 from field outbreaks. Out of these, 16 cases received from dog, five samples from cow, one each from goat and sheep. Of them, 14 cases were diagnosed as positive through all the tests mentioned above. Detail of the test results is given in table.20

Table 20: Result of different tests for the diagnosis of Rabies

Tests	Species	Sample tested	Sample positive	Remarks
Negri Body test / FAT/Biological test	Canine	16	8	
,,	Bovine	5	5	
,,	Caprine	1	-	
,,	Ovine	1	1	
Total		23	14	

Washing and Sterilization Unit

This unit is backbone of the CVL and so for microbiology unit. It provides the clean and sterilized glassware for media preparation and reagent preparation.

Serology

Serology unit of 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 for diagnosis, monitoring and surveillance of animal diseases mainly associated with viral and bacterial infection. Majority of the samples received in the unit are provided by RVLs, DLSOs, AQCPs, private practitioners, farmers and staffs of CVL. They include both types of samples collected for the purpose of disease outbreak investigation as well as the samples collected for routine serological work.This unit possesses capacity and facility of Competitive enzyme-linked immunosorbent assay (ELISA), IgM Capture ELISA, Indirect ELISA, Tube agglutination test, Agar gel immunodiffusion (AGID) test, Plate agglutination test (PAT), A solid phase immuno assay (Immuno comb) and Rapid test (SD Bio line)

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. This unit has tested 78, 028 serum against RP to date and has provided scientific evidence for "Rinderpest Infection Free" status to Nepal.

Similarly this unit is supporting to National Peste des petits ruminants (PPR) Control Program by testing sera collected from vaccinated sheep and goat to detect antibodies. This unit has tested 20,879 sera in fiscal year 060/061. On an average 66.74% of tested samples (20,879) remained positive indicating low heard immunity against PPR in goats. Therefore, mass vaccination against PPR is still required to increase heard immunity up to protective level. In total 37, 554 serum samples were tested to detected antibodies in response to PPR vaccine to date.

In fiscal year 2061/062 this unit tested 690 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 cases was 96.37, 97.97 and 94.78 in New Castle disease, Infectious Bursal disease and Infectious bronchitis respectively. These results indicate that vaccinated birds have protective level of antibodies within the flocks.

In addition to above activities, a total number of 780 poultry serum samples were tested by performing Plate Agglutination Test (PAT) for the diagnosis of Salmonellosis and 1135 samples for the diagnosis of Mycoplasmosis. Prevalence of salmonella and Mycoplasma infection were 2.69% and 22.90% in fiscal year 2061/ 2062. Mycoplasma infection was found as the most common problem according to our laboratory findings. A total number of 764 serum samples, collected from RP suspected performing C-ELISA to conform PPR in 2061/2062 tested sheep and goat. Out of tested 764 samples, 30.23% were found test positive for PPR.

Brucellosis is not significant problem among Nepalese cattle and buffaloes. Out of tested 210 serum samples, only 0.95% was positive for Brucellosis in PAT.

In summary 4995 serum samples were tested in 2061/2062. Results of test have been illustrated in respective tables.

Serum bank

Sera collected from different species of animals such as cattle, yak, buffaloes, sheep, goats, pigs, horses, dog and poultry from all over the country during 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 130000. 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 061/062, number of serum collected for PPR sero-monitoring were 4223.

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 disposal of unwanted sera is necessary.

Table 1: C-ELISA test for diagnosis of PPR in Sheep and Goats (Cases of Disease Outbreak, F/Y 2061-062

S.N.	District	Animal Species	Sample tested	PPR results	
				Positive	Negative
1.	Kathmandu	Goat & Sheep	105	17	88
2.	Pyuthan	,,	13	7	6
3.	Kailali	,,	83	27	56
4.	Rasuwa	,,	63	37	26
5.	Kavre	,,	10	8	4
6.	Makwanpur	,,	10	5	5
7.	Banke	,,	184	19	165
8.	Araghakhanchi	,,	10	4	6
9.	Dolakha	,,	4	2	2
10.	Dhading	,,	5	3	2
11.	Chitwan	,,	14	3	11
12.	Bajura	,,	57	45	12
13.	Kanchanpur	,,	5	0	5
14.	Lalitpur	,,	2	2	0
15.	Bhajhang	,,	11	8	3
16.	Morang	,,	86	31	55
17.	Sankhuwasabha	,,	13	0	13
18.	Palpa	,,	4	3	1
19.	Manang	,,	5	0	5
20.	Salyan	,,	18	1	17
21.	Udaypur	,,	6	3	3
22.	Nawalparasi	,,	10	0	10
23.	Dhausha	,,	31	3	28
24.	Kapilbastu	,,	4	1	3
25.	Saralahi	,,	11	4	7
	Total		764	231	533

Table 2: Plate Agglutination test for *Salmonella pullorum* & *Mycoplasma* in poultry (F.Y. 2061-062)

S.N.	District	Salmonella (PAT)		Mycoplasma (PAT)		Total sample tested
		Positive	Negative	Positive	Negative	
1.	Chitwan	12	351	124	41	528
2.	Lalitpur	3	35	-	-	38
3.	Kathmandu	-	97	-	-	97
4.	Taplejung	-	9	-	-	9
5.	Sunsari	3	43	-	-	46
6.	Kailali	1(G)	33(G)	-	-	34(G)
7.	Makwanpur	-	5(G)	-	-	5(G)
8.	Saptari	2	156	-	12	172
9.	Bhaktapur	-	13	-	-	13
10.	Dhading	-	15	-	-	15
	Total	21	759	124	53	957

Table 3: Sero-monitoring of different poultry diseases by Immuno-comb (F/Y 2061-062)

S. N.	Districts	IBD		ND		IB	
		+ve	-ve	+ve	-ve	+ve	-ve
1.	Chitwan	423	10	417	16	415	18
2.	Lalitpur	10	-	10	-	10	-
3.	Kathmandu	91	1	91	1	91	1
4.	Taplejung	9	-	1	8	2	7
5.	Sunsari	43	3	46	-	36	10
6.	Kailali	-	-	-	-	-	-
7.	Makwanpur	-	-	-	-	-	-
8.	Saptari	90	-	90	-	90	-
9.	Bhaktapur	10	-	10	-	10	-
10.	Dhading	-	-	-	-	-	-
	Total	676	14	665	25	654	36

Table 4: Screening of Poultry Diseases by Immuno-comb (F.Y. 2061-062)

S. N.	Districts	MG		MS	
		Positive	Negative	Positive	Negative
1	Chitwan	60	254	73	241

2	Lalitpur	-	10	-	10
3	Kathmandu	-	-	-	-
4	Taplejung	-	9	-	9
5	Sunsari	-	46	-	46
6	Kailali	-	-	-	-
7	Makwanpur	-	-	-	-
8	Saptari	-	90	-	90
9	Bhaktapur	1	9	2	8
10	Dhading	-	-	-	-
	Total	61	418	75	404

Table 5: Sample tested for Brucellosis (F/Y 2061-062)

S.No.	District	Species	Sample Tested	Number of samples	
				Positive	Negative
1.	Dang	Human	1	-	1
2.	Ramechap	Goat	2	-	2
3.	Kathmandu	C, B, D	38	1(Buff)	37
4.	Gorakha	Cow	1	-	1
5.	Chitwan	Cow	1	-	1
6.	Rasuwa	Cow, Goat	25	-	25
7.	Lalitpur	Cow, Pig	21	-	21
8.	Kavre	Cow, Buffalo	22	1(Buff)	21
9.	Kailali	Goat	24	-	24
10.	Makwanpur	Pig, Buff, Goat	12	-	12
11.	Banke	Cow, Buff	22	-	22
12.	Nawalparasi	Buff	10	-	10
13.	Rupandehi	Cow	11	-	11
14.	Sankhuwasabha	Cow, Buff	17	-	17
15.	Dhankuta	Cow	1	-	1
16.	Dhading	Buff	2	-	2
	Total		210	2	208

Table 6: Serum collection and Storage record (F.Y. 2061-062)

S.N.	District	Animal Species	No.of Serum Collected
	CVL.Tripureshwor		
1.	Rauthat	Goat & Sheep	150
2.	Parsa	,,	200
3.	Bara	,,	200
4.	Kavre	,,	150
5.	Dolakha	,,	100
6.	Ramechap	,,	100
7.	Rasuwa	,,	25
8.	Nuwakot	,,	75
9.	Dhading	,,	125
10.	Kathmandu	,,	87
11	Lalitpur	,,	60
12.	Bhaktapur	,,	33
13.	Chitwan	,,	150
14.	Sindhupalchowk	,,	100
	RVL.Janakpur		
1.	Dhanusha	,,	150
2.	Saralahi	,,	150
3.	Sindhuli	,,	150
4.	Makmanpur	,,	150
	RVL.Pokhara		
1.	Kaski	,,	25
2.	Lamjung	,,	50
3.	Parbat	,,	75
4.	Gorakha	,,	75
5.	Gulmi	,,	75
6.	Palpa	,,	50
7.	Rupandehi	,,	100
8.	Tanahun	,,	50
9.	Shyanja	,,	75
10.	Myagdi	,,	75
11.	Baglung	,,	75
12.	Arghakhanchi	,,	50
13.	Nawalparasi	,,	100
14.	Kapilbastu	,,	150
	RVL.Biratnagar		
1.	Morang	,,	75
2.	Jhapa	,,	77

3.	Dhankutta	,,	76
4.	Saptari	,,	76
5.	Sunsari	,,	75
6.	Udaypur	,,	55
7.	Siraha	,,	80
8.	Ilam	,,	75
	RVL.Surkhet		
1.	Dang	,,	100
2.	Surkhet	,,	50
3.	Banke	,,	75
4.	Bardiya	,,	50
5.	Rolpa	,,	50
6.	Pyuthan	,,	50
7.	Salyan	,,	50
8.	Jumala	,,	29
	Total		4223

Table 7: PPR Sero-monitoring recorded in vaccinated cases by C-ELISA (F.Y.061/062)

S.N.	District	Animal Species	Total number of sample			
			Tested	Positive	Negative	Av. Pos. Sero. Co.
	RVL, Biratnagar					
1.	Sunsari	Goat & Sheep	422	301	121	88%
2.	Jhapa	,,	418	280	138	85%
3.	Morang	,,	354	287	67	74%
4.	Udaypur	,,	315	243	72%	83%
5.	Saptari	,,	419	330	89	76%
6.	Siraha	,,	290	220	70	78%
	Total		2218			
	RVL, Janakpur	,,				
1.	Dhanusha	,,	740	361	379	68%
2.	Mohotari	,,	940	576	364	65%
3.	Saralahi	,,	908	468	440	74%
4.	Sindhuli	,,	785	401	384	82%
	Total		3373			
	CVL, Tripureshwor	,,				
1.	Chitwan		759	484	275	63%
2.	Makwanpur	,,	853	572	278	78%

3.	Kavre	,,	832	520	312	70%
4.	Sindhupalchowk	,,	576	336	240	68%
5.	Dolakha	,,	500	364	136	64%
6.	Nuwakot	,,	403	270	130	67%
7.	Dhading	,,	900	564	336	85%
8.	Gorakha	,,	300	188	112	61%
9.	Bara	,,	650	323	327	74%
10.	Parsa	,,	640	334	306	60%
11.	Rauthat	,,	1000	598	402	63%
12.	Ramechap	,,	393	203	190	86%
13.	Kathmandu	,,	122	71	51	70%
14.	Bhaktapur	,,	119	62	57	65%
15.	Lalitpur	,,	113	74	39	64%
	Total		8744			
	RVL, Pokhara					
1.	Tanhun	,,	200	140	60	74%
2.	Palpa	,,	300	196	104	68%
3.	Lamjung	,,	200	120	80	79%
4.	Shyangja	,,	200	120	72	81%
5.	Kaski	,,	300	216	84	76%
6.	Kapilbastu	,,	940	494	446	65%
7.	Nawalparasi	,,	840	565	275	69%
8.	Rupandehi	,,	900	650	250	82%
	Total		3880			
	RVL, Surkhet					
1.	Banke	,,	857	437	420	66%
2.	Bardiya	,,	814	484	330	69%
3.	Surkhet	,,	400	260	140	76%
4.	Dang	,,	850	450	400	63%
	Total		3220			
	RVL, Dhangadhi					
1.	Kailai	,,	940	643	297	87%
2.	Kanchanpur	,,	705	380	325	62%
3.	Dadeldhura	,,	540	315	225	88%
4.	Doti	,,	360	207	153	69%
	Total		2848			
	Grand Total		20879			

Table 8: Summary of Serological test (F/Y 2061-062)

S.N.	Disease	Sample tested	Test method	Test Results		Percent
				Positive	Negative	
1	PPR (Routine Test)	764	C-ELISA	231	533	30.23
2	Salmonella Pullorum	780	PAT	21	759	2.69
3	Mycoplasma Gallisepticum	177	PAT	124	53	70.05
4	Poultry IBD (Bursal)	690	ImmunoComb ELISA Ab.Test	676	14	97.97
5	Poultry ND (Newcastle)	690	ImmunoComb ELISA Ab.Test	665	25	96.37
6	Poultry IB (Bronchitis)	690	ImmunoComb ELISA Ab.Test	564	36	94.78
7	Poultry MG-MS	958	ImmunoComb ELISA Ab.Test	136	822	14.19
8	Brucellosis	210	PAT	2	208	0.95
	Total	4959		2509	2450	

Table 9: Record of PPR outbreak (F/Y 2061-062)

S.No.	District	Animal Species	No.of Tested samples	PPR Results		Remarks
				Positive	Negative	
1	Kathmandu	Goat & Sheep	105	17	88	
2	Pyuthan	,,	13	7	6	
3	Kailali	,,	83	27	56	
4	Rasuwa	,,	63	37	26	
5	Kavre	,,	10	8	4	
6	Makwanpur	,,	10	5	5	
7	Banke	,,	184	19	165	
8	Araghakhanchi	,,	10	4	6	
9	Dolakha	,,	4	2	2	
10	Dhading	,,	5	3	2	
11	Chitwan	,,	14	3	11	
12	Bajura	,,	57	45	12	
13	Kanchanpur	,,	5	0	5	
14	Lalitpur	,,	2	2	0	
15	Bhajhang	,,	11	8	3	
16	Morang	,,	86	31	55	
17	Sankhuwasabha	,,	13	0	13	

18	Palpa	,,	4	3	1	
19	Manang	,,	5	0	5	
20	Salyan	,,	18	1	17	
21	Udaypur	,,	6	3	3	
22	Nawalparasi	,,	10	0	10	
23	Dhausha	,,	31	3	28	
24	Kapilbastu	,,	4	1	3	
25	Saralahi	,,	11	4	7	
		Total	764	231	533	

Biochemistry Unit

Introduction

The Biochemistry unit of Central Veterinary Laboratory (CVL), Tripureshwor deals with analysis of urine and serum samples. The samples are either collected from the animals or received directly from farmers, private clinicians, District Livestock Service Offices (DLSOs), Regional Veterinary Laboratories. More often, serum samples are collected from field conditions during disease outbreaks. These samples are processed in laboratory based on the Standard Operating Protocol (SOP) of the unit.

Serum Analysis

Samples received in this way are subjected to biochemical estimation (quantitative analysis). The quantitative analysis of the serum samples includes estimation of inorganic as well as organic substances with the use of commercial kits. The inorganic substances include estimation of Calcium, Magnesium, Zinc and Phosphorous. Similarly, the organic substances include estimation of Protein, Albumin, Billirubin, Serum Glutamate Oxallate Transferase (SGOT), and Serum Glutamate Phosphate Transferase (SGPT)

During the F/Y 2061-062, total number of serum samples received was 825. The result of biochemical analysis has been presented in table 1

Urine Analysis

Both qualitative and quantitative biochemical analysis of the urine samples are carried out. In qualitative analysis, smell, colour, consistency (specific gravity), pH, presence of RBCs, different types of casts, crystals, fungi, pus cells and other substances coming along the way of test procedure are appreciated with naked eye as well as with the help of compound microscope. The quantitative analysis includes biochemical estimation of phosphorous, sugar, ketone bodies, albumin, total protein, urobillinogen and triple phosphate with the use of commercial kits (Uristix/Multistix)

The urine samples were mostly received from the cases of haematuria. The urine samples from dogs are received with the prescription to rule out the presence of urinary calculi, urobillinogen, protein and haemoglobinuria.

A total of 250 samples were received and tested during the F/Y 2061-062 consisting of 120 from cattle, 110 from dogs and 20 samples from pigs. Of the total samples, twenty-three urine samples were studied under compound microscope. Of them two samples were found negative for the occurrence of any abnormality. Of the positive samples, seven of them were appreciated for the presence of Red Blood Corpuscles

(RBCs), five of them found to contain crystals, four samples with pus cells, three samples with epithelia and two samples with bacteria.

Table 1: Total blood estimation and urine analysis of different species

S. N.	Sample tested	No. of samples	Species of animals		
			Dog	Cattle	Pig
1	Urine analysis	250	110	120	20
2	Calcium estimation	124	-	124	-
3	Phosphorous estimation	127	-	127	-
4	Glucose estimation	30	20	10	-
5	Zinc	139	-	139	-
6	Magnesium	112	-	112	-
7	Protein	50		50	
	Total	832	130	682	20

Haematology

Haematology unit receives samples from private clinicians, District Livestock Service Offices (DLSOs), Regional Veterinary Laboratories (RVLs), Central Veterinary Hospital (CVH) or samples collected during disease out breaks. The unit deals with both qualitative and quantitative evaluation of different types of anomalies present in the blood. The qualitative evaluation is carried out with blood samples in case of blood protozoan parasites. Both qualitative and quantitative tests are made with the blood samples for the evaluation of blood parameters. These parameters include Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cells (RBCs) count, Total Leukocyte Count (TLC), Total Platelets Count and Differential Leukocyte Count (DLC).

A total of five hundred and fifty six samples were received and tested during the F/Y 2061/062. Of them, eighty six samples were subjected for microscopic examination of blood protozoan parasite using Giemsa's method of staining technique. Of the 86 samples, only 21 samples were found positive for the presence of blood protozoa. Among the positive samples, eleven samples exhibited the cases of Babesiosis; eight samples were positive for Anaplasmosis and only two cases of Theilariosis.

Table 2: Parameter of tested Samples of various species

S. N.	Species	Total samples	Hb	PCV	ESR	TLC	DLC	Blood protozoa
1	Cattle	158	28	28	18	28	26	30
2	Buffalo	98	25	12	12	12	13	24
3	Dog	198	33	32	28	35	32	38
4	Horse	52	12	10	10	8	2	10
	Total	506	98	82	78	83	73	86

Besides the routine laboratory work, this unit also deals with the three years Khari Disease investigation progarmme

Teaching Laboratory Unit

Three months laboratory training

Laboratory technicians need more training on different laboratory techniques for effective animal disease diagnosis. A separate teaching laboratory unit has been established to conduct three months laboratory training for the junior technicians working at different places in the kingdom under the Department of Livestock Services (DLS). Most of District Livestock Development offices (DLSO) have primary laboratory facility but trained laboratory techniacians is our constraint.With this noble objective CVL is conducting three monthes extensive laboratory training to JT/JTAs from different regional laboratories and district livestock development offices. The response of this training has been very encouraging and useful for upgrading veterinary laboratory services.

Name of the participants completed three monthes laboratory training during FY 2061/062.

Serial No.	Name of the Trainee	Office Address	Post	Remark
1	Mr.Ram Prasad chaudhary	National Avian Disease Investigation Laboratory, Chitwan.	JTA	
2	Ms. Shakila Rai	Biological production laboratory, Tripureshor.	JTA	
3	Mr. Masindra Yadav	District Livestock Development Office, Jhapa.	JTA	
4	Mr. Uttam Nepal	District Livestock Development Office, Rupandehi	JT	
5	Mr. Hari Dutta Bhatta	Regional Animal Disease Investigation Lavoratory (RVL) Dhangadhi.	JT	
6	Mr. Anirudha Sha	Regional Animal Disease Investigation Lavoratory	JTA	

		(RVL) Janakpur.		
7	Mr. Anand Saru	Regional Animal Disease Investigation Lavoratory (RVL) Surkhet.	JTA	
8	Mr. Shasi Dhar Sedai	Veterinary Quality Contral Laboratory.	JT	
9	Mr. Uddhav Bahadur Air	District Livestock Development Office, Baitadi.	JTA	
10	Mr. Ganesh Prasad Bhattrai	Regional Animal Disease Investigation Laboratory (RVL) Biratnagar.	JTA	

Parasitology Unit

The parasitology unit of the Central Veterinary Laboratory has been involved in detecting parasites causing adverse affect in animals and resulting in the loss of production, especially in livestock as well as poultry. Routinely fecal samples, skin scrapings and blood samples of different species of livestock are examined by adapting standard protocol. They are done mainly for the identification of eggs/ovas of different nematodes, cestodes and other common parasites found in the gsstrointestinal system of livestock.

Qualitative test is done by the technique of double floatation for the detection and identificaion of the eggs of gastrointestinal parasites, whereas the quantitative test is performed by the modified Mc Master Counts method for counting the number of eggs per gram in the feaces, so as to assume the extent of parasitic burden. Furthermore larvae culture, for the indentification of nematodes and collection of skin scrapings, blood smears and ticks is done for the examination. Larva identifying work is being performed in this unit to facilitate the parasitic study conducted by Regional Veterinary laboratories and collaborative larva identification work is also being conducted with Animal health research division of NARC. In addition to the faecal samples of the domestic animals, faecal samples of wild animals, particularly of the deer family were collected for the central zoo for assessing their parasitic burden during the different months of the fiscal year 2061/62. Beside this the faecal samples of aalmas, dogs, cats and monkeys were also collected and examined during those months.

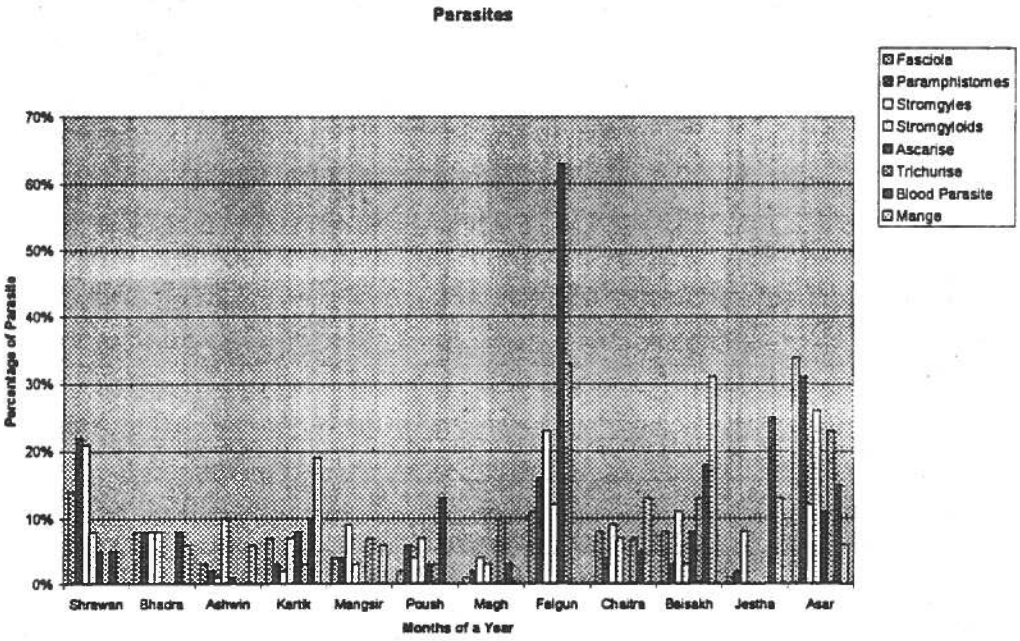
The district livestock services offices, private practitioners and the farmers themselves were actively involved during those sample collection.Regular communication and supply of standards protocols are being made to the district livestock services offices so as to provide our services to the farmers and also to maintain adequate sample flow.

This parasitological unit is also performing a collaborative research work with the zoology department of Tribhuvan University, Kirtipur and Institute of Agriculture and Animal Sciences (IAAS), Rampur. This unit is also involved in facilitating the graduate and postgraduate students of Himalayan Collage of Agricultural Sciences and Technology (HICAST) with their practical works along with the supervision of the research work.

In the fiscal year 2061/62, a total of 1633 fecal sample were collected and examined.The highest positive cases were of Fasciola followed by Ascarids+ Strongyles.

Table 1: Monthly distribution of samples examined for parasitic problem among different species of animals

S.N.	Species of animals	Sawan	Bhadra	Asbin	Kartic	Mangsir	Paush	Magh	Phalgun	Chaitra	Baisakh	Jestha	Asadh	Grand Total
1.	Cattle	51	47	21	36	55	23	62	81	92	81	120	79	750
2.	Buffalo	22	26	25	29	39	27	43	43	29	17	18	7	345
3.	Goat	21	24	9	11	22	29	16	13	24	16	24	40	249
4.	Sheep	7	-	-	9	-	-	-	12	-	6	10	-	44
5.	Dog	1	-	-	7	-	6	9	11	14	19	8	10	85
6.	Poultry	1	12	1	-	-	14	6	9	11	2	3	-	59
7.	Swine	6	10	1	-	3	-	3	-	2	3	11	18	57
8.	Other Species	-	-	-	-	2	2	7	-	11	-	20	2	44



The annual fecal sample examines for different parasite which has been tabulated above and its graphic presentation gives some idea for launching parasite control programme.

Table 2: Fecal sample examined and identified parisitc eggs under laboratory condition.

S.N.	Species of animals	Sample			Parasites identified in +ve cases			
		Total	+ve	-ve	Fasciola	Paramphostomes	Ascarids+ Strongyles	Others
1	Cattle	750	559	191	291	76	144	48
2	Buff	345	233	112	82	56	82	13
3	Goat	149	159	90	51	24	62	22
4	Dog	85	51	34	-	-	28	23
5	Poultry	59	29	30	-	-	22	7
6	Swine	-	33	24	-	-	26	7
7	Sheep	-	16	28	6	12	8	2
8	Others	-	18	27	=	=	15	3
	Total	1633	1110	523	430	168	387	125

Molecular Biology Unit

Molecular tools are increasingly important in modern animal disease research not only in other country but also in our country Nepal. In view of this Central Veterinary Laboratory (CVL) has established molecular biology unit in 2003. This laboratory has started to diagnose disease by DNA extraction by using RT-PCR technique. The use of the PCR technique amplify a specific DNA sequence prior to its detection with a DNA probe or by other means have introduced a new dimension not only to the diagnosis and epidemiological study of diseases, but also our understanding of the genetic relationships between and within species of infectious organisms.

This unit is using RT-PCR technique for detection of Haemorrhagic Septicemia (HS) by using specific primers to amplify a specific DNA sequence. For detection of this disease in this laboratory bacterial culture, bone marrow and tissue swab samples were used. These samples were brought to CVL from Dhading and Kathmandu valley districts of Nepal. Bacteria were cultured in Microbiology laboratory of CVL. Seed of HS were brought from Biological Product Division of Department of Livestock Services, Nepal. During this period samples were tested as a trial form. For the detection of HS the gene sequence of forward and reverse primers used were GCTGTAAACGAACTCGCCAC and ATCCGCTATTTACCCAGTGG respectively.

During fiscal year 2061 to 2062 (2004-2005) 24 total samples were tested for this disease. The results of RT-PCR were detected with the band formation in gel running. The positive control was found positive, negative control was found negative and some samples show band in gel running. From this we can conclude that our PCR- test is working for this disease.

In future this CVL has planned to detect many other bacterial, viral and parasitic diseases. Recently this CVL has planned to do PCR in Avian Influenza by using this RT-PCR technique.

NATIONAL AVIAN LABORATORY, BHARATPUR, CHITWAN

Introduction

With the decision of HMG/Nepal, National Avian Laboratory (NAL) was established in 2061 at Bharatpur, Chitwan. This has ever been the first specialized laboratory in the country under Department of Livestok Services (DLS). The laboratory has been functioning with limited available resources from the time of its inception. The new establishment was proposed in ninth five year plan keeping in view the problems faced by poultry industry run by private sector of the country. The industry has emerged as a fast growing sector under subsistence agriculture system of the nation with an investment of around Rs. 1600 millions. Its importance is exploited in the light of its significant contribution to Agriculture Gross Domestic Product (AGDP) and generation of employment opportunity.

National Avian Laboratory was established with an objective of delivering services related to health, nutrition, management and marketing of poultry. Named as National Avian Center earlier, it has been working, at present, as National Avian Laboratory. The construction work started with the proposal of setting up two-storey building in 1997-98 with the provision of accessory structures like laboratory animal house, biological pit, link road, drainage system and logistics. Successful construction of first storey building was completed by the end of 2002. As per the decision of DLS, the laboratory started functioning with the deputation of a Veterinary Officer and a Junior Technician pulling them from DLSO, Chitwan. With the registration of first sample on 12 February 2003, NAL began its ceremonial work in the field of poultry disease diagnosis.

At present NAL has, in its accommodation, the provision of biological pit, road channel around the main building with incomplete second-storey structure. Presently, most of the essential equipments are available with fully operational potential. The main sections of the laboratory are under the process of resetting and hopefully run in full-fledged way in shortcoming future.

Objectives

The National Center for Avian Disease Investigation started with the supreme objective of minimizing the risk associated with poultry industry so as to secure poultry sector of the country as a profitable and sustainable enterprise.

The overall objective of this center is prioritized as follows:

Immediate objectives

Develop facilities so that an accurate and reliable diagnostic service for avian diseases is provided.

Develop and recommend effective vaccination schedule based on epidemiological pattern of poultry disease occurrence.

Surveillance of avian diseases of zoonotic importance so as to formulate effective control strategy

Mid-term objectives

Isolation, characterization and preservation of field strains of different bacteria, viruses, fungi and protozoa causing diseases in poultry

Proximate analysis of feed to appreciate their nutrient value and to detect different toxic principle present in the feed

Environment study and accessment associated with poultry farming

Long-term objective

The long term objective includes reseach and development (R&D) associated with culture, isolation, identification and preservation of native strains of pathogen responsible for the avian diseases so that it can be utilized for the development of antigens, antisera, antibodies. With this type of approach, an indigenous supply of different types of test kits, poultry biologicals is ensured within the country which will address and meet with all the problems concerned with the poultry sector of the nation.

Main Sections of NAL

Sample Collection Unit

- Registration of Samples.
- Recording of History.
- Labeling of samples with Sample Number (NAL No).
- 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.
- Microscopic Examination.
- Immunohistochemistry.
- FAT.

Bacterial /Fungal Culture unit

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

Virology / serology unit

- Inoculation of sample into embryonating egg.
- HA and HI test.
- Plate and tube agglutination test.

Accessory Unit

- Washing, Cleaning & Sterilization Unit. .
- Changing Room.
- Consulting & Reporting Unit

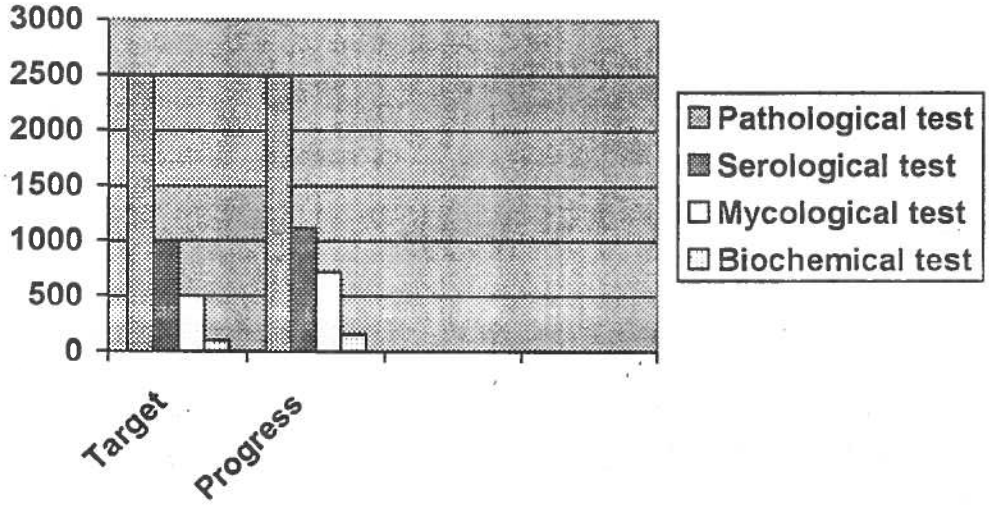
Manpower

NAL started functioning partially since February 2003. At the time of starting its operation, there was no staff of its own. One veterinarian and One Junior Veterinary Technician from adjoinig 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 Govenment of Nepal has approved the official status of this laboratory with required staffing. The status of manpower till date is as follows:

Total staffing approved: 11(7Technical & 4 Supporting staff)

S.N.	Designation	Class	Employee
1.	Senior Veterinary Officer	GII	Dr. T.R.Naeupane
2.	Veterinary Officer	GIII	Dr.P.S.Kushwaha
3.	Veterinary Officer	GIII	Dr. D.R. chapagain
4.	Veterinary Assistant (VA)	NGI	Mr.Endu Raya Yadav
5.	Veterinary Assistant (VA)	NGI	Mr.Shailendra Bhandari
6.	Junior Veterinary Assistant (JVA)	NGII	Mr.Ram Pd. chaudhari
7.	Junior Veterinary Assistant (JVA)	NGII	Mr. Binod kumar Saphy
8.	Assistant Accountant	NGII	Mr. Krishna kumar Maharjan
9.	Mukhiya	Non Gz.III	Mr.Rishee Ram Acharya
10.	Peon	-	Mr.Chunni Lal Yadav
11.	Peon	-	Vaccant
	Total - 11		

Graphic representation of targeted tests conducted in NAL at F/Y 2060/61



A

nnual Laboratory Investigation Report (F/Y 2061-062)

1. Microbiological test status

- **Microbial Identification**

Total sample received & cultured at different types of media- 718
Positive cases- 507
Druge sescitivity test- 456

- Different types of bacteria cultured and identified were *Escherichia, Salmonella, Pasteurallosis, Streptococcus, Staphylococcus* Spp. etc.
- The antibiotic sensitivity trend in terms of efficacy was ciprofloxacin, choloramphenical, enrofloxacin, tetracycllin, teramycin, cephalexin, neomycin, doxycycllin, erythromycin, cotrimexazol & amoxacillin.
- Diagnostic method/used in culture & Biochemical (motility, Gram's train, catalase, coagulase, Indole, MrVp& O/f) test.

2. Serological test status

- The plate agglutination test was done with 89 serum sample for sero-diagnosis of Salmonellosis.
- The HA test was done in virology unit of 9 cases of ND, IBD, AE&MD of Poultry samples.
- The immunocomb test was done for the diagnosis of IBD, ND & IB with a total of 30 Poultry samples.

3. Biochemical test status

- Estimation of Calcium was done with 145 serum samples collected from poultry with the help of Colorimeter.

4. Virological test status

- The egg inoculation test was done in virology unit of 19 cases of Poultry diseases (ND, IBD, AE & MD)

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, BIRATNAGAR (Eastern Region)

Introduction

Situated in Biratnagar, the sub-metropolitan city- 17, district- Morang, Regional Animal Disease Investigation Laboratory (RADIL) was established in the F/Y 1988-1989 AD. But until 1990-1991, the laboratory was running with slow pace with time and demand. It was hard to perform essential laboratory activities as per its objectives due to poor manpower deployment, insufficient equipments and inconsistence policy related to organizational set up. It was in the F/Y 1991-1992, RADIL, Biratnagar acquired its separate identity for the first time. There was provision of manpower, essential equipments and other logistics. Laboratory diagnostic activities were lunched as per specific objective of the laboratory.

The working area of RADIL includes 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 as follows.

1. High hills
2. Mid hills
3. Tarai

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 the principal livestock reared in this ecozone.

Mid hills: This region falls between high hills in the north and tarai in the south. Districts falling 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 specialties of this ecozone. Cattle, buffalo, goat and pigs are the main livestock species reared in this region. Poultry and rabbit industry are gaining popularity in recent years.

Tarai: Jhapa district of Mechi zone, Morang and Sunsari districts of Koshi zone and Saptari and Siraha districts of Sagaramatha zone occupy this ecozone. In general, crop constitutes the main farming activities; animal husbandry has become the second priority agricultural component. Cattle, buffalo, goat and poultry are the principal animals and birds that have solved livelihood of a fair number of populations in this

belt. The different components of subsistent livestock sector are developing graduaaly in the form of commercial enterprise especially in urban areas where basic development facilities like road, market, and electricity are available. Such components include poultry farming, dairy and piggery.

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

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:

Investigate and diagnose epidemics in the region.

Assist and support DLSOs in disease diagnosis and epidemic control.

Upgrade and strengthen DLSOs disease diagnosis capacities providing technical support.

Collate, analyze and predict animal disease situate in the region.

Human resource development.

Coordinate and support national animal disease control and eradication programme.

Support and facilitate national veterinary services and private veterinary services.

Research and development (R &D) addressing animal health issues in the region

Approved annual work programme (F/Y2061-062)

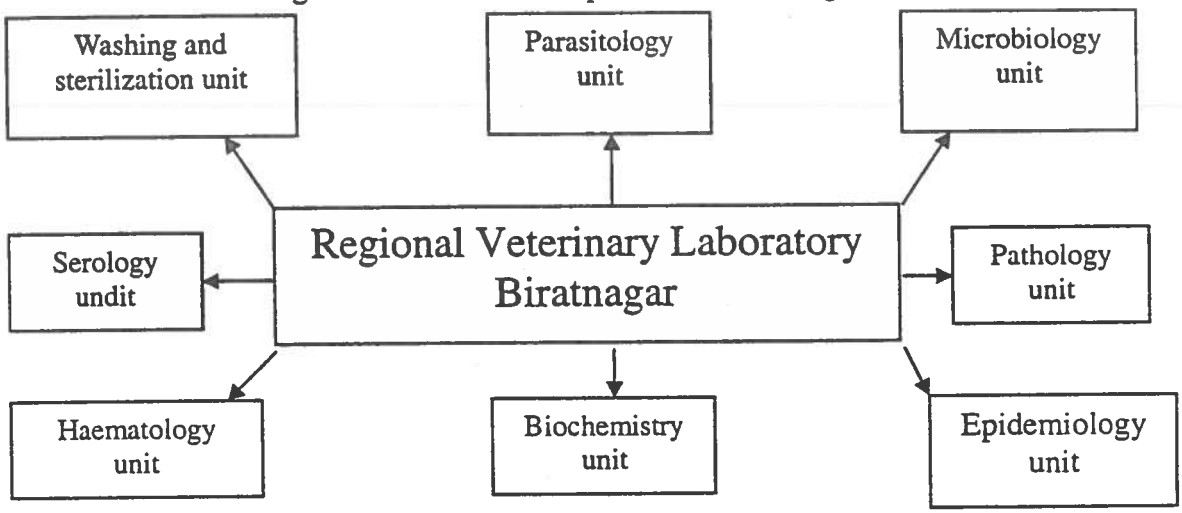
Table 1: Summary of progress report

S.N.	Programme	Unit	Target	Progress	Progress %
1	**Laboratory services**				
1.1	Parasitological examination	No.	1800	2143	100
1.2	Microbiological examination	No.	600	710	100
1.3	Pathological examination	No.	180	372	100
1.4	Serological examination	No.	500	800	100
1.5	Haematological examination	No.	300	413	100
1.6	Biochemical examination	No.	500	1404	100
1.7	Sample to be sent to other lab.	No.	200	462	100
2	**Investigation & surveillance programme**				
2.1	Study of infertility in cow	Times	6	6	100
2.2	Investigation of Sukharia disease in cattle.	Times	6	6	
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	Lab. training for JT/JTAs	Member	1	1	100
6	**Publication programme**				
6.1	Publication of quaternary epidemiological bulletin	Times	4	4	100
6.2	Material preparation for annual technical report	Times	1	1	100
7	**Purchase**				
7.1	Technical books	Times	1	1	100

Laboratory services

Laboratory services involve laboratory diagnosis of different types of diseases of different species of animals and birds. The tentative, presumptive and confirmative diagnoses of various diseases are made through post mortem examination, qualitative and quantative examination of different types of samples (autopsy and biopsy) and culture and isolation of microorganisms with the use of various laboratory procedures. These activities are performed through multidisciplinary set up of the laboratory. The technical set up has been shown in the figure 1. Most often the laboratory test results are verified and reconfirmed through CVL.

Figure 1: Technical set up of RVL, Biratnagar



Parasitological Examination

1. Examination of digestive tract parasites

Routine laboratory examination of faecal samples is carried out with both qualitative and quantitative analysis. Majority of the faecal samples are received from different DLSOs of the eastern region as well as through the farmers directly. Samples are also collected during surveillance and disease outbreak investigation in field condition. The faecal samples are processed mostly by sedimentation and floatation techniques to identify the eggs of gastrointestinal parasites. However, in certain cases, Mc Master Technique of faecal examination is carried out for quantitative analysis so as to ensure an appropriate line of treatment.

In the fiscal year 2061/062, altogether 1737 faecal samples from different species of animal such as cattle, buffalo, goat, dog etc. were received and examined in this laboratory in which 1358 samples (78%) found positive whereas 379 samples (22%) were negative for parasitic eggs. The result of coprological examination revealed that Fascioliasis has been the most commonly occuring parasitic infestation in cattle and buffaloes followed by different types of Nematoda infestation and Paramphistomiasis. Monthly distribution of different types of digestive tract parasites are presented in the table 2.

2. Examination of non-digestive tract parasites

Skin scrapings are collected from animals suffering from various skin lesions and examined for appreciation and 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 two minutes. The supernatant fluid is discarded and a drop of sediment is placed
on a clean glass slide and covered with a coverslip. It is then examined under low power microscope for the presence of parasites. Altogether 42 samples from different species of animals were received and examined in this laboratory. Mostly *Sarcoptes* mites were identified during skin scraping examination.

Similarly, the blood samples received in the laboratory were examined for blood parasites using Giemsa's staining technique. A total of 347 blood samples were examined for blood parasites in which 34 samples were found positive. Of the positive samples, the number of cases of Theileriasis was five and that of Babesiosis was twenty-nine.

Table 2: Result of coprological examination of digestive tract parasites on monthly basis

Months	Total sample		Trematodes		Nematodes			Others	Total
	+ve	-ve	Fasciola	Paramphistome	Trichuris	Hemonchus	Ascarids		
July	49	9	41	8	2	1	1	4	58
August	122	28	99	3	1	2	6	10	150
September	173	29	138	11	5	1	3	15	202
October	113	33	91	10	2	1	5	12	146
November	101	30	59	9	4	5	9	15	131
December	92	14	46	14	2	1	11	18	106
January	62	16	33	12	6	0	0	11	78
February	110	50	38	35	5	0	10	14	160
March	132	42	59	30	2	3	18	11	174
April	104	25	52	30	2	5	3	4	129
May	136	38	86	15	1	0	1	33	174
June	164	65	39	34	5	4	10	72	229
Total	1358	379	781	221	37	23	77	219	1737
Percent	78	22	58	19	26.21				

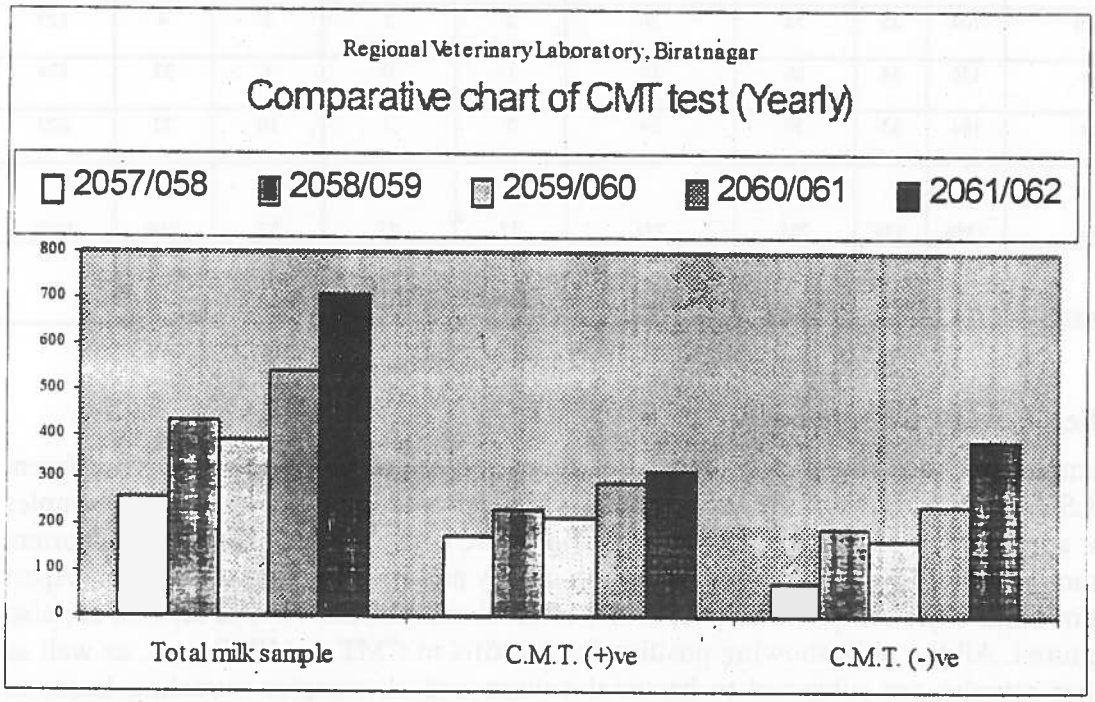
Microbiological Examination

Samples intended for microbiological examination are received either from different DLSOs or directly from the farmers. Microbial samples include mostly milk samples for appreciation of mastitis. However, samples are also collected during post-mortem examination at RVL and from field during survey and investigation of diseases. Apart from milk, other samples like pus, urine, swab obtained from various lesions are also cultured. All the milk showing positive for mastitis in CMT & MWT tests, as well as other samples are subjected to bacterial culture and all samples revealing bacterial growth on media are subjected to bacterial identification and antibiotic sensitivity test. Milk samples qualified positive during screening of mastitis through California Mastitis Test and MWT are subjected to bacterial culture. Samples that revealed bacterial growth on culture media are subjected to antibiotic sensitivity test. The result of CMT test has been presented in table 3

Table 3: Result of Calofornia Mastitis Test

Months	Total sample	Positive	Negative	Percent positive
July	54	25	29	46.29
August	82	36	46	43.9
September	64	34	30	53
October	30	14	16	47
November	35	7	28	20
December	11	3	8	27
January	53	37	16	70
February	106	31	75	29
March	103	29	74	28
April	48	27	21	56
May	70	48	22	69
June	54	32	22	59
Total	**710**	**323**	**387**	**45**

Figure 2: Year wise comparative chart of California Mastitis Test

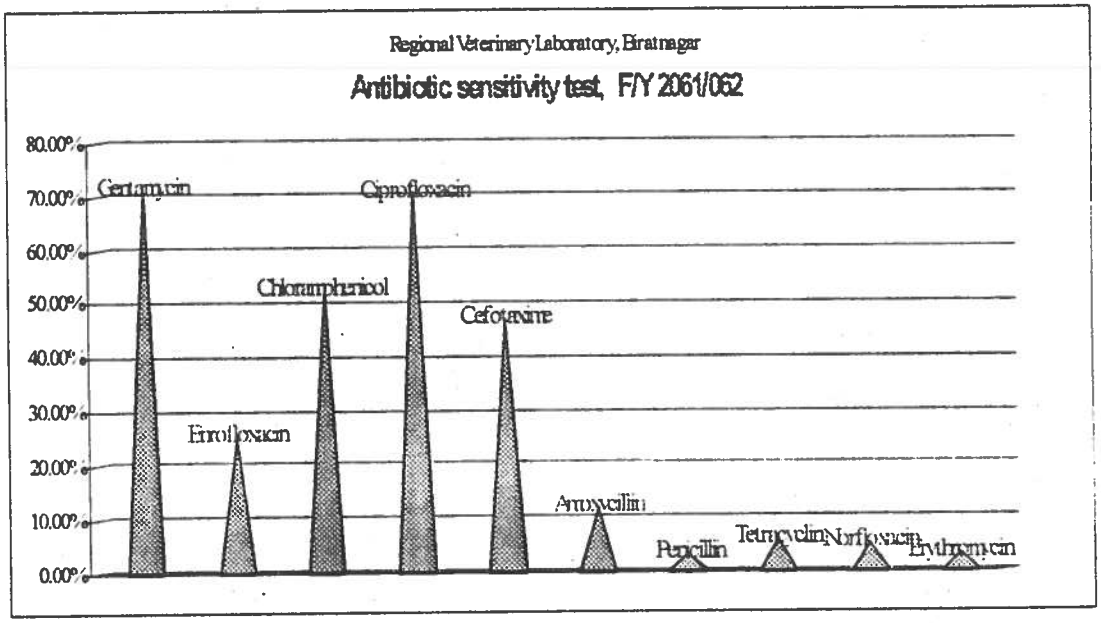


Types of media normally used for bacterial culture, isolation and identification

1. Nutrient agar 2. Mc Conkey agar 3. EMB agar 4. XLD agar 5. Blood agar 5. MS agar 6. Sabouraud's dextrose agar 7. Nutrient broth 8. Citrate agar 9. MR-VP media 10. TSI agar 11. Peptone agar 12. OF media 13. Oxidase agar 14. Liquid media for sugar

Figure 3: Sensitivity of different antibiotic with bacteria isolated from milk samples

(Antibiotic Sensitivity Test)



Basis of Bacterial identification

- Cultural characteristics on different media.
- Gram's staining
- Motility test (Hanging drop method)
- Colony characteristics
- Bio-chemical tests

List of bacteria Isolated from milk sample

The main bacteria isolated from milk samples intened for mastitis test are *Streptococcus, Staphyllococcus, E.coli, Proteus, Klebsiella, Pseudomonas, Enterobacter and Bacillus* spp.

Pathological Examination

Macroscopic examination (Post mortem examination) of dead birds and occasionally dead animals are done as pathological examination in the laboratory. Microscopic examination (Histopathological examination) is not carried out in the RADIL due to lack of essentials equipments. Tissues that are collected for histopathological examination are being sent to the Central Veterinary Laboratory, Kathmandu. During post-mortem examination, specimens such as impression smears, swab, and tissues are collected for required test.

Among the diseases diagnosed after PM examination of 372 birds, Chronic respiratory disease ranked the most common followed by IBD, Coccidiosis and

Ranikhet disease. Following are the lists of diseases which were diagnosed tentatively and the bacteria cultured and identified after bacteriological examination.

List 1: Tentative diagnosis of poultry disease on the basis of PM examination and laboratory tests

1. CRD 2. IBD 3.Coccidiosis 4. ND 5. Collibacillosis 6. Gangrinous dermatitis 7. Necrotic enteritis 8. Aspergillosis 9. Infectious coryza 10. Infectious bronchitis 11. Hydropericardium Syndrome

List 2: Bacteria isolated from sample obtained during PM examinations of bird

1. Salmonella 2. E.coli 3. Enterobacter 4. Streptococcus 5. Staphyllococcus 6. Other enterobacteria

Biochemical Examination

Routine examination of urine and analysis of blood serum to assess biochemical constituents are done in this laboratory under biochemical examination. Serum samples are collected from sites/farms selected for investigation of infertility, Sukharia disease and other disease conditions. Serum Samples are collected and analyzed for the estimation of total protein, glucose, phosphorus, zinc, SGOT, SGPT, Bilirubin estimation etc. using specific commercial kits. Urine samples are tested by using dipsticks (multisticks) as well as traditional biochemical methods. Examination of urine is 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. Bacterial identification is also done on the basis of bio-chemical tests like Catalase test, Citrate test, Indole test, TSI test, MR-VP test, Oxidase test, OF test, and sugar tests.

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 Hb is done by Sahli's haemoglobinometer method, PCV by microhaematocrit method, TEC and different WBC count 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 number of blood sample examined was 413.

Blood samples examined for blood protozoan parasites was 347 with 34 samples positive and rest of the samples were negative. The concentration of Hb and the PCV were estimated with the blood samples positive for blood protozoan parasites, result of which is given in table 4.

Table 4: Result of estimation of Hb and PCV in blood protozoa positive samples

Blood Parasite	Total	Avg. Hb gm%	Min. Hb gm%	Av. PCV	Min. PCV
Theileria spp.	5	6.0	3.7	23	10
Babesia spp.	29	5.3	4.5	19	13
Total	34	-	-	-	-
Blood Parasite	**Total**	**Avg. Hb gm%**	**Min. Hb gm%**	**Av. PCV**	**Min. PCV**
Theileria spp.	5	6.0	3.7	23	10
Babesia spp.	29	5.3	4.5	19	13
Total	34	-	-	-	-

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.

Table 5: Description of samples received by RVL, Biratnagar (F/Y 2061-062)

Location	Type of sample									Total
	Blood/Smear	Serum	milk	Stool	Urine/head	Swab	Skin scrapping	Cyst	Dead bird/fish	
DLSO, Sankhuwashabha		27								27
DLSO, Morang	114	10	9			1				134
DLSO, Ilam		75								75
DLSO, Saptari	6									6
DLSO, Jhapa		11								11
DLSO, Dhankuta	11	2		14			17	4		48
DLSO, Terahthum	9			20			1			30
DLSO, Bhojpur	22						8			30
DLSO, Khot ing		52								52
DLSO, Taplejung	7	13			Hrad	1	8		1	31
DLSO, Udaypur	1	11								12
DLSO, Siraha	6						10			16
AQCP, Rani		71							1	72
AQCP, Kankarbhitta	24	19	6	8	Urine		6			64
ARC, Tarhara									12	12
Purbanchal Poultry Farm		241							10	251
Sugar feed and hatchery		34							5	39
Total	200	566	15	42	2	2	50	4	29	906

Figure 4: Comparative description of different diagnostic technique applied at RVL over years, Biratnagar

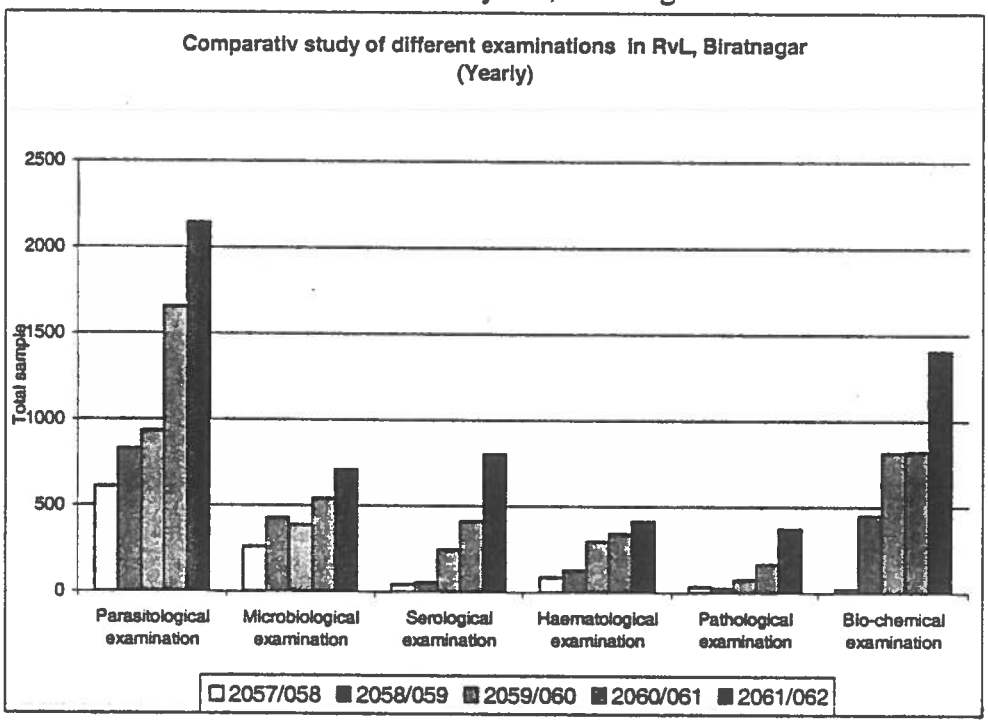


Table 6: Description of National PPR Control Programme (Seromonitoring) F/Y- 2061/062

DLSO	Udayapur	Sunsari	Morang	Siraha	Saptari	Dhankuta	Ilam	Jhapa	Total
Total vaccination target	10000	15000	15000	15000	15000	15000	15000	15000	115000
Total serum collection target	50	75	75	75	75	75	75	75	575
Total serum collection	54	75	79	80	76	76	77	77	594

Epidemiological reporting of Eastern Development Region FY- 2061/062

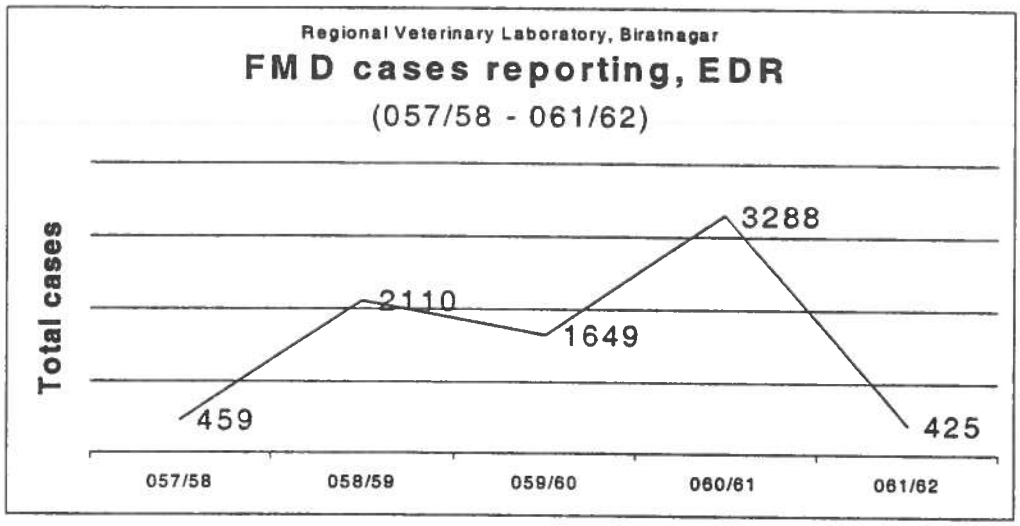
Annual progress report (F/Y: 2061-062)

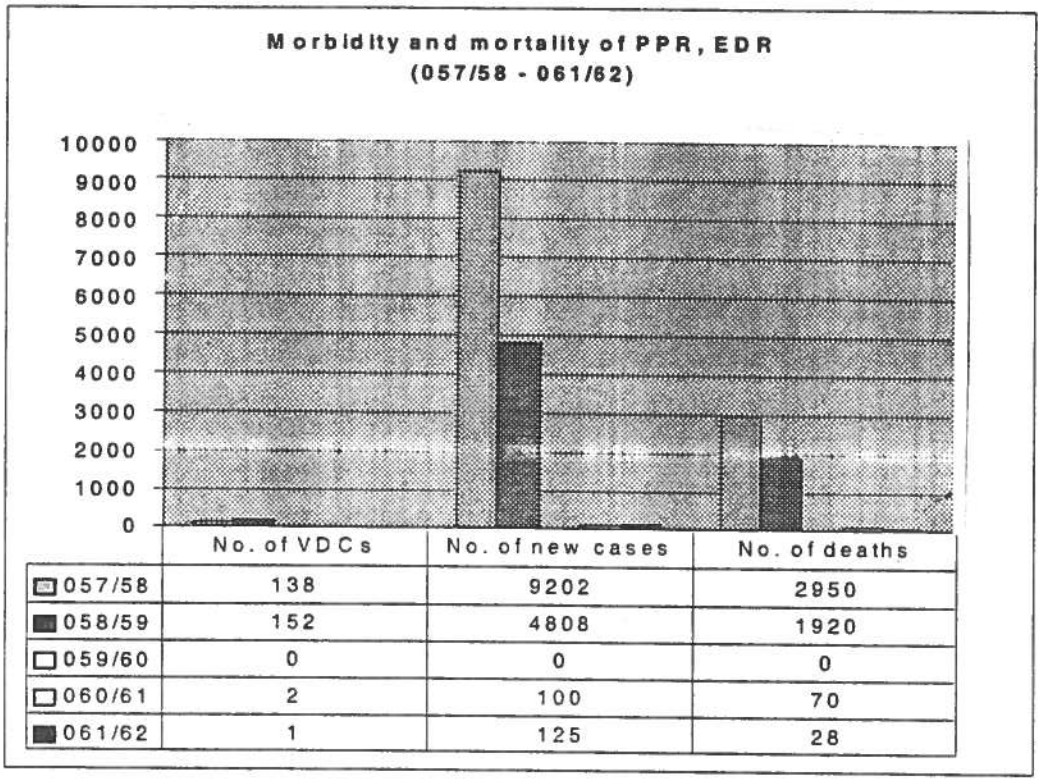
Total no. of animals treated, Eastern Dev.Region

S.N.	District	No. of cases where animal examined	No. of cases where no animal seen but sample examined	No. of cases where no animal seen and no sample examined	Total
1	Morang	11823	8272	9391	29486
2	Sunsari	15317	7577	11951	34845
3	Jhapa	10520	7251	4836	22607
4	Siraha	9290	6629	2745	18664
5	Saptari	10355	8637	8700	27692
6	Tehrathum	4107	4004	9292	17403
7	Dhankuta	1414	1196	2257	4867
8	Bhojpur	4788	602	3322	4712
9	Ilam	7488	6845	7940	22273
10	Panchthar	1814	2851	10466	15131
11	Udayapur	4636	1895	15396	21927
12	Okhaldhunga	2438	1974	8399	12811
13	Khotang	1922	1903	10907	14762
14	Solu	2359	3804	3447	9610
15	Taplejung	4226	605	9978	14809
16	Sankhuwasabha	2126	3352	10815	16293
	Total	94623	67397	129842	291862
	Percentage	32.42 %	23.09%	44.49%	

Situation of major diseases, Eastern Dev. Region (District wise) FY – 2061/062

S.N.	District	B.Q.	H.S.	Mastitis	F.M.D.	Rabies	Ephi.fever	Fasciolasis	Nematodia	Mange
1	Morang	34	8	939	115	20	-	10209	705	240
2	Sunsari	19	15	171	-	-	378	10470	4410	859
3	Jhapa	6	20	481	109	82	456	7895	2122	322
4	Dhankuta	-	3	45	5	20	84	1999	355	593
5	Bhojpur	-	-	110	41	-	-	7940	1908	1053
6	Sankhuwasabha	-	-	134	-	-	141	4480	4645	470
7	Tarhathum	-	11	115	-	239	38	3359	3020	322
8	Ilam	-	-	973	-	1	298	4194	1795	865
9	Panchthar	-	-	185	-	-	62	2504	3505	625
10	Taplejung	-	3	31	-	47	37	2279	4051	781
11	Solu	-	-	31	-	-	467	3012	243	147
12	Okhaldhunga	4	-	71	-	-	3	1424	507	91
13	Khotang	-	-	75	19	-	1	3405	1356	41
14	Udayapur	-	-	233	-	-	39	5408	2290	542
15	Siraha	6	106	182	136	2	303	2956	856	1278
16	Saptari	-	-	143	-	21	285	5109	1297	1770
	Total	69	166	3919	425	432	2462	76643	33063	9982





	No. of VDCs	No. of new cases	No. of deaths
057/58	138	9202	2950
058/59	152	4808	1920
059/60	0	0	0
060/61	2	100	70
061/62	1	125	28

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, JANAKPUR (Central Region)

Introduction

Regional Veterinary laboratory, Janakpur is one of the five regional veterinary laboratories under Directorate of Animal Health, Department of Livestock Services located in the central development region of the country. The laboratory works under the direct guideline of the Central Veterinary Laboratory (CVL). It works in the region providing the disease diagnostic facilities of different animal species. There are five different units in the laboratory- Pathology, Parasitology, Microbiology, Haematology and Biochemistry. Due to limited facilities available in the laboratory, samples intended for serological test and histopathological examination are referred to CVL.

In general, less sample flow to the diagnostic laboratories has been the established problem in the country. In particular, it has been a great problem to RVL, Janakpur. The major cause behind it would be comparatively easy access of the farmers of remote areas (form RVL, Janakpur) to other relevant laboratories. The second cause may be the less awareness concerning the importance of laboratory-based treatment among the farmers of terai belt.

Pathological Examination

The pathological examination includes post mortem examination of the dead animals. A total of 222 cases of post mortem examination were received during the f/y 2061/062. All the cases received were of birds. No cases of large or small ruminants and other species of animal were received. It is mentioned that all the samples of birds received were brought from the Janakpur municipality and its adjacent areas and districts. The status of poultry diseases in the area is presented in table 1

Table 1: Pattern of disease occurrence in poultry during 2061/062

S.N.	Tentative diagnosis	Total cases		Ranking on the basis of occurrence
		Number	Percent	
1.	Aflatoxicosis	19	8.56	5th
2.	Chronic Respiratory Disease	34	15.31	3rd
3.	Coccidiosis	66	29.73	Ist
4.	Gumboro Disease	16	7.21	6th
5.	Ranikhet Disease	11	4.95	7th
6.	**Salmonellosis**	21	9.46	4th
7.	Miscellaneous (Parasitic Gastroenteritis, Non-specific enteritis etc.)	37	16.67	2nd

Parasitological Examination

The total sample received in Parasitology unit was 1291. Of them, 12 percent of the samples were found negative with the presence of 59.64% of cases of Distomiasis followed by Nematode infestation 14.87%, Fascioliasis 9.68 and others 3.8%. The monthly flow of the samples alongwith the Parasitological test result has been presented in table 2.

Table 2: Monthly flow of samples with results

Month	No. of Samples received	Fasciola Spp.	Paramphistome Spp.	Nematoda	Others	Negative
Shrawan	12	1	2	3	0	6
Bhadra	75	4	17	23	4	27
Ashoj	218	13	127	58	5	15
Kartic	284	22	156	62	8	36
Mansir	40	4	20	2	2	12
Poush	75	2	40	10	5	18
Magh	105	15	75	5	6	4
Falgun	132	21	101	3	1	6
Chaitra	38	4	22	3	3	6
Baishakh	72	3	35	12	6	16
Jestha	106	13	72	7	7	7
Asadha	134	23	103	4	2	2
Total	1291	125	770	192	49	155

Similarly, the total samples intended for the external parasitic examination were fifty-one and all the samples were found positive. Details of the result are given in table 3

Table 3: Ectoparasitic Examination

Month	Total sample	Species of animals				Parasites diagnosed
		Buffalo	B. Calf	Ox	Goat	
Shrawan	3	1	-	-	2	Sarcoptic Spp.
Bhadra	5	-	2	-	3	Sarcoptic Spp., Psoroptic Spp.
Ashoj	3	-	-	1	2	Sarcoptic Spp.
Kartic	3	-	-	-	3	Sarcoptic Spp.
Mansir	4	2	-	-	2	Sarcoptic Spp.
Poush	5	-	2	-	3	Sarcoptic Spp., Psoroptic Spp.
Magh	3	-	-	1	2	**Sarcoptic Spp.**
Falgun	5	-	-	-	5	Sarcoptic Spp.
Chaitra	7	2	-	-	5	Sarcoptic Spp.
Baishakh	6	-	2	-	4	Sarcoptic Spp., Psoroptic Spp.
Jestha	4	1	-	1	2	Sarcoptic Spp.
Asadha	3	-	-	-	3	Sarcoptic Spp.
Total	51	6	6	3	36	

Haematological Examination

A total of 503 blood Samples were examined for different blood Parameters. Besides, examination of these samples for the presence of blood parasites was also done. Of them, 436 (86.67%) samples were negative and 67(13.33%) samples were positive. Details about the blood protozoan parasite examination are given in table 4.

Table 4: Blood Parasite examination

Month	**Total sample**	**Positive**				**Negative**
		Anaplasma	Babesia	Trypanosoma	Total	
Shrawan	11	2	1	-	3	8
Bhadra	29	4	1	1	6	23
Ashoj	53	3	3	-	6	47
Kartic	46	2	2	1	5	41
Mansir	25	1	1	-	2	23
Poush	32	1	2	1	4	28
Magh	83	4	4	-	8	75
Falgun	31	2	2	1	5	26

Chaitra	30	2	2	1	5	25
Baishakh	37	2	1	1	4	33
Jestha	90	5	5	-	10	80
Asadha	36	4	4	1	9	27
Total	503	32	28	7	67	436

Microbiological Examination

Mostly milk samples were taken for culture & sensitivity test. Culture of other samples was also done i.e. pus from ear, blood & others. Blood agar, mackonky agar, Nutrient agar were used for culture of organism.

Table 5: Microbiological examination

Month	No. of Samples	Positive
Shrawan	4	2
Bhadra	30	21
Ashoj	29	19
Kartic	33	27
Mansir	25	20
Poush	20	18
Magh	15	10
Falgun	70	50
Chaitra	23	19
Baishakh	18	16
Jestha	13	9
Asadha	72	53
Total	352	264

Table 6: Antibiotic Sensitivity Test on Isolated Organism

Antibiotic Disc	Percentage of Sensitivity
Ciprofloxacin	73
Gentamycin	62
Enrofloxacin	58
Oxytetracycline	25
Kanamycin	25
Cloxacillin	23
Ampicillin	22

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, POKHARA (Western Region)

Introduction

Western development region is situated in between 82° 30'-85° 15'E and between 27° 15'-29° 30'N. 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 extended in between central and mid-western development region in the east and west respectively.

Objectives

- Provide disease diagnostic services to the livestock and poultry farmers in the region
- Investigate epidemics in the region and assist, advice and support DLSOs
- Prepare the epidemiological profile of livestock and poultry diseases and maintain regional epidemiological database on animal health
- Supervise and assist in diagnostic services to basic and primary laboraotory units of DLSOs in the region
- Conduct and support laboratory and animal health related training for the paravets
- Coordinate national disease control and eradication programme in the region

Annual Work Program and Progress Report of F.Y. 2061/62 of Regional Veterinary Laboratory, Pokhara

S. N.	Programs and Activities	Annual target			Annual Progress	
		Unit	Target	Weightage (%)	Progress	Weightage (%)
1	Laboratory Services					
1.1	Parasitological Examinations	No.	700	2.19	1033	2.19
1.2	Microbiological Examinations	No.	400	3.50	501	3.50
1.3	Pathological Examinations	No.	500	5.77	968	5.77
1.4	Serological Examinations	No.	400	4.20	430	4.20
1.5	Hematological				277	

	Examinations	No.	200	1.75		1.75
1.6	Biochemical Examinations	No.	200	2.45	306	2.45
1.7	Sample collection and dispatch	No.	400	6.47	546	6.47
2	**Disease Investigation and Surveillance Program**					
2.1	Mycoplasma disease investigation	Times	12	16.83	12	16.83
.2	Investigation of Epidemic	Times	15	20.73	15	20.73
3	**Monitoring and Supervision**					
3.1	Monitoring and Supervision of district based Laboratories	Times	10	7.96	10	7.96
4	**Disease Investigation Workshop**	Times	1	3.32	1	3.32
5	**Training program**					
5.1	JT/JTA Lab diagnosis training	person	16	6.65	16	6.65
6	**Laboratory strengthening programme**					
6.1	Investigation of bovine reproductive disorders	Times	12	14.79	12	14.79
7	**Publication Program**					
7.1	Tri-monthly Epidemiological Bulletin publication program	Times	4	2.10	4	2.10
7.2	Annual Technical Report Publication	Times	1	0.44	1	0.44
8	**Purchase program**					
8.1	Scientific books	No.	4	0.87	4	0.87

Laboratory Services

Parasitological Examination

In the F/Y 2061-62 altogether 1033 faecal samples from different species of animals such as cattle, buffalo, sheep, goats, poultry and dogs were examined. Qualitative faecal examination of 985 samples and EPG count of 48 faecal samples were conducted. Fasciola, Paramphistomum, Coccidia, Strongyle, Strongyloids, Trichuris, Monezia and B.coli were major internal parasites identified. Detail description of results of monthly faecal examination during 2061/62 is presented in table 1.

Table 1: Result of monthly faecal examination F/Y 2061-62

Parasites	Months (As per Nepalese fiscal year)												Total	Percent Positive
	1	2	3	4	5	6	7	8	9	10	11	12		
Fasciola	5	4	6	0	5	0	0	2	3	9	10	0	45	9
Paramphistome	3	0	9	5	0	0	0	0	1	4	1	0	23	4.6
Ascaris	0	0	1	0	0	0	0	1	2	0	0	0	4	0.8
Strongyle	2	10	8	20	10	9	6	1	3	0	6	10	85	17
Strongyloides	6	4	7	22	0	0	3	0	9	0	6	5	62	12.4
Trichuris	1	0	0	0	0	0	2	0	0	6	0	1	9	1.8
Moneizia	0	0	2	0	0	0	0	2	0	0	1	0	5	1
Coccidia	23	16	6	10	24	27	18	16	43	36	16	4	239	47.9
Mixed infections	5	1	0	6	0	5	0	0	0	0	0	0	17	3.4
Others (B.coli)	6	0	1	0	0	0	0	2	0	1	0	0	10	2
Total Positive	51	35	40	63	39	41	29	24	61	56	40	20	499	100
Total Negative	6	23	19	30	35	43	26	81	65	103	92	11	534	

Eighteen skin scrapings from buffaloes, cattle and dogs were received for the examination of mites. The 10 positive samples revealed 9 Sarcoptic and 1 Demodectic species of mites in cattle/buffalo/goats and dogs respectively.

Microbiological Examination

A total of 501 samples were subjected for the microbiological examination. One hundred seventy eight SLST positive milk samples were cultured for bacteriological examination and the isolates were subjected for antibiotic sensitivity test. Out of 178 milk samples cultures, one hundred and thirty-five samples exhibited bacterial growth, result of which has been presented in table 2.

Thirteen milk samples collected from chronic cases of mastitis were also cultured in Saboraud's Dextrose Agar (SDA) for mycological culture and identification. Of them, seven milk samples were found to be positive for fungal mastitis constituting five cases of *Candida and two cases of Absidia* infestation.

Three hundred and twenty three pathological samples from poultry and other species of animal were subjected for the bacteriological culture, rest of which has been given in table 2. The results of the antibiotic sensitivity testing of bacterial isolates from the milk samples are presented in table 3.

Table 2: Bacterial species isolated from milk and other pathological samples

Bacterial Species	Number of isolates	
	Milk sample	Other pathological sample
Staphylococcus spp.	28	36
Streptococcus spp.	17	13
Bacillus spp.	31	58
Pasturella multocida		4
Proteus spp.	7	12

Micrococcus spp.	9	7
Enterobacter spp.	4	5
Salmonella spp		5
Escherichia coli	33	41
Actinobacillus spp.	1	1
Haemophilus spp.		1
Pseudomonas spp.	5	8
Mycoplasma spp		1
Total	135	192

Table 3: Results of antibiotic sensitivity test of bacterial isolates from milk samples

Antibiotics used	Percent sensitivity
Enrofloxacin	98.6
Gentamicin	81.0
Chloramphenicol	92.56
Nitrofurantoin	46.62
Co-trimoxazole	25.62
Tetracycline	34.62
Penicillin	19.36
Kanamycin	76.43
Amoxycillin	18.34

Pathological Examinations

Pathological examinations mostly consisted of post mortem examination (PM) of animals and poultry. Nine hundred forty poultry, 6 buffaloes, 4 Sheep, 3 rabbits, 8 piglets and 7 goats were brought for the postmortem examination. Three piglets were found to have died due to Pasturellosis, and 2 due to enteritis. Goats were found to have died due to Pasturellosis. The poultry diseases confirmed based on Postmortem and /or laboratory diagnosis is presented in table 4

Serological examination

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 2061/62, number of serum samples tested and their results are presented in table 5.

Hematological Examinations

Haematological unit of the laboratory is well equipped to determine a range of haematological parameters such as Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC), Differential Leucocytes Counts (DLC), Erythrocyte Sedimentation Rate (ESR), estimation of haemoglobin (Hb) and Packed Cell Volume (PCV). Some of the blood samples were also examined for the presence of blood protozoa and bacteria. A total of 189 blood samples from different species of animals were examined for different hematological parameters. Similarly a total of 88 blood

samples were examined for the presence of blood parasites. Of them, only six samples were found positive constituting one blood sample from buffalo for Theileria spp, three samples positive for Anaplasma spp in cattle, one sample positive for Babesia spp in cow and one sample positive for Trypanosoma spp in dog.

Table 4: List of poultry diseases diagnosed during PM examination and confirmed with laboratory various test methodologies

S.N.	Disease diagnosed	Number of cases	Percentage of cases
1	Infectious Bursal Disease	206	21.9
2	Coccidiosis	172	18.3
3	Colibacillosis	141	15.0
4	Leechi heart disease	79	8.4
5	Haemorrhagic enteritis	78	35.1
6	Vitamin/Mineral Deficiency	33	3.5
7	Chronic respiratory Disease	29	3.1
8	Salmonellosis	27	2.9
9	Omphalitis	27	2.9
10	Ranikhet Disease	19	2.0
11	Mycotoxicosis	16	1.7
12	Ascariasis	13	1.4
13	Fowl Cholera	6	0.63
14	Avian encephalomyelitis	5	0.53
15	Fowl pox	3	0.32
16	Marek's Disease	1	0.1
17	Miscellaneous disease	85	9.4
	Total	940	100

Table 5: Results of different serological tests

Animal/Bird species	Number of serum tested	Disease/Codition	Test	Result
Cow	11	Brucellosis	RBPT	1 positive
Buffalo	16	Brucellosis	RBPT	All negative
Goat	102	Brucellosis	RBPT	All negative
Poultry	61	Mycoplasmosis	PAT	16 positive
Poultry	154	Salmonellosis	PAT	38 positive
Poultry	8	Avian encephalomyelitis	AGPT	3 positive

Biochemical examination

Biochemical examinations include biochemical analysis of serum, and routine and microscopic examination of urine. Multistick strip is used for routine urine analysis. Microscopic examination of urine is done after centrifugation of the urine sampl

Biochemical parameters of serum samples were determined using spectrophotometer and commercially available biochemical kits.

During the F/Y 2061-062, a total of 306 samples consisting of serum and urine were examined in biochemistry unit. Biochemical estimation of 184 serum samples included calcium, inorganic phosphorus, magnesium and total protein. Out of 122 urine samples examined, 45 were diagnosed as the cases of haematuria, 33 samples as the cases of proteinuria and 44 samples revealed miscellaneous causes. Detail of the biochemical test result has been shown in table 6.

Table 6: Some biochemical values of Goats of Bandipur Goat Farm
Sample Size: 91

Parameters	Unit	Mean	Range Mean±2SD	Standard normal values
Calcium	mg%	8.68	6.70 -12.64	8.9-11.7
Inorganic Phosphotus	mg%	4.75	2.37-7.13	4.2-9.1
Magnesium	mg%	2.65	2.13 -3.17	2.8-3.6
Total Protein	g/dl	6.92	4.5 -9.34	6.4-7.0

Table 7: Epidemiological findings of animal disease outbreaks investigated during 2061/62

S. N.	Outbreak (Month/Year)	District	Animal Spp	Disease diagnosed	population at risk	Animal affected	Animal died
1	Shrawan 2061	Baglung	Buffalo	F.M.D.	22	4	0
2	Shrawan 2061	Tanahu	Goat	Pox	151	60	2
3	Bhadra 2061	Kaski	Pig	H.S.	25	5	2
4	Ashoj 2061	Livestock farm, Kaski	Buffalo	Alopecia	30	30	0
5	Ahsoj2061	Kaski	Poultry	N.D.	1600	1600	644
6	Ashoj 2061	Gorkha	Goat	External parasite/Abortion	70	14	7
7	Kartik 2061	Bandipur, Tanahu	Goat	G. I. Nematode	400	23	6
8	Kartik 2061	Myagdi	Buffalo	H.S.	46	11	8
9	Magh 2061	Kaski	Poultry	A. E.	1100	1100	435
10	Magh 2061	Shyanja	Buffalo	Scabies/ Mineral deficiency	20	7	2
11	Falgun 2061	Shyanja	Buffalo	F.M.D.	20	4	0
12	Chait 2061	Shyanja	Poultry	N.D.	950	950	256
13	Jestha 2062	Kaski	Poultry	Marek's	850	850	61
14	Jestha 2062	Myagdi	Buffalo	H.S.	240	7	3
15	Jestha 2062	Kaski	Goat	G.I. nematode	26	26	4

Sample collection and dispatch

A total of 546 serum, blood and tissue samples of different animal species and poultry were collected from the disease investigation areas in the western region.
Four hundred and forty seven various samples were subjected for the laboratory investigation at RVL, Pokhara.

Seventy four various samples were dispatched to Central Veterinary Laboratory, Kathmandu and 25 samples to National FMD & TADs Laboratory, Kathmandu for confirmatory disease diagnosis.

Human Resource Development

A total of 16 JT/JTAs from 16 DLSOs of western region were provided one week training on veterinary laboratory diagnosis.

Epidemiological bulletin Publication

During the F/Y 2061/62 four issues of Epidemiological bulletins were published to disseminate the information on the epidemiological disease situation of poultry and livestock in the region.

Disease Investigation and surveillance Program

Investigation of Mycoplasmosis in livestock

A total of 68 nasal swabs of goats suffering from respiratory disorders from Gorkha, Myagdi, Parwat and Gulmi were collected and subjected for Mycoplasma isolation. Similarly 9 pneumonic lungs from dead goats were collected and subjected for Mycoplasma isolation. One lung sample of goat revealed Mycoplasma. Fifteen poultry lungs were subjected for mycoplasma isolation however no mycoplasma could be isolated.

Investigation of Epidemics

Various disease outbreaks of animal and poultry were investigated during fiscal year 2061/62. A total of 15 epidemics were investigated. Of the outbreaks investigated, most were confirmed by laboratory while some confirmations were based on clinical signs and postmortem findings.

Animal Diseases profile from July 2004 to June 2005 in Western Region

Table 13: Animal Disease Profile of Mountain Districts.

Diseases	Mustang		Manang		Gorkha		Total	
	Case	Death	Case	Death	Case	Death	Case	Death
Bacterial Disease								
Anthrax	0	0	0	0	4	4	4	4
B.Q.	1	0	0	0	0	0	1	0
C.R.D	129	0	127	3	370	27	626	30
Infectious Coryza	8	0	0	0	0	0	8	0
Mastitis	0	0	5	0	44	0	49	0
Viral Disease								
E. Fever	0	0	15	0	0	0	15	0
Gumboro	0	0	0	0	500	65	500	65
FMD	0	0	0	0	98	0	98	0
PPR	0	0	0	0	20	7	20	7
Internal Parasite Disease								
Fascioliosis	10	0	0	0	10288	0	10298	0
Parasitic Gastroenteritis	5126	0	2364	0	0	0	7490	0
Intestinal Helminths	47	0	203	0	0	0	250	0
Paramphistomes	0	0	0	0	689	0	689	0
Ecto Parasitic Disease								
Mange	434	0	1750	40	755	0	2939	40
Ext-parasite	10223	0	2990	0	0	0	13213	0
Protozoan Disease								
Bovine Babesiosis	1	0	0	0	0	0	1	0
Coccidiosis	73	3	0	0	0	0	73	3
Other Diseases								
Foot lesion	0	0	388	4	0	0	388	4
Diarrhoea	492	11	865	0	3777	145	5134	156
Respiratory sign	114	0	0	0	16	0	160	0
Infertiflity	8	0	172	0	15	0	195	0
Anorexia	0	0	390	0	0	0	390	0
Wound	195	0	911	0	0	0	1106	0
Fever	0	0	555	0	0	0	555	0
Tympany	0	0	120	0	182	0	302	0
Abortion	23	0	45	0	36	0	104	0
Weakness	0	0	782	0	0	0	782	0
Red urine	10	0	66	0	22	0	98	0
Catration	138	0	858	0	0	0	996	0
Cough	0	0	374	0	1417	0	1791	0
Warbal Infestation	3	0	5	0	0	0	8	0
Skin lesion	12	0	9	0	353	0	374	0
Pneumonia	47	0	273	41	0	0	320	41
Indigestion	3	0	39	0	0	0	42	0
Retention of placenta	3	0	136	0	0	0	139	0
Fracture	0	0	10	0	0	0	10	0
Avitaminosis	0	0	100	0	0	0	100	0
Sudden Death	0	0	0	0	9	9	9	9
Degnala	0	0	0	0	17	3	17	3

Table 14: Animal Disease Profile of Terai Districts

Disease	Kapilvastu		Rupandhehi		Nawalparasi		Total	
	Case	Death	Case	Death	Case	Death	Case	Death
Bacterial Disease								
H.S	345	9	302	22	50	9	697	40
B.Q.	4	0	71	1	5	1	80	2
Mastitis	109	0	465	0	109	0	683	0
Enterotoxaemia	0	0	42	8	0	0	42	8
Pullorum Disease	0	0	534	96	0	0	534	96
CRD	880	90	2417	56	0	0	3297	146
Viral Disease								
FMD	236	3	356	0	330	15	922	18
E.Fever	8	0	910	0	0	0	918	0
Fowl Pox	607	80	22	2	0	0	929	82
Rabies	1	1	11	8	19	19	31	29
Ranikhet	1211	305	2700	110	0	0	3911	415
Gumboro	0	0	2237	31	0	0	2237	31
PPR	48	34	52	0	0	0	100	34
Endo Parasite Disease								
Fasciolosis	2254	0	4819	4	2044	1	9117	5
Parasitic Gastroenteritis	205	0	816	1	754	0	1775	1
Paramphistomiasis	0	0	562	3	0	0	562	3
Round Worm Infestation	0	0	79	0	0	0	79	0
Ecto Parasitic Disease								
Mange/Mites	302	0	344	0	10	0	656	0
Lice/Ticks	277	0	77	0	0	0	354	0
Warble Infestation	0	0	164	0	8	0	172	0
Protozoan Disease								
Coccidiosis	638	108	2757	31	44	0	3439	139
Thaileriosis	0	0	7	1	0	0	7	1
Bovine Babesiosis	0	0	3	1	0	0	3	1
Others Diseases								
Diarrhoea	1288	63	4351	22	546	6	6185	91
Respiratory sign	185	0	427	0	144	0	756	0
Infertility	183	0	398	0	117	0	598	0
Anorexia	0	0	338	0	15	0	353	0
Wound	276	0	567	0	112	0	946	0
Tympany	101	0	676	0	80	1	857	1
Abortion	137	0	290	0	64	0	491	0
Nervous Sign	52	0	715	10	14	0	781	10
Skin Lesion	161	0	238	0	43	0	442	0
Stomatitis	0	0	56	0	0	0	56	0
Red Urine	0	0	0	0	8	0	8	0
Sudden Death	9	9	14	14	3	3	26	26
Cough	70	0	276	0	36	0	382	0
Pyrexia	421	0	322	0	206	0	919	0
Poisoning	2	0	0	0	2	0	4	0
Prolapse	2	0	47	0	11	0	60	0
Retained Placenta	18	0	93	0	8	0	119	0
Pneumonia	21	3	0	0	93	0	114	3
Paralysis	8	0	0	0	2	0	10	0
Dystocia	18	0	41	0	23	0	82	0
Arthritis	0	0	60	0	1	0	61	0
Constipation	30	0	559	0	3	0	592	0
Castration	0	0	2225	0	193	0	2418	0
Actinomycosis	0	0	3	0	0	0	3	0

Table 15: Animal Disease Profile of Mid Hill Districts

Diseases	Tanahu		Kaski		Arghakhanchhi		Syanjha		Gulmi		Palpa		Parvat		Baglung		Lamjung		Mygdi		Total	
	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death
Bacterial Disease																						
H.S.	22	5	0	0	0	0	16	7	0	0	39	23	31	19	5	0	0	0	36	16	149	70
Mastitis	56	0	82	0	125	0	199	0	142	0	1416	0	45	0	16	0	24	0	142	0	2247	0
Fowl Cholera	0	0	0	0	0	0	0	0	0	0	0	0	209	0	1150	22	0	0	0	0	1359	22
CRD	207	1	0	0	17	0	100	0	14	0	781	118	67	0	0	0	0	0	385	23	1571	142
Enterotoxaemia	0	0	0	0	0	0	0	0	0	0	9	9	0	0	0	0	0	0	0	0	9	9
BQ	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	3	2
Viral Disease																						
FMD	156	4	151	12	0	0	394	10	0	0	1508	65	187	0	34	0	0	0	52	0	2482	91
E. Fever	0	0	0	0	0	0	0	0	0	0	375	0	0	0	0	0	0	0	74	0	449	0
Rabies	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	2	2
Fowl Pox	66	0	0	0	0	0	144	0	0	0	0	0	29	0	50	5	0	0	857	8	1146	13
Sheep and goat Pox	7	0	0	0	0	0	0	0	0	0	150	0	0	0	0	0	0	0	0	0	157	0
Parvo Enteritis	3	0	65	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	68	6
Gumboro	152	6	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	425	27	589	33
Ranikhet	0	0	0	0	0	0	150	52	0	0	0	0	0	0	0	0	200	100	0	0	350	152
P.P.R.	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
Endo Parasite Disease																						
Fascioliosis	4214	0	11359	0	1622	0	8239	0	5962	0	9833	0	2964	0	1248	0	4458	0	3773	0	53672	0
Paramphistomum	636	0	4497	0	242	0	3405	0	2187	0	2891	0	2041	0	1377	0	700	0	1127	0	19103	0
Parasitic gastroenteritis	2370	0	3558	0	2560	0	110	0	1074	9	8081	0	64	0	0	0	16	0	779	0	18612	9
Intestinal helminths	0	0	0	0	2359	0	42	0	0	0	0	0	0	0	0	0	0	0	1302	12	3703	12
Round worms	0	0	0	0	0	0	1636	0	0	0	0	0	0	0	0	0	0	0	0	0	1636	0
Ecto prasatic Disease																						

Mange/ Mites	43	0	1370	0	665	0	569	0	1331	0	1647	0	336	0	0	0	113	0	1534	0	7608	0
Lice/ Ticks	290	0	0	0	3344	0	2848	0	6174	0	2068	0	0	0	0	0	800	0	6313	0	28837	0
Protozoan Disease																						
Coccidiosis	1989	28	134	0	25	0	796	3	0	0	7791	386	230	0	1060	62	1477	128	320	16	13822	623
Bovine Babesiosis	45	1	0	0	0	0	0	0	0	0	214	22	0	0	0	0	0	0	0	0	259	23
Other Diseases																						
Diarhoea	1591	3	4156	0	872	0	541	0	1718	8	5564	46	863	0	466	0	263	0	2818	42	18852	99
Respiratory Signs	53	0	1137	0	230	0	198	0	470	0	2429	0	492	0	0	0	79	0	91	0	5179	0
Infertility	214	0	2067	0	208	0	291	0	195	0	1847	0	69	0	5	0	93	0	204	0	5193	0
Anoerexia	168	0	4522	0	179	0	355	0	0	0	0	0	0	0	0	0	0	0	0	0	5224	0
Wound	65	0	158	0	83	0	225	0	208	4	967	0	0	0	0	0	0	0	66	0	1772	4
Tympany	61	0	1491	0	141	0	120	0	379	5	0	0	0	0	0	0	0	0	354	0	2546	5
Cough	61	0	451	0	32	0	190	0	7	0	0	0	0	0	0	0	0	0	0	0	741	0
Opacity	11	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43	0
Abortion	53	0	1	0	42	0	13	0	98	0	894	0	6	0	2	0	59	0	127	0	1295	0
Nervous Sign	4	0	0	0	53	0	31	0	131	0	663	0	0	0	0	0	0	0	0	0	882	0
Skin Lesion	153	0	1581	0	370	0	5	0	301	0	1300	0	400	0	543	0	28	0	552	0	5233	0
Pyrexia	177	0	4178	0	0	0	117	0	0	0	0	0	0	0	0	0	0	0	0	0	4472	0
Stomatitis	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	0
Red Urine	122	0	100	0	2	0	91	0	20	0	298	0	99	0	10	0	68	0	108	0	918	0
Enzootic Haematuria	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0
Sudden death	46	46	1	1	11	9	0	0	81	81	599	42	0	0	0	0	6	6	28	28	772	213
Asthama	0	0	579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	579	0
Dystokia	35	0	0	0	45	0	77	0	0	0	1005	28	0	0	15	0	0	0	177	0	1354	28
Castration	0	0	0	0	1023	0	0	0	0	0	8781	0	0	0	0	0	0	0	1102	0	10906	0
Pneumonia	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0

Seromonitoring of PPR Vaccination Programme

During F/Y 2061/62 a total of 205000 goats and sheep were vaccinated against PPR in the western region. The RVL, Pokhara collected 1025(0.5%) serum samples for the seromonitoring and the serum samples were sent to the Central Veterinary Laboratory, Kathmandu for laboratory examination.

Details of Budget Expenditure (2061-62)

The details of budget expenditure of Regional Veterinary Laboratory, Pokhara for the year 2061/62 is shown in Table 14.

Table 14: Details of budget expenditure

Budget Line	Budget Heads	Approved Budget (NRs)	Expenditures (NRs)	Remarks
1.01	Salary	893223	835206.12	
1.02	salary suupl	62777	62776.72	
1.03	Transfer TADA	9000	6479	
1.4	Dress	11000	10835	
1.5	Feed	12000	11958	
2.01	Water and Electricity	106000	82212.85	
2.02	Telephone	42000	41999.13	
2.03	Office materials	501000	500997.64	
2.5	Repairs	183000	182932.20	
2.6	Fuel for other purpose	115000	111848	
2.7	Service cost	10000	4800	
2.8	Miscellaneous	17000	17000	
4.02	Medicine	90000	89629	
4.04	Program cost	50000	49317	
4.05	Program TADA	229000	229000	
6.01	Furniture	0	0	
6.03	Equipment	0	0	
6.04	Building	0	0	
	Total	2331000	2236990.66	

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

Mid-western region: an Overview

The mid western region is one of the five development region of Nepal situated in between the far-western and western development regions. It represents three zones namely, Bheri, Rapti, Karnali that encompass 15 districts. There are six municipalities and 577 village development committee in this region. This region represents 28.79% of the total area and 5.29% of the total cultivated land, 12.89% of the total forest area, and 12.63% of the total pasture land of the country. The land defragmentation in this region suggests that 40.35% of the total land is covered with forest area, 18.31% with pasture, 10.6% with agriculture, 4.14% as barren land and 26.6% with steep slopes, niches and snow land.

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 climatic contrast in the region. Geographical location includes mountain, hill and terai belt.

Mountain

The five district of Karnali zone makes the high hill mountain. The altitude of this region ranges from 738mt. in kalikot to 7334.7 mt. in Humla. Almost all part of Humla and Jumla is situated above 4000 meter from the sea level. Other three districts of this belt comprise both high and low altitude.

Hill

Hilly part of this region includes the district of Bheri and Rapti Zone. Among these districts Surkhet, Dailekh, Salyan, Rolpa and Puythan share similar altitude while Rukum and Jajarkot is located a bit higher in comparison to these districts.

Terai

The western part of the region confirms the terai belt. There are three districts that make the terai and inner terai area of the region. Bankey, Bardia and Deukhuri part of Dang district make terai.

The climatic variation of this region can be divided into four types as follows.

Temperate: Humla, Jumla and some of the highest part of Muju, Dolpa and kalikot.
Sub-temperate: lower part of Mugu, Dolpa and kalikot and throughout Rukum and
Sub-Tropical: Surkhet, Dailekh, Salyan, Pyuthan, Rolpa and Inner terai of dang
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 link roads used all along the year. Rest of district can be approached either by the treaking or aerial route Mugu and kalikot only by means of helicopter other means.

Information on Livestock and Livestock production in mid- western region (F/Y 2061-062)

S.N.	Animal species	Unit	Year 2005
1.	**Improved breed**		
	Cow	Number	10489
	Buffalo	,,	206129
	Goat	,,	360684
	Sheep	,,	42610
	Swine	,,	216891
	Broiler	,,	284036
	Layers	,,	783245
	Rabbit	,,	9650
	Chauri	,,	9184
	Horse	,,	1415
2.	**Local breed**	,,	-
	Cow	,,	1505326
	Buffalo	,,	665862
	Goat	,,	169073
	Sheep	,,	402014
	Swine	,,	265329
	Poultry	,,	2109903
	Rabbit	,,	5306
	Chauri	,,	18570
	Horse		12356
3.	**Improved verities grass cultivated area**	ha	726
4.	**Livestock product**	-	
	Milk	mt.	226831.4
	Meat	Ton.	74236.47
	Egg	Number	52864.75
	Wool	mt.	14473.25
	Ghee	mt.	1277.374
	Skin(leather)	Number	96766

Introduction

Regional Veterinary Laboratory, Surkhet serves for the animal health for the livestock population distributed throughout mid-western region of Nepal. The laboratory was established during the fiscal year (1988-89 (2048-049) with the view of developing it as a reference veterinary diagnostic and investigation centre representing all the 15 districts of Bheri, Rapti and Karnali Zone of Nepal. It is located at Itram village of Birendra Nagar municapality, the district as well as regional headquarters. Before the truely establishment of this laboratory, the pre-requisite infrastructure like reconstruction of building and procurement of equipment were undertaken by the existing regional directorate of livestock service (RDLS) through its annual program. The laboratory did not have its own staffing at the time of establishment and was run by the assistance of RDLS staff up to 1991-1992. In the succeeding years, the laboratory got its own manpower and started functioning as an independent body. The present manpower in the laboratory comprises two veterinarians, three mid level technicians and six supporting staff.

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

The process of rennovation has been added regularly in a more technical way to help in the routine work of the laboratory. The most remarkable of such steps was achieved during 1997-1998 with the technical support provided by strengthening of veterinary services for livestock disease control (SVSLDC) project. SVSLDC project had been continuously supporting the laboratory for its upliftment so that its physical and technical capabilities boosted along with others. RVDL, in this way, developed itself especially as a reference laboratory for investigation of goat diseases. In recent years, the laboratory is serving with disease surveillance and investigation and as epidemiological centre for mid-western region. Although, upgrading of the laboratory has been realized too slow, some of the encouraging results and sound achievement in the direction of investigation have been appreciating.

Objectives

To provide diseases diagnostic services to all type of livestock's owners.

- Monitor and assist in diagnostic services to basic and primary laboratory in the region.
- Investigate epidemics of animal diseases in the region and assist DLSO technically.
- Prepare the epidemiological profile of livestock and poultry diseases
- Investigate the diseases of animal species relatively important in this region
- Study the surveillance of animal disease of economical and zoontic importance
- Monitor and supervise district laboratories and serve as referral laboratory to them.

Laboratory Activities

Parasitology unit

This unit involves in the examination of fecal sample, skin 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 lung worm infestation in goats. In edition to this many fecalsample 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, 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 these serum samples collected from livestock and poultry are dispatched to CVL and other referral laboratories for prescribed diagnosis. This unit provides plate agglutination test for some of the diseases like *Salmonella pullorum*, micoplasama, brucellosis, tuberculosis. Seromonitoring of vaccinated goat and poultry is also performed by sending the serum sample to CVL.

Table 1: Description of Serological works conducted at RVL, Surkhet

S.N.	Date	Sample	No.of Sample	Investigation for Suspected Dis.
1.	2061.9.18	Serum	1600	P.P.R.
2.	2061.9.18	,,	257	P.P.R., Mycoplasma.
3.	2061.11.16	,,	100	Enterotoxaemia
4.	2062.3.8	,,	300	P.P.R., Brucella & Mycoplasma.
5.	2062.5.1	,,	454	P.P.R.
6.	Total		3165	

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

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 (especially 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 bulletin were published every year distributed in the region to concerned offices .

Supporting unit

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.

***Washing 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.

Serological examinations

Apart from the serological samples collected for PPR, mycoplasma and other tests for the diagnosis of ovine abortion. Altogether 272 samples were collected during F/Y 2061-062 PPR and were confirmed by CVL. The serum samples collected from Pyuthan, Dang, Salyan, Rolpa was intented for Mycoplasma test and Brucellosis.Other causes behind abortion in the mentioned districts could not be appreciated.The sample collected from the case of respiratory problems were dispatch to CVL.

Approved annual work program of fiscal year 2061/062 and summary of achievement of RVL Surkhet

S.N.	Program and activities	Unit	Target	Progress	% Progress
1	Laboratory service Program				
1.1	Parasitological Examination	Number	1200	1545	100
1.2	Microbiological Examination	,,	450	497	100
1.3	Pathological Examination	,,	250	265	100
1.4	Serological Examination	,,	600	614	100
1.5	Hematological Examination	,,	350	430	100
1.6	Biochemical Examination	,,	400	593	100
1.7	Sample dispatched	,,	350	593	100
2	Disease investigation and surveillance program			-	
2.1	Investigation of goat respiratory diseases	Time	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			-	
5.1	Computer training	Head	2	2	100
6.1	Quarterly epidemiological bulletin publication	Time	4	4	100
6.2	Annual technical report publication	,,	1	1	100
7	Purchase of scientific books and journals	,,	15	15	100
	Total programm cost	942		945	
	Total administration cost	1258		1072430/50	
	Total cost	2210		2017430/50	

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 272 samples were examined for

blood protozoan and other hematological parameters. Out of which 180 sample were from our regular investigation program of diseases of respiratory system in goat.

Table 2: Result of Parasitological examination

Month	Fasciola	Strongyles	Strong yloides	Moniezia	Protozoa	Paramp histomes	Trichuris	Ascarid	-ve	Total
Janaury	31	7	5	-	5	12	1	3	11	61
February	25	10	21	-	3	9	2	4	9	77
March	59	53	2	-	8	11	20	2	32	173
April	19	45	3	-	6	3	10	3	37	117
May	16	35	-	-	8	7	8	5	53	128
June	33	69	-	-	29	25	23	14	67	231
Jully	15	55	-	-	11	29	13	5	41	143
August	38	22	-	-	4	21	6	3	26	91
September	26	16	5	-	1	10	1	0	10	67
October	17	22	4	12	2	6	0	5	12	63
November	28	27	2	-	1	7	2	6	17	73
December	24	13	1	-	10	6	0	4	12	41
Total	331	178	43	12	88	145	87	54	327	1265

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 It was done for the 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.

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. During F/Y 2061-062 altoghter 376 samples were examined from the respiratiory disease of goat. Of these, 97 samples were procured from post mortem examination of poultry birds and 279 samples were collected from goats suffering from respiratory diseases. The result of culture and isolation is presented in table 3.

Table 3: Result of bacteriological culture of nasal swab

Bacterial species	Number Isolated	% of bacteria isolated
staphyllococcus epidermis	43	11.43
staphyllococcus aureus	48	12.76

streptococcus	51	13.56
proteus sps	39	10.27
pasturella haemolytica	26	6.91
E.coli	37	9.84
Bacillus	27	7.18
Positive	271	72.07

Table 4: Result of Haematological examination

H/B Estimation %	PCV%	TLC	DLC	Giemsa stain
57(6-11%)	57(30-40%)	57(5.9)	26 (N-29,E9.8, B-0-1,L-40, M-7-8)	26 (negtive) not seen any blood protozoa
100(8-12%)	54(25-36%)	60(4.9)		(negtive)
141(9-12%)	60(26-29%)	40(5.1)		(negtive)

Table 5: Description of diseases diagnosed tentatively during PM examination

Diseases of the poultry were summarized in the following manner .Altogether 299 cases of poultry were examined during the year 061/062.

S.N.	Diseases diagnosed	Number of cases	Percentage
1.	Colibacillosis.	19	6.35
2.	Coccidiosis.	67	22.4
3.	Salmonellosis.	34	11.37
4.	Bacillus	14	4.68
5.	Litchi heart disease.(Infectious anemia)	13	4.34
6.	Haemoragic enteritis.	23	10.7
7.	Ascitis	27	9.03
8.	Deficiency syndrome.	11	3.67
9.	Chronic respiratory disease.	6	2
10.	IBD	52	17.39
11.	Mycotoxicosis.	6	2
12.	Avian leucosis.	9	3.01
13.	other	18	6.02
	total	299	100

Regional Veterinary Laboratory Surkhet
Laboratory investigation report
2005 Jan to 2005 Dec

S.N.	Disease/Activities	Animal spp.	District	Sample		Test detail			Remarks
				No.	Tested	Method	+ve	-ve	
1.	Mastitis	C,B	S,D,B,	83	126	CMT	83	43	
2.	Microbiological identification	P,G,S	S,D,B,		56	CMT and PM sample	34	22	staph, Bacillus spp, E-coli, streptococcus.
3.	Anti-microbial sensetivity		S,D,B,	64	64	Antibiotic sensitivity test	-	-	ciprofloxacin-75%, Genta-65%, chloram-30% , Amoxy-15%,penicilin-10%
4.	salmonellosis	S,G,P	S,D,B,	93	93	S .kit	57	36	salmonella kit used
5.	Brucellosis	S.G	S,D,B,	76	76	B.R.T	-	-	B.R.T.
6.	Mycoplasma	p	S	36	36	M.kit	-	-	M.kit
6.	Faecal examination	B.C,G,S ,P	S.D.B,	1265	1265	sedimentation & floatation	938	327	Fasciola-374, strongyles-178, strongyloids-67,moniezia-12 , paramphistomum-145, trichuris-87, ascaris-54, coccidiosis-88 other-28
7.	Haematological examination	S.G	S,D,B,	259	259	Hb/PCV/TC/ DC	-	-	Hb-59, PCV-105, TC-45, DC-50
8.	Blood parasite	S,G	S,D,B,	172	172	Giemsa staining	-	-	-
9.	post mortem examination	P	S	102	102	PM observation	-	-	IBD, CRD, Coccidiosis, collibacillosis, licthiherd ?, other.
10.	sample dispatch to cvl for further investigation	S,G	S,B,D,Br	455	455	PPR,Mycoplasma	-	-	-
10.1	serum	B.C.S.G	S.D.B,	215	-	BRT,Myco...	-	215	-
10.2	skin scraping	S,G,B	salyan	12	12	10% KOH	8	4	mange
10.3	straw sample	-	-	-	-	-	-	-	-
10.4	Fungle growth sample	-	-	-	-	-	-	-	--

Abbreviations used:
District code - S: surkhet, D: dang, B: bankey, Br: bardiya
Animal sp. code - C: cattle, B: buffalo, S: sheep, G: goat, P: poultry,

Disease situation of mid-west on the basis epidemiological reporting (Jan 2005 - Dec 2005)

Disease	species	Cases	Death	Vaccine	Treatment
1. Liver fluke	cattle	3219	32	-	3187
	Buffalo	3940	6	-	3934
	goat	2183	17	-	2166
	sheep	1738	36	-	1702
	yak	26	3	-	23
2. Internal parasite	cattle	953	-	-	953
	Buffalo	984	-	-	984
	goat	1831	-	-	1831
	sheep	373	-	-	373
	pig	241	-	-	241
	equine	61	-	-	61
	donkey	454	-	-	454
3. Respiratory signs.	cattle	651	-	-	651
	Buffalo	271	-	-	271
	goat	781	-	-	781
	sheep	161	-	-	161
	pig	21	-	-	21
	equine	91	-	-	91
	dog	5	-	-	5
4. Rabies	dog	566	9	557	-
5. Skin lession	cattle	623	-	-	623
	Buffalo	361			361
	goat	1093			1093
	sheep	501			501
	pig	69			69
	equine	31			31
6. CRD	chicks	671	49	-	622
7. Gumboro	,,	2460	193	400	2267
8. N.D.	,,	1634	186	1270	2448
9. Coccidiosis	,,	3932	42	-	3890
10. Red urine	cattle	524	-	-	524
	Buffalo	127	-	-	127
	goat	21	-	-	21
11. Infertility	cattle	852	-	-	852
	Buffalo	262	-	-	262
	goat	224	-	-	224
	Sheep	29	-	-	29
12. Mastitis	cattle	166	-	-	166
	Buffalo	338	-	-	338
	goat	89	-	-	89
	Sheep	4	-	-	4
13. F.M.D.	cattle	135	-	-	135
	Buffalo	85	-	-	85
	goat	58	-	-	58
	Sheep	17	-	-	17
14. Cocci	cattle	247	-	-	247
	Buffalo	38	-	-	38
	goat	15	-	-	15
	poultry	439			439
15. Ephemeral fever	cattle	39	-	-	39
	Buffalo	51	-	-	51
16. H.S. / B.Q.	cattle	158	-	1035	158
	Buffalo	165	5	1352	160

REGIONAL ANIMAL DISEASE INVESTIGATION LABORATORY, DHANGADHI (Far-western Region)

Introduction

Far Western Development region covers only nine districts that fall under two zones viz; Seti & Mahakali. It is the smallest development region among the five development regions. The region shares borders with the Tibetan China to the north and Indian state border of Uttranchal and Uttar Pradesh to the south west.

Geographically the region is divided into three parts namely, Mountains, Hills and Terai. The mountainous districts comprises of Bajura, Bhajhang, and Darchula. The livestock rearing mainly consists of migratory flocks (seminomadic as well as transhumans type) of sheep and goats because the steep slopes of the mountains are not suitable for other agricultural practices. Cattle, Yak, Nak, Chauri, and Chyangra are the important livestock species raised in these districts.

In the four hill districts viz; Baitadi, Dadeldhura, Doti and Accham livestocks are kept mainly for the production of milk and milk products like ghee which is the main source of income to the farmers. Accham is particularly important due to the fact that the **Acchame** cow, the smallest indigenous breed of cattle is found in the district. It has been classified as endangered breed of cattle as its population is rapidly declining due to lack of conservation policy of native breeds.

Kailali and Kanchanpur constitute terai belt in the region. These districts bear high potential for the development of livestock components in the form of industry. Almost all kinds of livestocks species like cattle, buffalo, sheep, goat, pig, poultry, duck, etc. are raised. Pig farming is more common among the tharu communities who constitute one of the ethnic groups in these districts. Goat raising and poultry keeping are steadily growing in the form of commercial enterprise. Poultry farming is rapidly growing component in particular.

Disease status

There are a number of infectious diseases which remain as constraints to the development of the livestock sector of the region, apart from mangemental, nutritional and other factors. Major infectious diseases include FMD, PPR, Swine Fever, and HS. The parasitic diseases caused due to Liverfluke, Strongylus, Strongyloides, Haemonchus, Paramphistomes etc. infestation involve a sizeable financial loss.

A few economically important diseases of poultry in this region are New Castle, Infectious Bursal Disease, Coccidiosis, Chronic Respiratory Disease (CRD), Inclusion Body Hepatitis (IBH) also called Leechi Heart Disease, Collibacillosis, Mycotoxicosis etc. The disease like IBH is basically introduced from India due to open Indo-nepalese border.

Apart from the aforementioned diseases, there are few zoonotic diseases of major public health concern. Rabies and Japanese Encephalitis (JE) have been frequently creating havoc in human health which indirectly affects livestock development. While

Rabies is found to occur sporadically throughout the year affecting livestock and humans, a fairly good human population is affected with JE during the months of Shrawan and Bhadra claiming lives every year. In recent outbreak of Japanese Encephalitis more than 200 people have been admitted to different hospitals in Kailali and Kanchanpur districts, out of which 65 have already died. Mostly children below the age of 14 years are said to be affected. The effect of JE borne zoonosis has led the future of pig farming at the stake due to consecutive outbreak of this disease per year. As pig acts as the amplifier host of the JE virus, a large number of pigs are killed every year.

Veterinary medical care is hardly practiced based on the laboratory findings. It is, in most instances, based on the history, complaints laid by the farmers, and the clinical findings. Also more than a decade elapsed since the inception of Regional Veterinary Laboratory, Dhangadhi in 2049-050. It has not been able to provide diagnostic services to all the districts of the region. The major constraint is the insufficient flow of pathological samples from the districts livestock services. Nevertheless there has been considerable improvement in recent years in comparison to earlier days as some of the DLSOs and the quarantine offices are now submitting increasing number of samples for the shake of laboratory based treatment.

Activities of Regional Veterinary Laboratory, Dhangadhi

RVL, Dhangadhi is located in the heart of Dhangadhi municipality of Kailali district. It was established as a reference laboratory in the region.

The multidimensional role of the laboratory is ensured through a series of activities conducted in livestock development sector. RVL serves these functions through its several units. It does routine laboratory work and conduct work related to investigation programme mandatory to it. The technical set up of the laboratory is presented in figure 1.

Figure 1: Technical set up in RVL, Dhangadhi

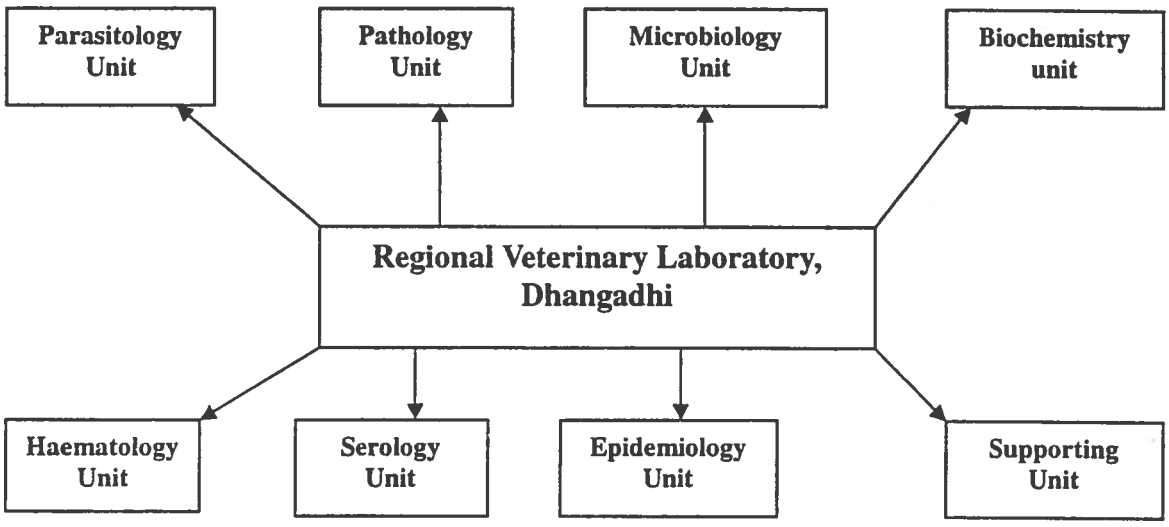


Table 1: Approved Annual Work Program and Summary of Achievement of RVL, Dhangadhi (F/Y 2061-062)

S.N.	Programs and Activities	Unit	Annual Target		Annual Progress	Progress Percentage
			Quantity	Weightage		
1.	Laboratory Service programme-					
1.1	Parasitological Examination	No.	2000	5.65	2791	100
1.2	Microbiological Examination	No.	350	2.83	343	98
1.3	Pathological Examination	No.	450	5.49	423	94
1.4	Serological Examination	No.	350	3.39	358	100
1.5	Hematological Examination	No.	400	3.23	406	100
1.6	Biochemical Examination	No.	400	4.52	377	94.25
1.7	Dispatch of samples (CVL and other Lab	No.	200	3.23	253	100
2.	Disease Investigation and Surveillence Programme					
2.1	Investigation and surveillance of kid mortality	Time	12	13.17	12	100
2.2	Investigation and surveillance of Khari Disease.	Time	12	28.27	12	100
2.3	Investigation of Epidemic Diseases	Time	6	8.89	6	100
3.	Inspection and Supervision Programme					
3.1	Inspection and Supervision of District Laboratories	Time	6	4.36	6	100
4.	Annual workshop on Animal Disease Investigation)	Time	1	2.02	1	100
5.	Training Programme:					
5.1	District Level sample collection and dispatch training	Time	3	1.62	3	100
5.2	Refresher Lab.	Head	12	6.54	12	100

	training for J.T, J.T.As					
6.	Publication Programme:					
6.1	Quarterly Epidemiological bulletin publication	Time	4	1.94	4	100
6.2	Annual Technical Book Publication	Time	1	0.40	1	100
7.	Purchase Programme					
7.1	Purchase of scientific Books and Journals	Time	1	0.81	100	100
7.2	Purchase of Anaerobic bacteria culture jar	Time	1	3.63	100	100

Percent Progress of Animal Health Services Programme: 99.23
Weightage of progress: 99.38%

Laboratory Services

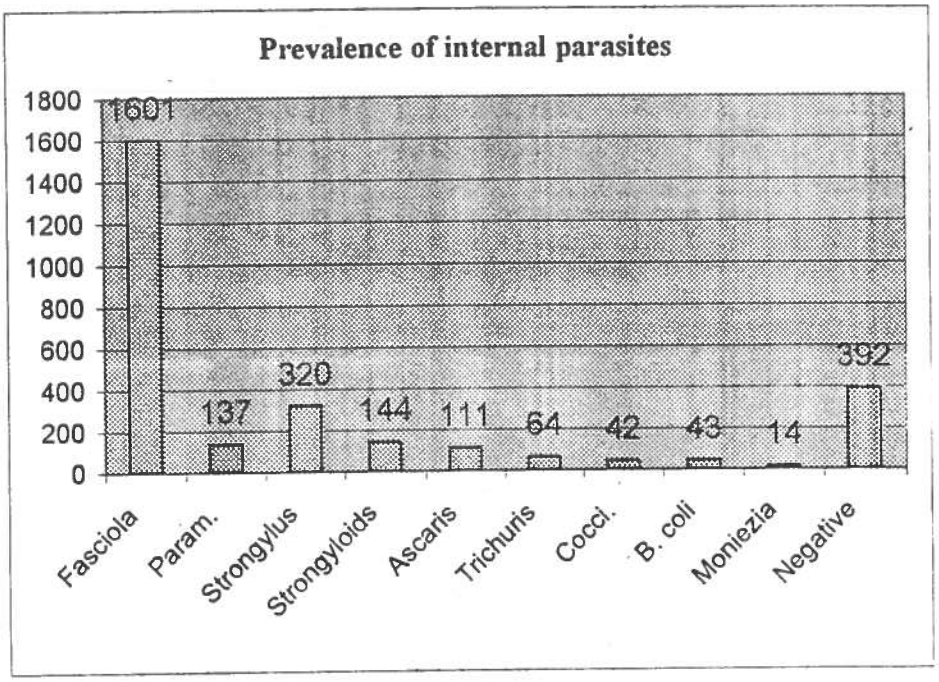
Parasitological Examination

The major flow of faecal samples is maintained by DLSO, Kailali. Apart from this source, samples were also collected from field during disease outbreak investigation and the investigation programme mentioned in our approved annual programme.

Altogether 2867 samples were tested for different parasitic conditions of livestock and poultry populations. Out of 2867 samples, 2475 (86.33%) samples were positive and only 392 (13.67%) were negative for the occurrence of any parasites. Analysis of coprological test result revealed that the most common digestive tract parasite was Fasciola followed by others of nematode group, viz. Strongylus, Stronglyloides, Ascarids, and Trichuris. Besides, Coccidia and Moniezia have also been identified in some species of animals. However, it is mentioned that cases of mixed parasitic infestations are frequently identified. The prevalence of internal parasites in livestock population is presented in the bar diagram listed as figure 2.

The present laboratory test result reveals that economic loss incurred by the farmers due to low production and productivity of animals is attributed, to a great extent, to infestations of aforementioned digestive tract parasites. It also suggests the level of awareness among the farmers of this region is low regarding problems related to animal health caused by such parasites.

Figure 2: Bar diagram representing the prevalence of common digestive tract parasites



Serological Examination

A total of 334 serological samples were received and tested for various diseases. Most of the serum samples were collected from goats for the diagnosis of PPR, Mycoplasma, Brucellosis and other diseases/conditions responsible for abortion. The samples thus procured were forwarded to CVL, result of which has been presented in table 2. The serum samples procured from poultry birds were examined for two important diseases namely, Salmonella and Mycoplasma. Similarly, serum samples collected from buffaloes under Khari disease investigation program from Baitadi and Darchula districts were sent to CVL for biochemical analysis and test against Brucellosis.

A serum sample collected from pig suspected for swine fever during an outbreak in Tribhuvanbasti, Jhalari, and municipality area of Mahendranagar in the month of March 2005 was also forwarded to CVL for serological examination result of which the result is still awaited. Details of the serological tests conducted at RVL, Dhangadhi has been shown in table 3.

Table 2: Result of Serum samples sent to CVL

S.N.	District	Animal spp.	No.of sample	Test requested	Result		Remarks
					Positive	Negative	
1	Kailali, AQCP	Goat	17	PPR	2	15	
2	Kanchanpur	Goat	5	PPR	0	5	
3	Bajhang	Sheep	11	PPR	8	3	

4	Kanchanpur	Swine	1	Swine fever	-	-	Not confirmed
5	Kanchanpur	Goat	11	PPR	-	-	Not confirmed

Table 3: Result of Serological examination performed at the RVL, Dhangadhi

S. N.	Animal species	Number of samples	Type of test performed								Samples sent to CVL
			Salmonella		Mycoplasma		Brucellosis		Tuberculin		
			+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
1	Bovine	71					0	35	0	9	27
2	Sheep/goat	194			3	25	0	87			79
3	Poultry	110	2	54	2	56					0

Haematological Examination

Haematological examination includes TLC, DLC, TEC, PCV, ESR, Hb estimation, and examination of blood smears for the presence of blood protozoan parasites. The sources of blood samples includes different animals during cases of epidemic outbreaks in field condition, samples referred by the DLSO Dhangadhi and the animals selected for preplanned investigation programme of the laboratory.

Interpretation of the test results of the blood samples collected from anevealed that these samples invariably showed low Hb concentration and very high ESR value. Hb value in these animals ranged between 5.4 - 7.6 g/dl and the ESR value recorded to as high as 65 mm/hr.

A total number of 159 blood smears were examined for blood protozoan parasites with 156 negative samples and only three positive samples. The positive samples included two cases of Babesiosis and only one case of Theileriosis. The result of haematological test has been shown in table 5.

Table 5: Haematological tests conducted at the RVL, Dhangadhi.

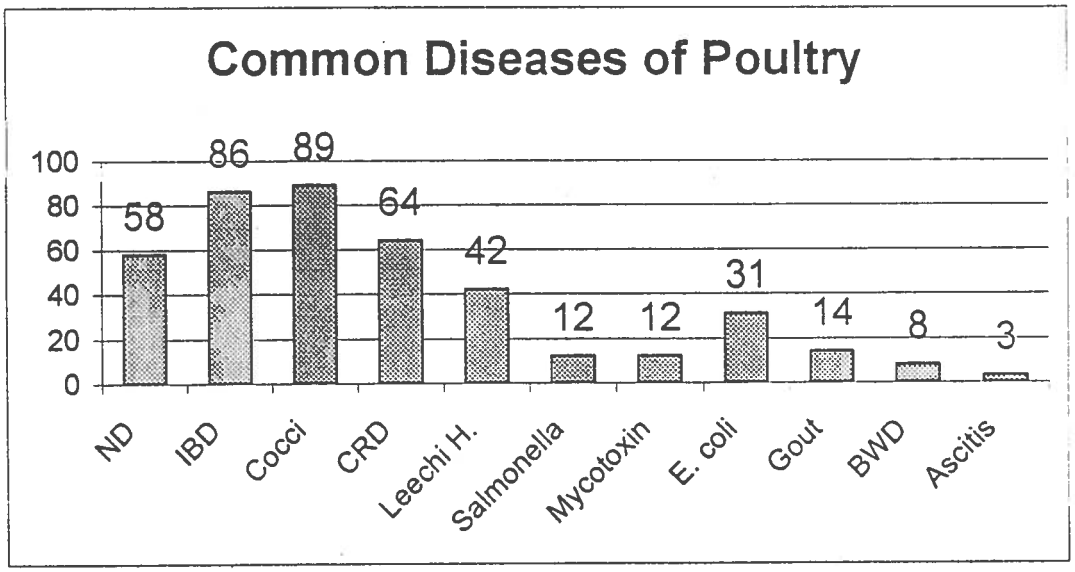
Type of Test	Number	Remarks
Total Leukocyte Count	2	
Total Erythrocyte Count	2	
Differential Leukocyte Count	66	
Packed Cell Volume	96	
Haemoglobin estimation	96	Generally low in Khari diseased animals
Erythrocyte Sedimentation Rate	4	High in Khari diseased animals
Blood parasites	159	3 positive, 2 Babesia, 1 Theileria

Pathological Examination

Necropsy examination includes to a great extent, the case of poultry followed by few cases of other animals and birds. In many instances necropsy of large animals is done

in field condition. Such type of PM examination includes cases of disease outbreak and cases important from vetrolegal view point. Tentatively diagnosed cases are confirmed through various laboratory procedures at RVL or in many cases relevant pathological samples are forwarded to CVL and other relevant laboratories. The trend of poultry disease occurrence in the area has been represented in the bar diagram in figure 3.

Figure 3: Bar diagram showing common Poultry Diseases of Far Western Region



The commenest problem among the poultry birds was identified as Coccidiosis followed by Infectious Bursal Disease, Chronic Respiratory Disease, New Castle Disease, Leechi Heart Disease and Colibacillosis. Less common but important diseases/conditions were diagnosed as the cases of gout, Bacillary White Diarrhoea, other Salmonella infections and Ascitis

Apart from poultry post mortem examination was done with nine goats and only one pig. The diseases/conditions diagnosed in case of goats were Pneumonia, Jaundice, Kumri and Parasitic infestations and that in case of pig was Swine Fever.

Microbiological Examination

The samples subjected to microbiological examination comprise milk, nasal swab, vaginal swab, and swab from visceral organs like liver, lungs, intestine etc. of various animal species. The media used commonly for microbial culture were Nutrient agar, Mc conkey agar, Blood agar, and Saboroud Dextrose Agar. Bacteria and fungi were identified on the basis of colony characteristics, morphological features and Gram's staining property. Due to limitation of the facility available in the laboratory, biochemical tests for further verification of different bacterial species could not be performed fully. However, a few biochemical tests like catalase test could be done in few cases. During the F/Y 2061-062, a total of 334 pathological samples were received in the laboratory result of which has has been shown in table 6.

Table 6: Description of pathological samples with test results

Animal Spp.	Type of sample	Number	Major bacteria identified
Cattle	Milk	59	Streptococcus Spp, Staphyllococcus Spp, Corynebacterium Spp, Pseudomonas Spp. and Enterobacter Spp.
Buffalo	Milk	82	Streptococcus Spp, Staphyllococcus Spp, Corynebacterium Spp, Pseudomonas Spp. and Enterobacter Spp.
Buffalo	Skin	7	Blastomyces and Candida Spp.
Goat	Nasal swab, vaginal swab	92	Streptococcus, Staphyllococcus and Bacillus Spp.
Poultry	Liver	50	Escherichia coli, Streptococcus, Staphyllococcus, Corynebacterium Spp.
	Lungs	19	Escherichia coli, Streptococcus, Staphyllococcus, Corynebacterium Spp.
	Intestinal swab	13	Escherichia coli, Streptococcus, Staphyllococcus, Corynebacterium Spp.
	Egg	19	Negative

Biochemical Examination

Biochemistry unit deals with biochemical exploration and estimation of essential components responsible for disease processes. For this pupose urine and serum are tested using commercially available kits. Due to limited resources available in this unit, qualitative examination of urine samples was done mostly. Estimation of Calcium could be only done with serum samples. The qualitative parameters of urine appreciated include detection of urobilinogen, protein, pH, blood, specific gravity, ketone bodies, glucose, bilirubin etc. Microscopic examination was carried out only when the case history is suggestive of infectious diseases of urinary tract so that different types of casts and cells are explored in the urine. Detail about the biochemical test in given in table 7.

Table 7: Description of biochemical examination with test results

Type of sample	Number	Test used	Remarks
Urine	27	Multistix	11 large, 2 moderate ketone
Serum	14	Commercial kit for calcium estimation	Biochemical estimation of Calcium but the result was unreliable

Biochemical examination of calcium was done with serum sample collected from buffaloes suffering from Khari disease. The test result was not satisfactory because variable results were found with same kit when the test conducted more than once. However, the result of on the basis of average observation suggested the level of serum calcium ranging between 3-18mg/dl.

Epidemic investigation

Description of epidemic investigation has been presented in table 8

Table 8: Location of epidemics

S.N.	District	Month	Disease outbreak
1.	Kanchanpur	Poush	Swine Fever
2.	Bajura	Magh	FMD
3.	Bajura	Phalgun	PPR
4.	Doti	Chaitra	FMD
5.	Bajhang	Chaitra	PPR
6.	Darchula	Jestha	FMD
7.	Dadeldhura	Asar	RD & IBD

Table 9: PPR seromonitoring Programme

District	No. of Vaccinated Goat and sheep		Collection of Serum samples (Number)		Remarks
	Target	Progress	Target	Progress	
Dadeldhura	20,000	20,000	100	100	The target set for serum collection was 0.5 percent of total vaccination. It is evident that progress in Bajura is apparantly lower than the set target.However number of serum collected is more than 0.8 percent of the vaccination accomplished.
Kanchanpur	15,000	15,000	75	75	
Kailali	30,000	30,000	150	154	
Bajura	65,000	24,819	325	200	
Bajhang	20,000	20,000	100	100	
Achham	15,000	15,000	75	75	
Baitadi	15,000	15,000	75	76	
Doti	30,000	30,000	150	150	

Publication of Quarterly Epidemiological Bulletin

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

quarterly and annual bulletin comprises the detail of disease existence of this region, it will only be the repetition of the same thing to present it here.

Manpower of Regional Veterinary Laboratory, Dhangadhi

S.N.	Name of the staff	post	Class	Sanctioned	Availa -ble	vacant
1.	Dr. Bimal Kumar Nirmal	Senior VO	GII	1	1	-
2.	Dr. Raju Gautam	VO	GIII	1	1	-
3.	Mr. Shyam Prasad Pathak	JT	NGI	1	1	-
4.	Mr.Anil Man Sob	JTA	NGII	1	1	-
5.	Mr. Hari Singh Bhandari	JTA	NGII	1	1	-
6.	Ms.Menaka Shrestha	Typist	NGI	1	1	-
7.	Mr. Mithan Lal Bist	Store keeper	NGII	1	1	-
8.	Mr. Khem Raj Joshi	Accountant	NGII	1	1	-
9.	Mr. Netra Bahadur Kathayat	S. man	NGIII	2	1	1
10.	Mr. Shankar Prasad Paudel	Driver	None	1	1	-
11.	Mr.Daman Bahadur Shahi	Peon	None	1	1	-
12.	Prem Bahadur Chaudhary	Peon	None	1	1	-
13.	Total			13	12	1

Investigation of Illness and Mortality in Buffalo in Dhading District- A report

Vinay Kumar Karna[1], Rebati Man Shrestha[2]

Abstract

The farmers of Tandi Bainsi village-8 of Jyamrung Village Development Committee in Dhading district noticed sudden illness in buffaloes with febrile reaction, off fed, diminished water intake, low milk yield and finally death after one month of morbidity. Within the period of one month, some 13 buffaloes died creating a roughly estimated financial loss toll of NRs. 2.86 lakhs. After investigating the situation, it was found that the causes behind death of the buffaloes were Blood Protozoan Parasites (Babesia and Anaplasma spp.) worsened the condition with Subacute Haemorrhagic Septicaemia acquired through vaccination of sick buffaloes with (H.S+B.Q.) combined vaccine (Manufacturer: Central Biological Production Laboratory, Tripureshwor, Nepal) preserved in freezing chamber of home refrigerator. Therefore, the objective of the present paper is to explain how improperly preserved vaccine and vaccinating an ailing animal are responsible for vaccine failure and disease development.

Methodology

1. Epidemiological Information

General

Housing System

The buffalo shed was typical of village type. In majority of cases, buffaloes are kept in permanent sheds located in close proximity of dwellings. Such sheds typify thatched huts in terms of construction. In some cases, buffaloes are kept in temporary sheds during daytime located in the vicinity of dwellings. These sheds are provided with thatched roof with walls either open or temporarily partially covered. The floors are made of earth and are provided with green leaves as bedding material to avoid cold waves. During night hours, buffaloes are kept in the sheds located at ground floor of two-stored wooden houses. It was learned that buffaloes kept in temporary sheds during daytime suffer a lot during inclement weather.

Feeding Practice

Free range grazing is practised negligibly. They practise stall-feeding only. The common roughages comprise locally available tree leaves and range grasses. Maize flour is fed as concentrate feed to milch buffaloes.

[1] Veterinary Officer, Central Veterinary, Tripureshwor
[2] Chief veterinary Officer, Central Veterinary, Tripureshwor

Health Management

There is less access of qualified veterinary medical service. Occasionally, on demand, Village animal health workers and junior technicians serve their animals. There is negligible practice of deworming and vaccination among the stocks. In any kind of illness, farmer use to go to District livestock service office (DLSO) for fecal examination and follow treatment regime as per advice. Similarly, farmers do not vaccinate animals against any disease. Use of vaccine found to practice in disease condition.

Breeds, breeding practice and origin of the buffaloes

Majority of buffaloes domesticated in the village are native breeds. Some of them were crossbreed of Murrah buffalo. There is no access of artificial insemination service and hence natural service is practiced. One buffalo bull has been found to serve all the buffalo available in the village.

All the sick buffaloes were of local origin. However, the dams were either bought from Indian border side villages or Nepalese border side villages of Rupandehi, Sarlahi, Rauthat and Parsa districts. The purpose and idea of buffalo rearing was found according as the preference of ethnic group. The lower caste (Damai) raise female buffalo calves, served by locally available bull and after parturition, they immediately sell their buffaloes to fetch a good sum of money. Conversely, the upper caste (Brahmin) use to rear milch buffalo and they sell calves within the village or adjacent villages.

Clinical

Case history

Some 13 buffaloes and one cow were found ill at eight different sheds during the period of investigation. Of them, three buffaloes were really showing illness while others including cow were found apparently healthy. Following are important chronological events behind the illness and mortality among the buffalo population.

- Illness starts with off fed and denial of water intake.
- Febrile reaction of fluctuating nature. Generally, the temperature ranged between 100-104°F.
- After few hours of clinical episode, animal looks apparently healthy and resume normal feeding and water intake followed by illness. This episode found to occur in cyclical order.
- Vaccination against HS+BQ at the initial stage of illness. Interrogating the shopkeeper sold the vaccine to the farmers, it was learned that the vaccine was preserved in freezing chamber of the home refrigerator, which leads to freezing of the vaccine content.
- Some of the buffaloes show constipation followed by semisolid defecation with foeted odour but not diarrhoea.

- The dying animals stretch their anterior portion of the body and bellowing just before death after 23- 28 days of illness.
- The treatment trial was noticed varying farmer to farmer. During the initial stage of illness, some of the farmers followed Basipen (Ampicillin)/Bistrepen injection @2.5 gm I/M for 5 days. Some of them followed Meriquin injection @10 ml I/M for three days as advised by the available technicians. Hopelessly, they filed complaints in DLSO. Three of the farmers followed further treatment as advised by district veterinarian. The treatment regimen was Sulfadimidin injection @ 80 ml I/V with plasma expander (Lactated Ringer) for two consequent days followed by the same dose S/C. Simultaneously, Berenil@ 1.66 gm/buffalo I/M was also tried with the least success. No complete recovery was seen in any animal.

Clinical findings

- The temperature ranged between 100-103.8°F.
- The vaginal mucous membrane was pale pink suggesting anemia in six of the buffaloes examined. Rest of the buffaloes was having pink mucous membrane.
- Prescapular lymph node was normal.
- Rumen motility ranged between 0-3/2 minutes.
- In some of the buffaloes, respiratory difficulties and scant watery nasal discharge recorded.
- Presence of hard tick in the buffalo sheds.

Post mortem examination

One buffalo was learned died 12 hour past of our arrival in the village and therefore post mortem examination could not be approached. The DLSO technician and the local butcher involved in the post mortem, however, collected a few organs in 10% formalin provided by the DLSO.

2. Sample collection

- Blood samples in EDTA as well as in plain vaccutainer- 24 (Twelve in either tube)
- Fecal sample- 12
- Liver, lungs, spleen, intestine in 10% formalin- One set

3. Laboratory Examination and Test results

The twelve sera samples were tested for the occurrence of Brucellosis by plate agglutination method. All the sera were negative. However, tube agglutination test was not practiced for the fact that the clinical findings showed irrelevancy to the occurrence of Brucellosis. Neither of the faecal samples showed any negotiable parasite and its load. However, few eggs of some of the common nematodes were seen which was interpreted clinically, not important.

Histological examination of lungs revealed mild inflammatory responses. The lesions present in the intestine and spleen were not negotiable. Obvious pathological changes were noticed in the liver with fatty degeneration of hepatocytes, hemorrhages and chronic inflammatory reactions. There was the presence of adult parasites in the section, which could be interpreted as trematode (Amphistomes) parasites.

Table 1: Result of blood smear examination

S.N.	Farmers' name	Species of animals	Blood Protozoa identified	Remarks
1.	Lekh Nath Pathak	Buffalo	Anaplasma + Babesia	Milch
2.	Padam Pathak	Buffalo	Anaplasma + Babesia	Milch
3.	Sujan Pathak	Buffalo Heifer	Anaplasma + Babesia	Apparently healthy
4	Lekh Nath Pathak	Buffalo Heifer	Anaplasma + Babesia	Sick
5	Padam Pathak	Cow	Anaplasma + Babesia	Apparently healthy
6	Purna Nepali	Buffalo Heifer	Anaplasma + Babesia	Healthy
7.	Sujan Pathak	Buffalo	Anaplasma + Babesia	Milch (Sick).
8.	Lal Bahadur Nepali	Buffalo	Anaplasma + Babesia	Apparently healthy
9.	Santa Bahadur Pathak	Buffalo	Anaplasma + Babesia	Apparently healthy
10.	Purna Nepali	Buffalo	Anaplasma + Babesia	Sick
11.	Moti Ram Pathak	Buffalo	Anaplasma + Babesia + Theileria	Apparently healthy. Dumb-bell but Gram + ve like organism in the blood smear.
12.	Jeeb Nath Pathak	Buffalo	Anaplasma	Apparently healthy

In the course of blood smear examination, one of the smears among the twelve showed the presence of only one Gram +Ve (Stained with Giemsa's stain), dumb-bell like organism under the microscope. It was interpreted as the presence of bacteria in the blood. Consequently, the blood sample was then kept in for bacterial culture using *blood agar* media. After 18 hours of culture, a scant quantity of the culture was made in smear and stained as per Gram's Staining technique. Upon examination of the smear under microscope, a pure culture of Gram –Ve, dumb-bell shaped bacteria was explored. It was interpreted as the organism of *Pasteurella* spp. However, biochemical test was not performed with the bacteria and interpretation was finalized on the basis of media specificity and cultural as well as staining characteristics.

Discussion

So far thirteen buffaloes died during one-month period. The duration of illness and ultimate death was found to vary from 23-28 days. This lays the idea of non-occurrence of sudden death or diseases of per acute or acute nature. It seems a sub acute nature of illness. Given the laboratory test results and the situation fortified with the use of improperly preserved vaccine in ailing animals, may abstract an idea

for the occurrence of sub acute hemorrhagic septicemia. Further, non response to antibiotic therapy, anemic animals, under dosing of Berenil, presence of hard ticks and rainy season help to formulate the idea of blood parasite infestation also which is also evidenced with laboratory test report. Though we could not grab the opportunity of examining a buffalo heifer died. On macroscopic examination of the liver, lungs, spleen and intestine, which were provided by the technician, complete fibrosis of liver with the presence of amphistomes recorded. In this single case, the cause of death might be chronic parasitism (Distomiais). In other cases, parasite might be one of the most important predisposing factors along with inclement weather and unhygienic farm practice calling the aforementioned condition.

Conclusion

Given the above facts and figures, it may be concluded that the buffaloes in the village were died due to blood protozoan diseases with supremeposed infection of Pasteurella causing Sub-acute form of Haemorrhagic Septicaemia that was driven among the buffalo population due to vaccination of diseased animals with improperly preserved H.S.+ B.Q. vaccine. Conclusively, this exemplified a very good case of vaccinal hazard in field condition.

Acknowledgement

We would like to extend our heartiest thanks to Mr. Gyan Bahadur Bogati, senior technician, Histopathology unit, CVL who was a member of the investigation team along with Mr. Dhan Pat Yadav, senior technician, DLSO, Dhading, sincere efforts of whom made the investigation study possible.

A Case study Of Marek's Disease in Layers

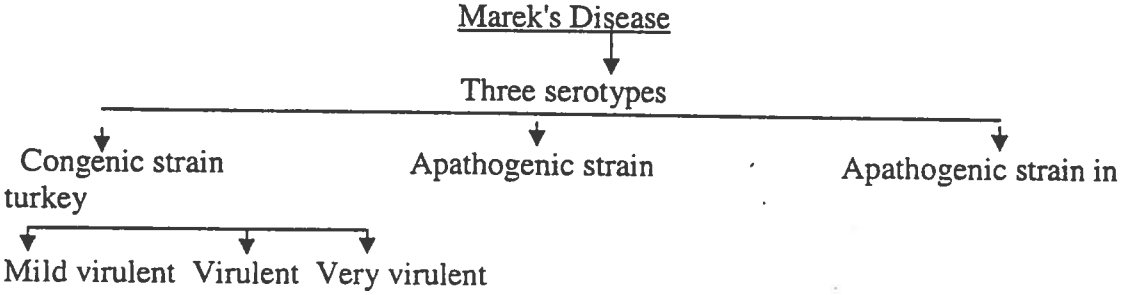
D. R. Chapagai[1]

Abstract

A study related to Marek's disease was conducted in Chitwan district based on clinical and laboratory diagnosis. For this purpose, two hundred and thirty Post Mortem (PM) samples submitted to National Avian Laboratory (NAL) were taken into consideration alongwith the provision of farm visit for clinical examination of the disease that included 39 poultry farms. Among the 230 PM samples, pathological lesions of Marek's disease (MD) were recorded in 19 cases. The othere 39 farms was visited & the antimortem was done by visualize the farm with individual affected birds shown by the poultry farmers. During the farm visit, birds at 13 different poultry farms exhibited symptom of MD which was then subjected to post mortem as well as laboratory examination. It was concluded that Marek's disease has been one of the commonest problem among layer flocks in Chitwan district causing a sizeable financial loss to farmers.

Introduction

Marek's disease (MD) is a viral disease caused by DNA Herpes virus. It is the disease of proliferation of the lymphoid tissue affects most of the visceral organs & tissues mainly affected the peripherl nerves. The virus produces four types of antigen called mild MDV, virulent MDV, very virulent (VV) MDV and very virulent plus (vv+) MDV strains.



Historical bacground

The marek's disease was known first time by Jozef Marek's about a century ago in 1907. Outbreak were reported in 1914 in USA, Netherlands , UK and many other countries.The disease gradually increased in severity mortality 20% in pullet flock in 1922. A marked increased in mortality in between 1925 and 1937. Due to marked mortality and loss of chickens industry concerns promoted the fedral fund to construct a new laboratory. An initial project launched in 1939, were directed towards the genetics and pathology of fowl paralysis in chickens.

[1] Veterinary Officer, Ntional Avian Laboratory

Methodolody

1. Case History

The history of flock was given in the entry registration form. By external clinical symptoms & visualize the carcase it was more or less conformed the M.D. disease in sever condition.The P.M. of birds, which show the tumour in the visceral part with paralitic sign are conformed. The following_Metholody are used to diagnose the disease.

Clinical symptoms and signs

The following symptoms and signs were observed in poultry birds suffering from MD during farm visit.

- Light weight (emaciation) of the birds
- Paralysis of the limbs, wings, cervical nerves if present torticolis of neck.
- Incordination of movements. .
- In case of torticolis neck gets twisted and the head may be turned upside down.
- The iris of eyes may become whitish and have deep eye.
- Tumors may develop in the skin and feather follicles.
- The keel bone is pointed and sharp as knife.
- Reluctant to move and stay in one place.
- Loss of appetite.
- Loss of brightness of feathers as well as birds.
- Birds lie on its side with one leg stretched forward and other backwards.

2. Post mortem Examination

On PM examination, presence of tumors in different visceral organs was the most striking lesion in majority of birds. Besides, following pathological changes were seen during post mortem examination.

Nerve: Loss of glycerine shining of sciatic nerve.Loss of cross striation and grey discoloration.Edematous appearance (2-3 times enlargement of nerves). Small nodules are found on palpation.

Visceral organs: Lymphomatous lesions(tumor and tumor like growth) can be found in the gonad (ovary), lungs, liver, heart, pancreas, kidney, spleen, bursa, thymus' proventriculus, intestine, iris of eye and skeletal muscle.Sometimes white or grayish firm mass is ofen found in liver lungs and proventiculus.Nodular may also seen in liver with circular type.In ovary marked envolvement of cauliflower like appearance. Proventiculus and lungs become thickned and firm which is detected by palpation.In heart one or more nodular tumor may be seen in myocardium or pin point foci may be seen in the epicardium.

Skin: The whitish nodule in dressed carcass and scab like lesions with brownish crust formation is found in few cases.

Eye: Grey eye (loss of pigmentation in the iris) Slight haemorrhage and corneal edema were observed.

3. Laboratory finding

In case of subclinical stage blood was taken from the suspected birds & 0.2 ml of blood inoculated by yolk sac route in 4-6 days old chicken embryos. The pathological changes were observed routinely. The CAM of chicks dying after 24 hours of inoculation can be examined but not found in laboratory. The pock lesions of MD virus were seen after 11 days of inoculation. The embryos also showed atrophy of muscles & curling of neck & limb.

Discussion

MD is not curable disease. Main transmision is by infected premises where day old chicks become infected by the oral and respiratory routes. Dander from feather follicles of MD infected chicken for more than one year.Young birds (chicken) susceptible to horizontal transmission.Susceptibility decreases rapidly after the few days or month. Virus can survive for 4-12 month in dropping & litter.The most common mode of infection is by inhalation of infective particles .Unvaccinated flock may show viraemia at 8th weeks of infection.Hens are more susceptible than male.

It is differentiated with avian leucosis complex . In ALC the birds are sexually matured and it occurre in laying stage .In case of MD birds are sexually immature. Most of the cases were found age of 3.5-5.5 month .In case of M.D. Nerves are affected and paralysis but not in ALC.

It is well to prevent the birds from MD is effective vaccination. The vaccination program is most widely and economic method of prevention.The serotype I cell associated vaccine gives better response. The cell free and cell associated forms of HVT are available but the cell associated HVT has been most effective and widely used.MD. Vaccines are given to day old chicks or before hatched. Both vaccine are given by s/c or I/m inoculation.Vaccine are also effective at day18 of incubation.Revaccination at 7-12 days can be given .It is popular in Europe and USA.The maternal antibodies reduce the effectiveness of cell-associated vaccines. The shorter the interval between vaccination and exposure to the virulen field virus, thepoor the level of protection because at least 7 days is required to gain the immunity after vaccination.The stress which appears to interfere the vaccinal immunity.The immunive suppressive stress may play an important role in MD outbreaks in vaccinated flocks.The onset of egg production.IBD, Reovirus, deserves condition interfere to the vaccinal immunity.

Conclusions

M.D. is not curable disease,better to prevent by vaccination as well as better management of the farms.Advise was given to farmers to saperate the affected birds ,to burn the dander and feather,to change the litter & to provide the immino modulative therapy in supplement.

References

SAIF' Y.M., Diseases of Poultry, 11th edition 2003, Lowa state press pp. 407-448 wigh, DC. Hirsh, N.James Macllachlan, R.L.Walker, Veterinary microbiology -2nd edition David Sainsburry, Poultry Health &Management, 4th edition, Important poultry diseases- Intervet

Investigation on Infertility in Cows of Eastern Terai Rrgion of Nepal

S. N. Dev[1], K. P. Sah[2]

Abstract

The present study was coducted to investigate the causes behind the problem of infertility in the cows reared under rural managemental system in Morang and Sunsari district of Nepal from the fiscal year 2001/02-2003/04. After site selection, all the information regarding general and health management, breeds and breeding system and the problems faced by the farmers in terms of low production and productivity of their farm animals were recorded and accordingly gynaecological examination of the animals suffering from infertility was done. Similarly, one hundred and sixty-one anoestrus cows were selected for laboratory based examination to assess haemoglobin (Hb) and serum level of calcium, protein, phosphorus and copper. Biochemical estimation revealed the serum level of Hb, calcium, total protein and phosphorus below normal while that of copper was in normal range. Based on laboratory test results, line of treatment was formulated and ensued in the animals under investigation study with promising results in 56% of animals. It was concluded that nutritional deficiency may be one of the cause of infertility in cows of the areas under study.

Background

Infertility problem accounts for more than half of all losses resulting from diseases of cattle. It has been considered as one of the major problems in Nepalese dairy cattle as it costs serious financial loss per annum attributed to feeding, medication and managerial costs on one hand and simultaneous losses incurred due to low production on the other. An optimum financial benefit can be ensured if a cow calves once per annum and for this a cow must be free from gynaecological disorders. Therefore every effort should be directed to bring a cow into estrous after two months of calving and should conceive by the end of three month post calving.

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 micro elements 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 association of nutritional deficiency with infertility problem.

[1] Senior Veterinary Officer, RVL, Biratnagar
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Introduction

Infertility is a multifactorial syndrome. The causes of infertility may be broadly classified as infectious and non-infectious. The major non-infectious cause includes deficiency of various types of nutrients (low energy intake, deficiency of major trace minerals and vitamins), post-partum loss of body weight, environmental stess (extreme heat, extreme cold) etc. Infertility due to nutritional causes is usually characterized by failure of estrum or cessation of the estrous cycle and only under certain condition; 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). In this way, there remains a scope to improve breeding efficiency of the indigenous stocks through balance feeding regimen, scientific way of management and efficient health coverage. The infectious cause includes all the diseases/conditions created due to bacterial, viral, fungal and parasitic infestations.

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.

Objectives

Study the incidence of infertility among indeginous cows in the area under investigation.

Assess the nutritional status of infertile animals.

Evaluate therapeutic nutrition.

Materials and Method

Relevant format was designed and primary information related to reproductive performance was gathered by direct interviewing the farmers following door to door survey in the selected sites. For the present investigation, a series of planned activities was designed as progressed accordingly.

1. Selection of sites, screening the animals and clinical examination

For the shake of convenience, areas having most of the cow suffering from infertility problem was selected. Information on site of investigation has been presented as table 1. Then screening of cows showing reproductive disorder was done, information on which has been provided in table 2. Every year, 10 apparently healthy cows having normal estrus cycle and free from reproductive disorders were also selected. The screened animals were then studied for breeding history, body score, milk yield and other relevant information through a set of questionnaire. Besides, physical examination of the reproductive organs of the anoestrus animal was done to determine the anatomical condition of ovaries, uterus, cervix and vagina for any defect.

2. Sample collection and laboratory Examination

Blood samples were collected from anestrous as well as healthy cow from the jugular vein under aseptic condition for the estimation of haemoglobin and serum separation. The serum was then stored at – 20°C for biochemical studies. Nutritional assessment of all anoestrus and control animals were done.Estimation of haemoglobin, total protein and phosphorus was done as per information provided with the specific kits.

Table 1: Sites of investigation

Year	District	VDCs
2001-02	Morang	Jhorahat, Sorbhag,Tankisinbari
2002-03	Morang Sunsari	Jhorahat, Sidhraha Pu. Kusaha Duhabi
2003-04	Sunsari	Chhitaha, Chimari

Table 2: Screening of cows for investigation

Year	Type of animals		
	Anoestru	Heifer with delayed puberty	Post-partum anoestru
2001-2002	51	30	21
2002-2003	50	32	18
2003-2004	60	39	21
Total	161	101	60

For the estimation of copper, serum samples were sent to the Central Veterinary Laboratory, Kathmandu.

3. Treatment trial

All anoestrus cows were dewormed with broad spectrum anthelmintics namely, Oxyclozanide and Levamisole (Brand name: Oxyzan-L).

All the anoestrus cows were supplemented with mineral mixture (Brand name: Agrimin forte) at the dose rate of 30 gm/day for 40 days.

4. Evaluation and follow up

Gynaeco-clinical examination of treated cows on 21 and 42 days post treatment alonwith blood collection for reassessment of mineral level in serum.

Evaluation of treatment trial and follow up works were carried out regularly. Apart from this, those animals that came into heat after medication were inseminated.

Result and Discussion

The results of laboratory tests conducted during the investigation are presented in tabular form as follows.

Table 3: Result of biochemical estimation of different serum constituents in anestrous and control cows before treatment

	Hb gm%			Total protein gm%			Phosphorus mg/dl			Copper Mg/dl			Calcium Mg/dl		
	Mi	Av	Ma	Mi	Av	Ma	Mi	Av	Ma	Mi	Av	Ma	Mi	Av	Ma
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	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

Effect of mineral mixture therapy

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

Table 4: Effect of mineral mixture therapy

Year	Number of animals		Percent
	Anestrous	Exhibited estrous	
2001-02	51	31	61
2002-03	50	27	54
2003-04	60	32	53.33
Total	161	90	55.9

Table 4 reveals 55.9% of animals exhibited oestrus 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, 21, and 42 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. The result has been summarized in table 5

Table 5: 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 percent)	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. The physiological changes studied in the ovary of treated animals are presented in table 6.

Table 6: Effect of mineral mixture therapy on the ovary from 0 day to 42 day

Animal type	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

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 estimated values of Hb and other biochemical constituents in the serum of anoestrus rural cows before treatment are presented in the table 3 in the annexure. 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 has 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.

Similarly, 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.

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 and 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.

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Reference

Arthur, G.H.; Noakes, D.E.; and Pearlon, H. (1989).Veterinary control of herd fertility. Veterinary Reproduction and Obstetrics, 6th edition, Bailliere Tindal, London.pp356-358

Bhaskaran, R. and Khan, A.C.K. (1981). Role of blood serum inorganic phosphorus in post parturient anoestrus cows. Livestock advisor 6 (10): 33-35.

Blood, D.C., Radostitis, O.M. and Henderson, J.A. (1983). A test book of the diseases of cattle, sheep, pigs, goats and horses, sixth ELBS edition.

Dyson, D. (March 2000) Strengthening of Veterinary services for livestock disease control project. – Interim report.

Dyson, D.; Sharma, B.; and Pant, G.R. (2000) Investigation on infertility in cattle and Buffaloes in Nepal. Veterinary review, NAARC, KTM, Nepal.15:7-9

Sharma, M.C.; Joshi, Chinmay; Saxena Neetu; and Das Gunjan. (2004), Role of minerals in reproductive performance of livestock. Livestock International , volume 8, Issue 5, PP 5 – 10.

Dutta, J.C.; Baruah, R.N.; dutta, L. and Talikdar, S.C. (1988) Blood biochemical studies in anoestrus and normal cycling cattle. Indian Vet. J. 65: 239-241

Ivanov, A.I.; Sorikin, M.M.; Cheredkor, S.N.; antyukov, M.A. and Semenov, B.Ya. (1970) The effect of nutrition on reproductive function in cows and bulls. Nauch. Trudy. Belerossk. Mauchnoissled. Vet. Inst. 8; 149-150 (Cited from Anim. Breed. Abstr. 40: 3068.

Kumerasan, A. (2002) Repeat breeding syndrome in Cattle and Buffaloes, therapeutic approach at field level. Pasudhan. 16(4):1

Luktute, S.N. and Sharma, C. (1978), Studies on the incidence of true anoestrus in rural cattle and buffaloes. Indian Vet. J. 55: 940-942.

Larson, L.L.; Mebruek, H.S. and Lowry, S.R. (1980). Relationship between early postpartum blood composition and reproductive performance in dairy Cattle .J.Dairy Sci., 63:283-289

Naidu, K.V. and Rao, A.R. (1981): Incidence of infertility among crossbred cattle of Andra Pradesh. Indian J. Anim. Sci. 51:829-831.

Reddy, V.N.V. (1998) Reproductive Disorders in Dairy Cows. Vijaya scientific publication, Bangalore, India.

Rao, A.R. (1997) Reproductive Disorders in Females. Reproductive disorders In Indian Livestock, ICCAR. New Delhi.pp356-358

Sharma, M.C.; Umashankar; Gapped, O.P.; Verma, R.P. and Mishra, R.R. (1984): Biochemical studies in cyclic, anoestrus and repeat breeking crossbred cows. Indian J. anim. Reprod. 4:51-53.

Sinha, B.P.; Sinha S.N. and Sinha,B.(1987) Incidence of anoestrous in crossbred cattle in field and farm condition. Livestock Advisor: 13(3):43-48

Hydropericardium Syndrome in Broilers in Pokhara Valley

S.P. Devkota[1], V.C. Jha[2]

Introduction

Hydropericardium Syndrome (HPS) is an emerging disease of broiler birds characterized by high morbidity and mortality which involves liver along with fluid accumulation in the pericardial sac. The mortality rate ranges from 10-80 percent. In some outbreaks mortality may reach upto 100 percent. In India, it is called Leechi Heart Disease because the heart surrounded by Hydropericardium lesion appears similar to a peeled leechi fruit. In Pakistan, it is commonly known as Angara Disease because of its first appearance in Angara Goth, Karanchi.

Literature review

Hydropericardium Syndrome involves mainly broilers of 3-6 weeks of age. Rare outbreaks in broiler breeder flocks and commercial layers were also recorded. The mortality and severity of lesions may be higher in immunosuppressed birds particularly with concurrent infections of Infectious Bursal Disease, Marek's Disease and Chicken Infectious Anemia. Prevalence and severity of HPS outbreaks are related to the density of the poultry in an area. The HPS agent is highly pathogenic and spreads readily form flock to flock and farm to farm laterally through oral-faecal route.

Originally, HPS was attributed to a toxicity or nutritional deficiency. An infectious agent was suggested as a possible cause when it was discovered that the disease could be reproduced by inoculating young broilers with bacteria free liver homogenates from affected birds. In late 1988, it was determined that an RNA virus was an aetiological agent. More recently, HPS virus has considered to be a member of group I adenovirus serotype 4 as the causative agent. The adenovirus is a DNA virus.

In natural outbreaks, the affected birds may not exhibit any clinical signs other than sudden heavy mortality. However, in the terminal stage, the birds become dull and depressed and show a characteristic posture, with their chest and beak resting on the ground and with closed eyelids. These birds show yellow, mucoid droppings.

The most striking postmortem lesion is the presence of upto 10 ml of clear transudate in the pericardial sac. Generalized congestion and pulmonary edema are evident and the liver and kidneys are usually enlarged, pale and friable. Histological lesions in the heart reveal mononuclear cell infiltration, severe vascular changes, and severe edema leading to disruption of muscle bundles, hemorrhages and degenerative changes. The liver shows focal areas of necrosis and mononuclear cell infiltration. Centrilobular or diffuse degeneration and necrosis of hepatocytes, swelling of the hepatocytes due to severe fat accumulation which in some cases leads to rupture of cell membrane. The hepatocytes are shrunken in some areas and have pyknotic nuclei. Degenerating hepatocytes almost always contain basophilic intranuclear inclusion bodies,

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surrounded by a clear halo or filling the entire enlarged nucleus. Kidneys reveal marked swelling of the tubular epithelium, necrosis and extensive haemorrhages. Changes in the Bursa of Fabricius, thymus and spleen include lymphocytosis and cyst formation, leading to depletion of lymphocytes in the medullae of follicles of Bursa of fabricius.

Materials and Methods

During the fiscal Year 2061-62 (2004-05 AD), various outbreaks of a disease in broiler flocks in the Pokhara valley was reported to the Regional Veterinary Laboratory, Pokhara. During the investigation, postmortem examination were performed and for further confirmation the histopathological samples like heart, liver, Bursa of fabricius in 10 percent buffer formalin were sent to the Central Veterinary Laboratory, Tripureshwor, Kathmandu.

Case history

The symptoms as stated by the farmers were sudden accelerated mortality with characteristic posture, with their chest and beak resting on the ground with closed eyelids.

Necropsy examination

Out of 956 postmortem cases of birds conducted in the laboratory, 79 cases were tentatively diagnosed as hydropericardium and hepatitis.

During the postmortem examination these birds showed fragile swollen liver with pale uneven surfaces. The pericardium was filled with straw coloured fluid in large quantity. The heart size was increased with slight petechial haemorrhages on the myocardium. Spleenomegaly, congested lungs, nephritis and swollen or atrophied bursa of fabricious were other pathological lesions found out during the postmortem examination.

Laboratory diagnosis

Histopathological samples collected during postmortem examination included heart, liver and Bursa of fabricius. They were preserved in 10 percent buffer formalin and referred to Central Veterinary Laboratory, Tripureshwor, Kathmandu. The result of histopathological diagnosis has been mentioned below.

Results

The histopathological findings of the samples sent to the Central Veterinary Laboratory were as follows:

- Heart: Revealed severe myocardial haemorrhages, degeneration and edema of individual myofilbrils, infiltration of huge number of mononuclear cells as well as pericardial membrane is also heavily infiltrated with lymphocytes.

- Liver: Haemorrhage and severe fatty degeneration of the hepatocytes. Severe infiltration of mononuclear cells, but absence of inclusion bodies.
- Bursa of Fabricious: Bursal follicles were highly haemorrhagic, some of the follicles were showing lymphoid cells depletion in the centre.

The symptoms, postmortem findings and the histopathological findings revealed that the outbreak were due to Hydropericardium Syndrome. All these cases supported that there were several outbreaks of HPS in Pokhara valley. In these outbreaks at about 55 farms with more than 33000 birds population were at risk.

References

Calnek, B.W. (ed.) Diseases of Poultry, Tenth edition, Iowa State University Press, Iowa, USA, pp:1019-1022.

Chandra, R., Rao, V.D.P.; Govez-Villamandos, J.C. ; Shukla, S.K; Banerjee, P.S. (edited) Diseases of Poultry and their control. (2001) International Book Distributing Company, Lucknow. UP, India. pp: 188-195.

Chauhan, H.V.S. and Roy, S. Poultry Diseases, Diagnosis and Treatment (1998) Second edn. New Age International (P) Limited, Publishers, India. pp: 177-179

Karna, V.K. Inclusion Body Hepatitis-Hydropericardium Syndrome in Broilers in Nepal: A Case Report, Nepalese Vet. J. 27:95-98.

An outbreak of Hemorrhagic Septicemia in Buffaloes of Myagdi District- a report

V.C Jha[1], S.P. Devkota[2]

Introduction

Haemorrhagic septicemia occurs in cattle, yaks, camels and water buffaloes and to lesser extent in pigs and horses. *Pasturella multocida* (Carter type B) is the cause of haemorrhagic septicemia in Asian and North American countries (Radostits et al, 2003). Haemorrhagic septicemia is most prevalent in rainy seasons and factors like increased work (fatigue), poor nutrition, changes in management, temperature and seasonal variation predispose to development of clinical disease.The present report deals with an outbreak of haemorrhagic septicemia in buffaloes of Myagdi district.

Materials and Methods

In November 2004 an outbreak of a disease in buffalo was reported from Arthunge Village Development Committee (VDC) of Myagdi district. Investigation was carried out after receiving the information of the outbreak. The technical staff of the District Livestock Services Office, Myagdi visited the outbreak area and based on clinical symptoms, tentatively diagnosed the case of outbreak as haemorrhagic septicemia. The staff could only collect the long bone from a dead buffalo. The long bone preserved in ice was brought to the Regional Veterinary Laboratory for the laboratory investigation. Epidemiological data such as total population, number of affected animals, dead animals and recovered animals were recorded.

In the laboratory the bone marrow from the long bone was procured aseptically and subjected to bacteriological culture on blood agar and MacConkey agar aerobically. The organism isolated was further subjected to biochemical tests and antibiotic sensitivity tests (Cowan, 1974).

For pathogenicity test in mice, ten fold serial dilutions of an eight-hour old nutrient broth culture of the isolate were made up to 10-3. Each dilution was injected intraperitonially to groups of two mice at the rate of 0.5 ml each and observed to determine mortality among them. The same quantity of plain nutrient broth was injected in control group of two mice.

Results and Discussion

In a population of 46 buffaloes in the investigation area, the number of affected buffaloes was 11 aged between 1.5-6 years. The affected buffaloes showed clinical symptoms of high temperature (105 to 106°F), anorexia, general weakness, depression and dysponea. Respiratory distress and edema in the lower jaw and throat

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were the prominent clinical signs. Out of 11 affected buffaloes 8 died within 12 to 48 hours after onset of clinical symptoms. The dead animal could not receive appropriate treatment in time. Three buffaloes could recover after treatment with Enrofloxacin @ dose rate of 15 ml I/V and Diclofenac/paracetamol injection 20 ml I/M for five days. The morbidity and mortality rates were 23.9% and 17.4% respectively.

The bacteriological cultural examination of the bone marrow sample revealed moderate, round, grayish and nonhaemolytic colonies on the blood agar plates and no growth in MacConkey agar plates. Gram staining of the colonies revealed Gram negative coccobacilli. The Giemsa staining of the colonies revealed typical bipolar organisms.

As the pure colonies were isolated in the first attempt, the isolate was subjected to biochemical tests. They were found positive for catalase, oxidase, indole production, nitrate reduction and were negative for motility. The results of the antibiotic sensitivity test showed that the isolate was sensitive to enrofloxacin, tetracyclin and amoxycillin and resistant to cotrimoxazole and penicillin.

The results of mice inoculation revealed that the isolate was highly pathogenic to mice. There was 100% mortality within 48 hours in all groups of mice which were injected with broth cultures containing different dilutions of the organism. No mortality was seen in the control (two in number) mice which were injected with the plain nutrient broth.

The findings of this investigation suggest that the buffaloes had suffered and died from haemorrhagic septicemia. The high case fatality (72.7%) in this outbreak might be due to ignorance of the farmers about the disease, delayed reporting about the outbreak and non-access of appropriate treatment in time to seriously affected animals.

After confirmation of the disease the remaining cattle and buffaloes in the village were immediately vaccinated against Haemorrhagic septicemia using Alum Precipitated H.S. vaccine.

References

Cowan, S T (1974). Manual for the identification of medical bacteria, 2nd edition, Cambridge University Press, London

Radostits, O M, Gay, C C, Blood, D C and Hinchcliff, K W (2003), Veterinary Medicine, Ninth Edition.

Investigation of reproductive disorders in cows and buffaloes in western region

V. C. Jha[1] & S. P. Devkota[2]

Introduction

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

Infertility in animals is associated with microbial pathogens, anatomical and cogenital anomalies, hormonal imbalance, nutritional deficiency, hereditary defects and extreme climatic conditions.

Jha (2005) reported that out of the 118 serum samples of repeat breeder and aborted cows examined, 0.8% samples were positive for the presence of antibody of brucellosis, 9.3% for leptospirosis and 50.8% for infectious bovine rhinotracheitis/Infectious pustular vulvovaginitis and no samples positive for chlamydiosis.

The present study attempts to investigate the extent of infertility problem and to isolate, identify the specific and nonspecific causes responsible for infertility in cattle and buffaloes of western region. The treatment responses of nutritional supplementation and/or antiseptic and antibiotics in infertility cases have also been evaluated.

Materials and Methods

In collaboration with Livestock Services Offices of Rupendehi, Kaski, Syanja and Tanahu districts, the infertility camps were attended and also the dairy farms having cows and buffaloes suffering from infertility problems were visited. Clinical examination and rectal palpation were performed and where appropriate pathological samples were collected for laboratory investigation.

Five vaginal swabs and 24 uterine mucus samples were collected from the cows and buffaloes suffering from endometritis and/or vaginitis and/or cervicitis with a history of repeat breeding and/or abortion. The vaginal and uterine mucus samples were preserved in the Cary Blair transport media until the samples were subjected for Campylobacter and other bacteriological procedure in the laboratory. For Trichomonads the cervical and/or uterine mucus samples were examined on a slide

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under a microscope. Blood samples were collected for serological examination of brucellosis.

During farm visit, some of the cows were found to having inactive ovaries and a few of them found to suffer from endometritis and/or cervicitis on clinical examination. They were treated at hand with following treatment regimen.

Anoestrus

- Vitamin AD3E injection 5 ml I/M on alternate days (Total two doses)
- Minerals powder 50 gm/ animal/day orally for 10 days.
- Tonophosphon injection 10 ml I/M on alternate days (Total two doses)
- After above treatment Janova 3 capsules on 11 th day and 3 capsules on 12 th day given orally.

Repeat breeder

- Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine.
- Gentamycin injection 25 ml I/M daily for three to four days.
- Minerals powder 50 gm/ animal/day orally for 10 days.

Aborted animals

- Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine.
- Terramycin LA injection 20 ml I/M on alternate days (total 3 injections)

After five months the area was visited to evaluate the reproductive status of the treated cows and buffaloes.

Results and Discussion

The reproductive problems recorded among the breed type are presented in table 1. The Disease condition found during rectal examination of cows and buffaloes are presented in table 2.

Table 1: Reproductive problems recorded among cattle and buffloes

Species/Breed	Age group	Number of animals examined		
		Anestrus	Repeat breeding	Abortion
Cow/Jersey	2-11 yrs.	14	19	8
Cow/Holestein	3-13 yrs.	2	6	2
Buffalo/Murrah	3-10 yrs.	2	1	-
Buffalo/local	2-7 yrs.	2	6	1
Total	63	20 (31.74%)	32 (50.79%)	11 (17.46%)

Table 2: Disease condition found during rectal examination of cows

Reproductive disorder	Pathological condition			Codition of ovary			Normal
	Endometritis/Pyometra	Vaginitis/ Vulvitis	Cervicitis	Inactive	Follicular cyst	Lutea l cyst	
Anoestrus	-	4	1	12	-	-	3
Repeat breeding	4	12	4	-	4	2	7
Abortion	2	2	-	-	-	-	6
Total	6	18	5	12	4	2	16
Percent	9.52	28.57	7.93	19.04	6.34	3.17	25.39

Table 3: Bacterial species isolated from the uterine mucus samples

Bacterial species	No. of isolates
Bacillus spp	23
Escherichia coli	10
Staphylococcus spp	9
Streptococcus spp	2
Micrococcus spp	2
Enterobacter spp	2
Proteus spp	2
Pseudomonas spp	2
Total isolates	50

It can be seen in table 3 that the most common isolates from the vaginal and uterine mucus were *Bacillus species* followed by *Escherichia coli* and *Staphylococcus species.* The pattern of bacterial isolates found in this study indicates that most of the isolates, which does not seem to be pathogenic in healthy condition, may be pathogenic when the reproductive organs are under stress or injured Singh et al (1996) reported that the *Bacillus spp.* was more frequently isolated followed by *Escherichia coli* and *Staphylococcus aureus* from cervico-vaginal mucus of repeat breeder cows. Deshmukh and Markandeya (1995) reported that ascending types of microbial infections and residual microflora of genital tract of cows under certain conditions lead to low grade endometritis resulting into repeat breeding.

Table 11: Antibiotic sensitivity test of some bacterial isolates

Isolate	Number of isolates tested	AM	EX	G	CF	CX	CO	C	K	O
Escherichia coli	10	7	10	10	10	5	7	10	8	6
Pseudomonas spp.	2	1	2	2	2	0	1	2	2	1
Staphylococcus spp	9	2	9	7	7	6	4	8	6	4
Bacillus spp	23	14	23	18	23	7	14	23	15	7
Streptococcus spp.	2	2	2	2	2	1	0	2	2	0
Micrococcus spp	2	1	1	2	0	1	1	2	1	0
Proteus spp	2	0	2	2	1	0	0	0	1	1
Enterobacter spp	2	1	2	2	2	0	0	2	1	0

AM = Amoxycillin, Ex = Enrofloxacin, G = Gentamycin, CF= Cefotaxime CX= Cloxacillin CO= Cotrimoxazole C=Chloramphenicol, K= Kanamycin, O = Oxytetracycline

Among the isolates examined for antibiotic sensitivity tests, most of the isolates were susceptible to gentamycin, chloramphenicol, enrofloxacin and cefotaxime. Majorities of the tested isolates were found to be resistant to amoxycvillin, oxytetracyclin and cloxacillin. Based on this finding the gentamycin, chloramphenicol, enrofloxacin and cefotaxime can be promising drugs to treat the infectious cases of infertility caused due to bacterial infections.

The cervical and uterine samples tested for Trichomonads microscopically, none of the sample was found positive. The cervical and uterine samples subjected for campylobacter isolation were found negative.

The 12 serum samples subjected to the Rose Bengal Plate Test for the detection of antibodies of Brucella were all negative.

It can be seen in table 5 below that out of 63 infertility cases treated, only 44 treated cases could be followed up after an interval of five months. Out of 44 followed up cases, 27 (61.36%) cases were found to be concieved. The treatment response of the cows and buffaloes was found promising. Therefore the treatment therapy applied in this study may constitute judicious line of treatment in cases of infertility due to infectious causes and in particular infertility due to microbial origin.

Further future works in detail needs to be done on the identification of various causes of infertility including infectious causes and development of its strategic control measures.

Table 5: Treatment response in cows and buffaloes with reproductive disorders

Gynaecological disorder	Treatment applied	Number of animals		
		Treated	Followed up (after 5 months)	Pregnant
Group-1 Anoestrus	Vitamin AD3E injection 5 ml I/M on alternate days (Total two doses) Minerals powder 50 gm/ animal/day orally for 10 days. Tonophosphon injection 10 ml I/M on alternate days (Total two doses) After above treatment Janova 3 capsules on 11 th day and 3 capsules on 12 th day given orally.	20	14	9
Group-2 Repeat breeder	Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine. Gentamycin injection 25 ml I/M daily for three to four days. Minerals powder 50 gm/ animal/day orally for 10 days	32	22	13
Group-3 Abortion	Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine. Terramycin LA injection 20 ml I/M on alternate days (total 3 injections) After 3 to 4 months a follow up visit was made to know the reproductive status of the treated cows and buffaloes.	11	8	5
	Total	**63**	**44**	**27**

Reference

Deshmukh, VV and Markandeya, NM (1995) A study on antibiotic sensitivity pattern of bacterial isolates from repeat breeding cows, Int. J. Anim. Sci. 10: 337-338

Jha, VC (2000) Study on infectious causes of infertility in crossbred & exotic cattle in Nepal. Annual Report 1999/2000, Animal Health Research Division, Tripureswor: 19-23.

Jha, V C (2005) Study on infectious causes of infertilityand its management in crossbred & exotic cattle in Nepal.Nepalese Veterinary Journal, Vol. 28: 25-31

Khanal, DR (1996) Proceedings of First National Workshop on Livestock and Fisheries Research in Nepal, May 7-9, Khumaltar, Lalitpur.

Sankhi, K.P. (1999) Souvenir, Sixth National Veterinary Conference, Nepal.

Singh, N.P. Chaturvedi, V.K. and Singh, DP (1996) Bacteriological studies on repeat breeder bovines, Indian Vet. J. 73: 462-463.

Investigation of diseases of goat under commercial rearing system

U. P. Shah[1]

Introduction

Goat rearing is one of the traditional occupations particularly popular among the rural poor community of Nepal. Statistical data reveals that 5.4 millions of goats in Nepal (DFMAS 1991) are mostly reared under conventional rearing system. The efforts made in the past to develop goat farming as commercial enterprize has posed positive impact in some parts of the country. In the process of commercialization, some of the newer diseases have been introduced with the introduction of improved breeds of goats. These diseases need to be diagnosed properly to adopt appropriate preventive and control measures.

Objective

Keeping this fact in view, the regional veterinary laboratory (RVL) Surkhet alongwith others has been assigned to investigate the diseases of goat under commercial rearing system with the following objectives from the F/Y 2055-56.

- Trace out the viral diseases met with goat population under commercial rearing system
- Confirm the viral diseases of goat based on the laboratory examination
- Establish the Pathophysiological parameters of viral diseases in Nepalese breeds of goat.

Materials and methods

1. Retrospective study

The retrospective study involves recording of information on previous cases. There have been records of problems/diseases of goats in some of the districts of the region namely, Surkhet, Banke and Dang.

2. Prospective study

Site selection

Selection of site was cofirmed utilizing the tools of participatory rural appraisal (PRA). However, basic criteria like easy accessibility to the site, nearby service centre/sub centre or DLSO, users' group, group strength, priority in terms of disease problems were taken into consideration during site selection. In this way, Sivashakti Dangapari Goat Breeding Society located at Satabaria-4 of Dang district was selected for present study. This user group keeps its recognition in having local breed of goat named Dangpari with strength of 22 active members with 317 goat population. RVL will consider other sites as well in future based on the criteria mentioned above.

Activities

Recording usual problems faced by the group members in goat keeping utilizing PRA tools, information derived from retrospective study, monthly herd inventory record and monthly visit to the investigation was scrutinized and followed accordingly.

[1] Veterinary Officer, RVL, Surkhet

Laboratory examination

Relevant samples were collected during the scheduled as well as emergency visited during outbreaks. These samples were decided to put into appropriate laboratory procedure.

Analysis of the available information correlating with laboratory test results

All the problems with goat rearing related to health and management was investigated using PRA tools and a way of clinical examination of the sick goats. The history and clinical findings so obtained were compared with the laboratory test results. Weightage paid by diiferent diseases/conditions were prioritized based on the disease information list for Surkhet, Dang and Banke districts present in the laboratory and the laboratory diagnostic results.

Results and discussion

Following are the result of present investigation.

Round worm

It is one of the most frequent problems found in the both of the districts and the percentage ranges from 34-42%.

Diarrhea

It was found another main problem in goats and parents ranges from 11-15%.

Digestive disorders

It includes indigestion, tympani, stomatitis, dysentery etc.

Skin problems

It includes mange, dermatitis, abscess etc.

Other problems

Besides the aforementioned problems, other problems include retention of placenta, posterior paralysis (kumri) cases of dog bite, abortion, respiratory problems and so forth.

Constraints

The major constraint realized was that the disease information system is based on tentative diagnosis. Only few cases have been confirmed through laboratory diagnosis. The samples collected during field visit are underway of detailed laboratory investigation.

Acknowledgement

The regional veterinary laboratory expresses its sincere thanks to the DLSO Surkhet, Dang and Bankey for their support in the present study. Similarly, I am grateful to Central Veterinary Laboratory, Animal Health directorate and not the least to my staffs for their kind cooperation and advice.

Khari Disease Investigation- a report

Rebati Man Shrestha[1], Raju Gautam[2] and Karuna Sharma[3].

Introduction

Khari is a chronic debilitating disease principally affecting the buffaloes of the two districts of the far western region namely, Baitadi, and Darchula. While the disease was initially confined to buffalo population in these districts, recently it has been found to affect the cattle population as well. The disease is reported to have been in existence for more than three decades however, no conclusion has been derived regading the exact etiological factor so far. Locally the disease has synonyms such as *Chaukhari* and *Dhulkhule*. These names have been given to the disease because affected animals have deformed hooves as the disease affects the hooves of the animals wherefrom ash like dust/powder appears and the animals became lame. The diseased animal progressively becomes weak, debilitated, drop in milk production to almost nil and shows signs of lameness. There is development of furrow or groove on the ground portion of the hoof from which grayish ash like powder is shed. The affected animal becomes so emaciated that animal looks hide bound. The body score becomes poor. Diseased animals exhibit characteristic posture which is evidenced with animals standing on abducted elbows and the shoulders appear to be stretched away from the thoracic region. Skin lesions include alopaecia, scale formation and extrusion of scally dust. Existence of the condition among large ruminants for such a long period without any known disease entity has remarkably affected the animal husbadry practice in the region. Such condition has incurred serious financial loss affecting their socio-economic status.

In order to address this serious problem an investigation programme was launched during the fiscal year 2061-62 with joint effort of Regional Veterinary Laboratory (RVL), Dhangadhi and Central Veterinary Laboratory (CVL), Tripureshwor. Khari disease investigation programme was designed as a project which will be continued for three consecutive fiscal years.

At the end of the first year of the investigation we have been able to successfully complete the basic survey through visit and observation of the diseased animals in order to obtain basic epidemiological data concerning the disease. During the visit, a large number of samples have been collected from the diseased animals. A few salient features or findings on processing of the epidemiological data, and collected samples will be presented in the following paragraphs while will discuss the results or the findings.

Materials and methods

1. Retrospective study

Existing reports and articles of previous workers on Khari disease was studied and relevant information was collected and analysed. It helped in formulationg the operational calendar and the part of study that was not considered in the past was recognized and taken into consideration.

1: Dr. Rebati Man Shrestha, Chief, CVL, Tripureshor.
2: Dr. Raju Gautam, Veterinary Officer, RVL, Dhandadhi.
3: Dr. Karuna Sharma, Veterinary Officer, CVL, Tripureshor.

2. Epidemiological survey

Benchmark survey of the disease was conducted using preset questionnaire (structured interview with the farmers) developed by the RVL, Dhangadhi. Epidemiological information was also gathered during field visit for on different occasions. It was found that adult animals aged 8.7 years or above were most commonly affected. However, occurrence of the disease in younger animals (age 1-3 years) was not uncommon. The disease was found to bear correlation with se in that the females were found to be affected more than the male of a species. The incidence of the disease was found in buffaloes at the rate of 80.35 percent and in cattle it was 19.64 percent.

The present finding on disease incidence rate fairly varies than the earlier investigation reports. It was previously reported that the disease condition was prevalent merely among adult buffaloes. Description on prevalence of the disease is presented in table 1

Table 1: Epidemiological status of the Khari disease in cattle and buffalo

S. N.	Species	Sex		Total	Average age
		Male	Female		
1	Buffalo	1	44	45	8.7 year
2	Cattle	1	10	11	

3. Sample collection and laboratory analysis

Relevant samples were collected from the ailing animals of different villages of Darchula and Baitadi districts during field visit. The samples included were blood in EDTA, sera, hoof and skin scrapping, grass, hay as well as soil. These samples were processed in the laboratory for bacterial as well as mycological culture, biochemical estimation, serological diagnosis, proximate analysis and other essential chemical analysis.

Different kinds of samples more than one hundred in number were collected from the affected animals of. Most of the samples were tested at RVL and rest of the samples or the samples intended for multidisciplinary laboratory approach were sent to CVL and other reference laboratories of the country. In this context, effort has been made to approach Indian Veterinary Research Institute (IVRI) to explore the scope of other laboratory diagnostic approach. The details of various samples collected are presented in table 2.

Table 2: Description of the samples

S. N.	Type of Sample	Number
1	EDTA Blood	19
2	Blood smear	23
3	Serum	56
4	Skin Scraping	8
5	Hoof dust	14
6	Soil	3
7	Hay	2
8	Fodder	1
9	Grass	4

Result & Discussion

1. Bacterilogical Examinations

The result of bacteriological examination revealed the presence of different types of fungi. However, contamination of the hoof samples may not be denied because the floor of the animal shed in most cases was wet and damp with least scope of access of sunlight as well as poor ventilation. The result of bacterial examination has been presented in table 3.

Table 3: Result of bacterial examination

S. N.	Animal Species	Type of sample	Media	Result
1.	Buffalo	Hoof scrapping	Sabouard	Blastomyces Spp. and Candida Spp.
2.	Buffalo	Hoof Scrapping	Selective	Absidia Spp

2. Haematological Examination

Blood samples were collected in EDTA and also blood smears were prepared from fresh blood samples. The scope of occurrence of blood protozoan parasites was ruled out and the serum samples were analysed for various haematological parameters. Descrition of the test result has been presented in table 4.

Table 4: Result of Haematological estimation

S.N.	Haematological Parameters	Number of samples	Average value	Range
1	Haemoglobin	25	6.56 g/dl	5.2-8 gm/dl
2	Packed Cell Volume	14	35%	
3	Erythrocyte Sedimentation Rate	5	43.6 mm/hr	28-65 mm/hr
4	Lymphocyte	27	44%	35%-67%
5	Neutrophil	27	50%	26%-58%
6	Monocyte	27	4%	2%-8%
7	Eosinophil	27	2%	

Haemoglobin estimation carried out at RVL using Shali's method showed its level in different animals lower than the normal value. However, fractions of the same set of blood samples tested in CVL using particle cell counter showed the Hb content within normal range. Also the value of Packed Cell Volume (PCV) calculated in CVL using the same equipment suggested the normal value and therefore, the present finding needs to be compared with Microhaematocrit method. However, individual variation in test results in not an uncommon phenomenon and it needs to be verified through repeated sampling and testing. The Erythrocyte Sedimentation Rate (ESR) was however surprisingly high. This leads to the idea of chronic nature of the disease. However, various kinds of urinary problems and other disease conditions prevailing in the ailing animals including neoplassia should be taken into consideration.

3. Biochemical Examination

Biochemical estimation of serum samples was carried out to assess the serum level of micronutrients in the blood of affected animals. Therefore, estimation of calcium, phosphorus, magnesium, zinc and total protein was carried out.

4. Examination of ectoparasites

Skin scraping examination for the presence of mange mites revealed that approximately 65% of the animals were affected by Sarcoptes Spp. Out of eight samples of the skin scrapings tested for mite infestation five of them were were found positive. It was concluded that skin lesions scale formation, alopaecia and excoriations. It may be mentioned that the animals suffering from Khari disease are prone to mange infestation which may be, in turn, responsible for anaemia in such animals. It may also be ruled out that the general debility and hoof lesions in the animals may not have coorelation with mange inferstation. The detail of the examination has been given in table 5.

Table 5: Result of skin scraping examination

S. N.	Animal spp.	No. of skin scrapings	positive	Identified organism
1	Buffalo	6	4	Sarcoptes mange
2	Cattle	2	1	Sarcoptes mange

Proximate analysis of Hay

Locally known as *Gajio*, hay is the principal source of feed to the animals of these two districts during lean period (October-May). It is prepared from locally available green roughages which are cut, sun dried during September-October and stored by staking in the form of a tower. A few information on some of the locally used grass for hay preparation laid by Natural Products Research Laboratory (NPRL) has been given in table 6. Similarly, result of proximate analysis of samples of some of the local grasses sent to Nepal Agriculture Research Council (NARC) has been presented in table 7.

Table 6: Information on grasses used for hay making (NPRL)

S.No.	Local Name	Scientific Name	Percentage composition in hay
1	Atharne	Pogonatherum paniceum	60%
2	Ghorade	Heteropogon contortus	30%
3	Others	Could not be identified because of absence of flower in the plant.	10%

Table 7: Result of proximate Analysis of Grass samples (NARC)

S.N.	Sample type	DM %	CP %	NDF %	ADF%	Lignin
1	Hay	88.63	6.14	82.69	67.13	9.17
2	Hay	86.15	11.05	56.29	55.19	29.31
3	Grass	65.87	4.06	62.68	55.15	9.76

It is mentioned that Dabadghao & Shankarnarayan (1970) in India found the crude protein content of a grass *Heteropogon* Spp was five percent when untreated and 5.8 percent when treated with nitrogen. The digestibility of the protein present in this grass is low. The grass is reported to be palatable in the early vegetative stage, but becomes disgusting with maturity (L. Beauv. ex Roem. and Schult). The phosphorus accounts for 0.09-0.15% of total dry matter, the figure far below the requirement of the dairy cattle. The calcium content is barely sufficient, ranging from 0.23 to 0.30% the dry matter.

The same laboratory has also analysed the toxic principals present in the hay. It has been reported that the alcohol extract present in the hay subjected to acute toxicity test on mice fed at the rate of 500mg/kg body weight resulted into death of 25% of the mice. However, mice fed the extract at the rate of 300mg/kg body weight remained alive.

Examination of Soil

Soil samples were also collected and sent to soil laboratory for various tests to measure important parameters. Also the soil test record of the Darchula district was obtained from the Regional Soil Laboratory, Dhangadhi. The result of the soil test of the laboratory as we obtained it from the soil laboratory is given in the table 8.

Table 8: 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	Type of soil sample	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

It is encouraging to note that the phosphorus content of the soil samples of Darchula and Baitadi and the same analyzed for a few grasses is low that may suggest the correlation of phosphorus deficiency with Khari disease.

Result of Drug Trials

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

Group 1: Injection Ivermectin twice monthly interval

Group 2: Minamil @ 25gm per day for 15 days in a month for two consecutive months.

Group 3: Injection Ivermectin and Plus Minamil as suggested in Group I and Group 2.

Group 4: Control (No treatment)

No improvement was observed in control group and only a slight improvement was recorded with animals of group 2. However, the skin lesions in these animals were hardly improved. Similarly there was an encouraging improvement in the skin lesion with animals under group 1. The skin lesions disappeared completely and regrowth of hair were seen but no improvement in condition.

Much encouraging result was recorded with the animals of group 3. This treatment regimen followed in group 3 with supplementation of some amount of concentrate feed provided improvement of the skin lesions and also the body condition and condition of hooves were improved slightly.

Annex

Working Plan of Khari Disease Investigation Programme for the Fiscal Year 2062-063

1. Types of samples referred to IVRI

- Heparin blood, Blood smear
- Serum for biochemistry and mycotoxin assay
- Soil
- Hay for selenium and Arsenic assay, mycotoxin and other Phytotoxin detection
- Individual species of grasses used in the preparation of hay for species identification and any phytotoxin associated with the species so identified.
- Liver, Kidney tissues in ice

2. Types of samples and prescribed tests to performed at the CVL, Tripureshwor

1. Serum

- Total Protein
- Zinc
- Calcium
- Phosphorus
- Magnesium

2. Blood Smear

- Blood protozoan
- Structure of RBC, Platelets, WBC
- Differential count

3. Hay

- Species identification
- Phytotoxin detection
- Proximate analysis

3. Types of samples and tests conducted to performed at RVL, Dhangadhi

- Blood for Haemoglobin by Shalis method, PCV
- Blood Smear
- Serum for Ca. and P. estimation
- Milk for Microbiology, Biochemistry, Physical

- Skin scrapings and hoof dust for mange mites and microbiology.

4. Works to be jointly executed by RVL and the concerned DLSO

- Clinical examination of the diseased animal, and history taking
- Necessary sample collection and dispatch
- Drug trial and Constant monitoring of the animals under investigation
- Record keeping, compiling of the findings for necessary interpretations.

5. Criteria of animal monitoring under drug trial

Monitoring of animals at 7-15 days interval for improvement in the following aspects

- Skin lesion and hair condition
- Musculoskeletal condition and lameness, hoof dust and crack, furrow in the ventral aspect of hoof.
- Milk Yield, and its physical and chemical status.
- Behaviour, and appearance
- Clinical examination for anaemia by visual examination of conjunctiva and mucus membrane.

6. Lists of important drugs to be used for trial

1. Vermic (Ivermectin)
2. Cypermethrin
3. Minamil
4. Cofecu
5. Balanced feed
6. Tonophosphon
7. E care Se

Investigation of Kid Mortality in Goats of the Far Western Region

R. Gautam[1]

Background

Goat rearing is an important component of livestock production. In recent years goat rearing has been gaining popularity in far western region of Nepal. The present goat population of the region is projected as 616115 (Statistical information on Nepalese Agriculture, 2002-03, MOAC), which constitutes 9% of the total goat population of the country. Goat is the second largest source of meat in our country next to buffalo and contributes about 19.47% of the total meat production in the country (Statistical Information on Agriculture, 2002-03). The present annual growth rate of goat population is seven percent and an average of 400,000 goats is added to the existing population per annum. However, goat rearing is facing newer challenges in the field of management and health care together with its growing population.

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

Objectives

1. Short Term objective

Study the prevalence rate of infectious and non infectious causes of kid mortality.

Study the epidemiological pattern of kid mortality so that effective control measures can be suggested.

Strengthen the diagnostic capability of the RVL, Dhangadhi.

2. Long Term objective

To identify the major causes of kid mortality

Disseminate the investigation report to the field veterinarians, technicians and other stakeholders.

1Veterinary Officer, RVL, Dhangadhi.

Methodology

1. Farmers' selection

A total of ten innovative farmers including six from Kailali and four from kanchanpur districts were selected for gathering information related to present study. For this purpose, a set of questionnaire was developed and presented to the farmers at the initial stage of investigation and also requested to give monthly progress information.

2. Sample Collection

Relevant samples were collected from the ailing kids and were set for laboratory procedure to find out the diseases/condition responsible for kid mortality.

Result and Discussion

1. General information

The information obtained from the selected farmers revealed an average of 29.8 kids was born per farm in a year. Mortality among pre-weaning female kids was found greater than the same among male kids. The information has been presented in table 1. This information contradicts the observation made by Neopane (1996), Singh et al (1990), Gebrelul et al (1994) and Aryal et al (2001) that reported higher survival rate of female kids. However, Mittal (1976), Majumdar et al (1980) and Sharma et al (1981) reported the non singnificant effect of sex on survival rate of kids during pre-weaning period.

Table 1: Pattern of kid mortality

S.N.	Pattern	Number
1.	Number of kids born	209
2.	Number of Male kids born	114
3.	Number of female kids born	95
4.	No. of male kids death	15
5.	No. of female kids death	22

The pre-weaning death among kids was found to be related with the type of birth. The highest mortality rate was recorded among the kids born as triplets followed by twins while the least mortality was recorded among kids born single (table 2). The present finding agrees with the report of Aryal et al (2001), Sharma et al (1981), Prasad (1983) and Singh et al (1990) who reported that singles had higher survival rate than the twins or the triplets. Fifty percent mortality in case of triplets as shown in table 2 seems to be very high. This may be due to smaller sample size of the triplets under investigation. Therefore, the present finding needs to be verified including more observations. It is expected that it will be verified by the end of the next year.

Table 2: Pattern of kid mortality with type of birth

S.N.	Type of birth	Number of kids		Mortality rate
		Born	Died	
1.	Single	99	9	6.06%
2.	Twins	98	22	15.3%
3.	Triplet	12	6	50%
	Total	209	37	17.7%

Seasonal variation in kid mortality rate has been found to exist with highest mortality during the summer-rainy season, followed by winter and low mortality during spring and the autumn. This finding agrees with the report of Aryal et al (2001). The reason for higher mortality during rainy and winter season could be attributed to inclement weather posing stress on the kids rather than the occurrence of any specific disease condition. The result of seasonal correlation with kid mortality has been presented in table 3.

Table 3: Seasonal correlation with kid mortality

S.N.	Season	Number of death	Percent
1.	Winter (Marg.- Magh)	6	16
2.	Spring (Phalgun – Baisakh)	3	8.1
3.	Summer/Rainy (Jestha – Shrawan)	23	62.1
4.	Autumn (Bhadra - Kartik	5	13.5

Mortality was observed to be related with age factor. It was highest between 15-30 days, followed by death between 1-3 months of age as shown in table 4. This observation is contrary to the findings of Aryal et al (2001) who reported highest mortality in kids less than 15 days of age revealing a decreasing trend in the mortality with the increasing age of kids.

Table 4: Mortality in relation with the age of kids

S.N.	Age of Kid	Number dead
1.	Less than 15 days	6
2.	16 to 30 days	12
3.	1 to 3 months	11
4.	Above 3 months	8

2. Laboratory Findings

During the investigation period, samples collected from different farm were examined and the results obtained through parasitological, haematological, and microbiological examination are given below. Since, this is not the final report, which is expected to come out at the end of the current fiscal year, only the summary of the laboratory result is presented here.

Parasitology

Most of the faecal samples collected from the kids under investigation were found infested either with a single or mixed nematodal parasites. Out of 26 faecal samples examined so far, twenty-one of them were positive for different nematodes. The major round worms identified were *Strongylus* and *Trichuris* Spp.

Haematological Examination

Haematological examination of samples collected from clinically ill kids was carried out to assess the alteration in major blood parameters such as PCV, Haemoglobin, and blood protozoan parasites, the result of which has been shown in table 5.

Table 5: Result of haematological examination

S.N.	Test	Average Value
1	Haemoglobin	9.1g/dl
2	PCV	20.4%

Microbiological Examination

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

Diseases involved in Kid Mortality

The most common cause of death of a kid was identified as Pneumonia, followed by diarrhoea, paralysis and anuria. It has been given in table 6.

Table 6: Diseases involved in kid mortality

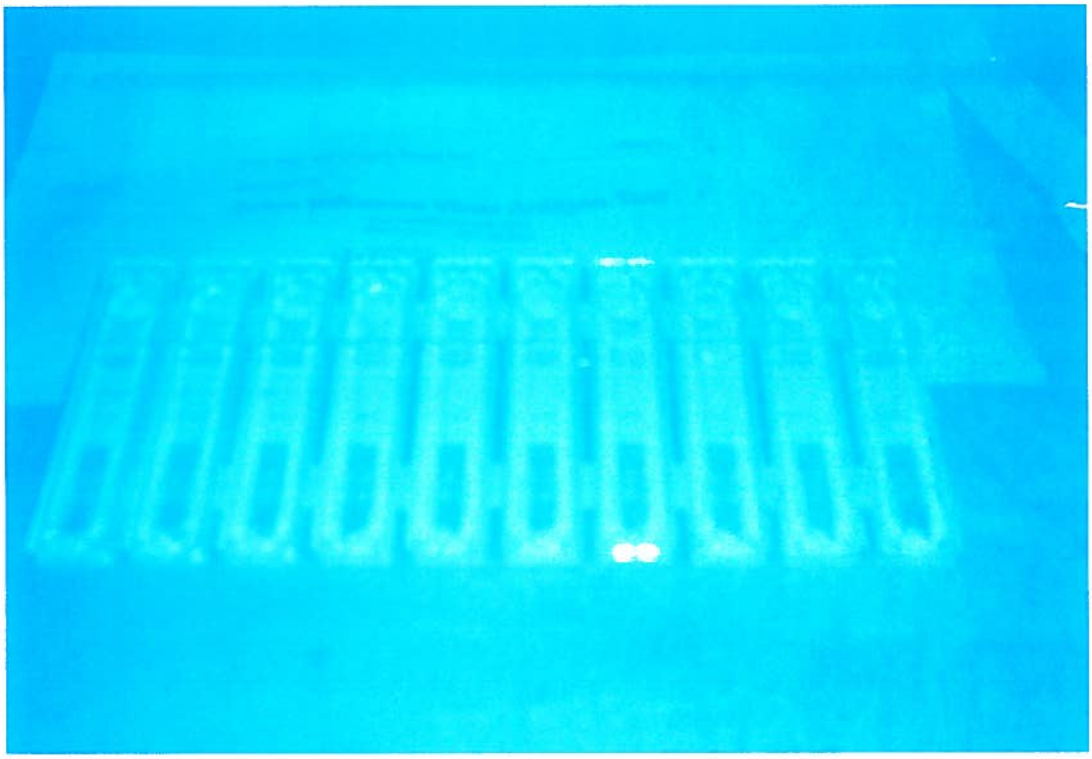
S.N.	Type of Disease/Condition	No. of Death
1.	Infectious Pneumonia	13
2.	Verminous pneumonia	9
3.	Paralysis	4
4.	Anuria	3
5.	Diarrhoea	7
6.	Others	1

Pneumonic conditions in the kid were identified of infectious and parasitic aetiology depending on the clinical symptoms and presence or absence of febrile condition.

Conclusions

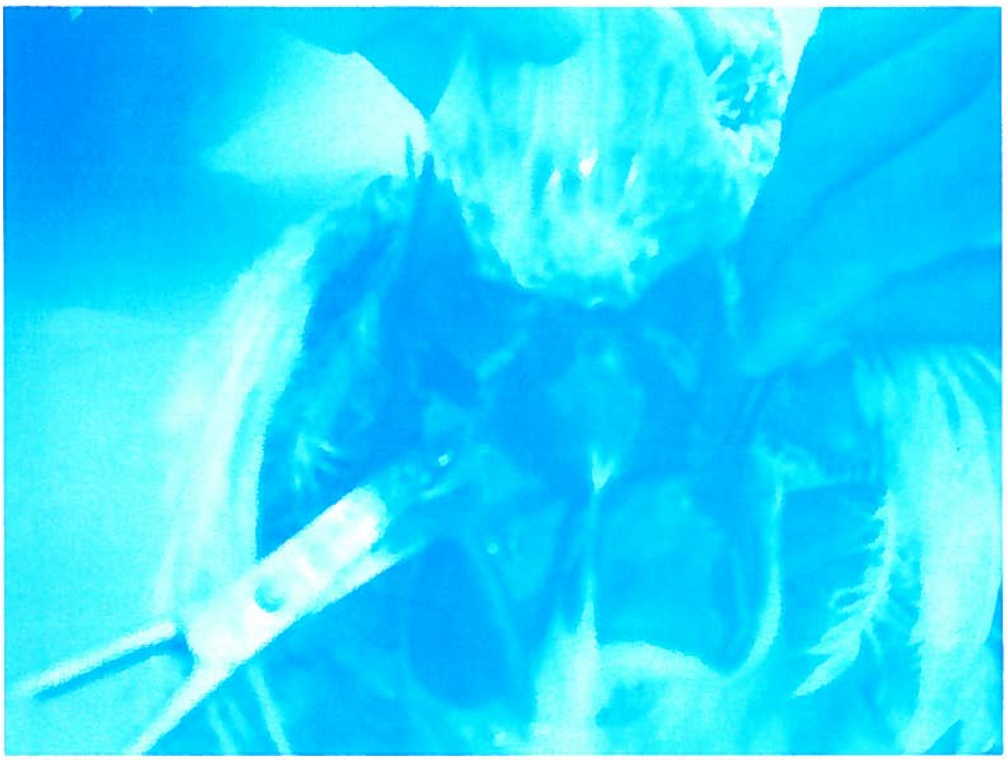
It is observed that kid mortality is positively related with the type of birth which suggests lower survival rates with more number of kids (twins or triplets). Higher mortality was observed during the rainy and winter season and the common diseases identified to cause
death were pneumonia, diarrhoea, anuria, paralysis etc

Three months laboratory training certificate distribution programme at CVL hall



Laboratory diagnosis of Avian Influenza with Sd-Bioline method

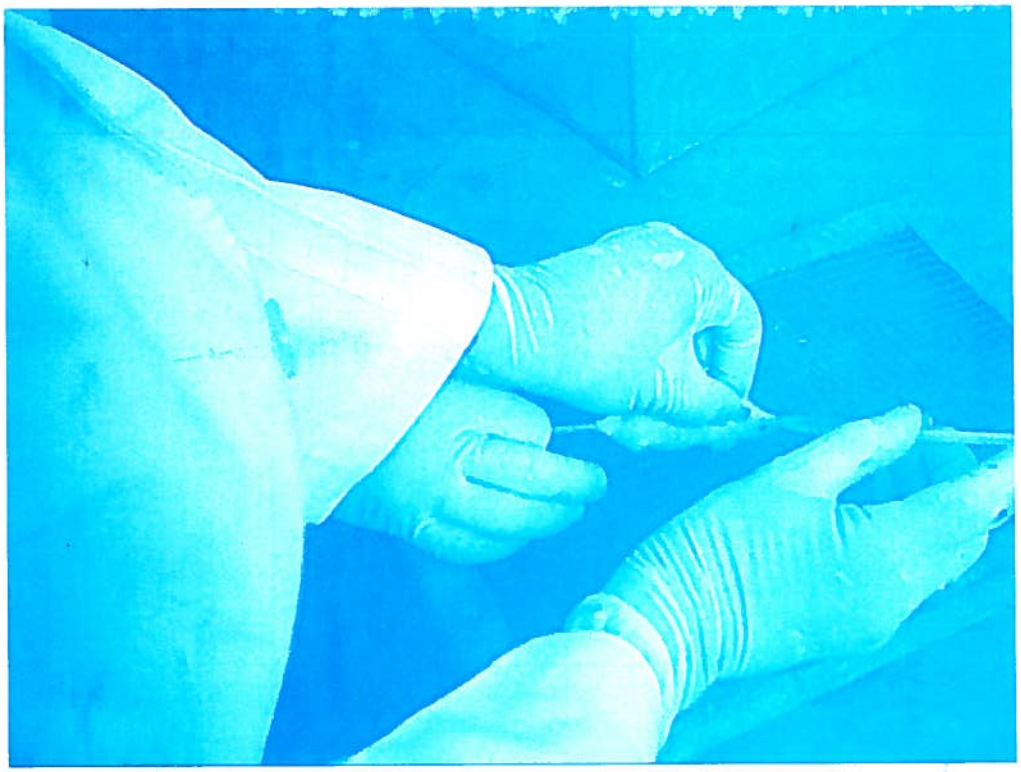
Wild birds for postmortem examination (AI diagnosis)



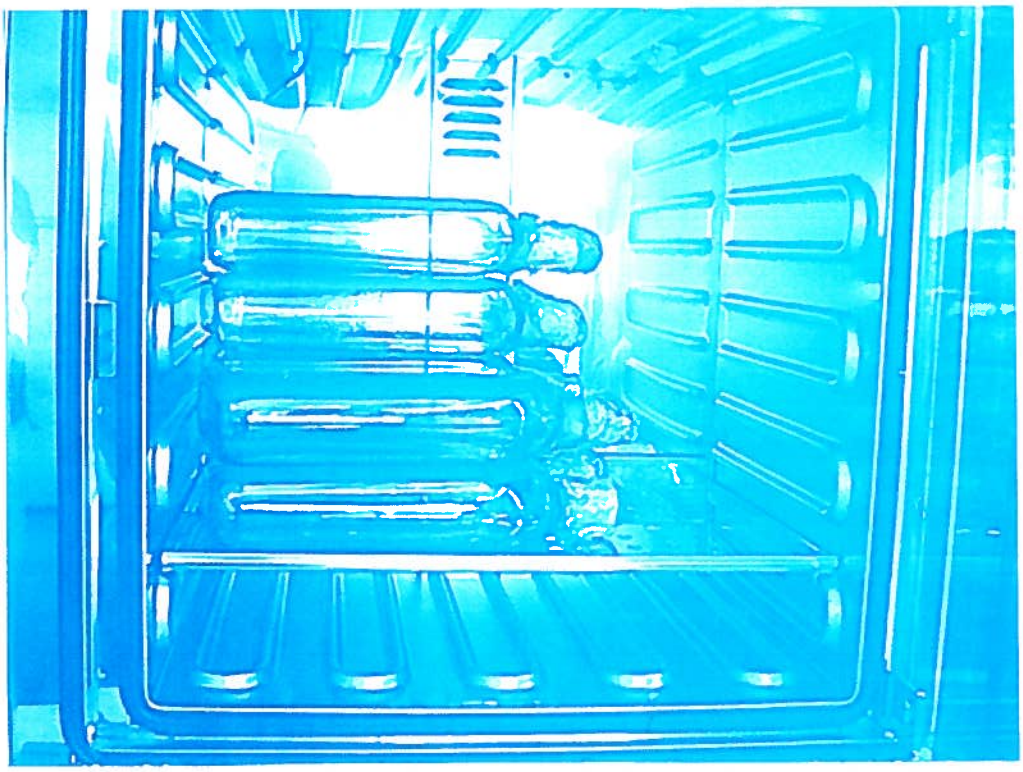
Leechi heart disease showing hydro pericardium.

Demonstration of post mortem examination for the participants of three r
laboratory training

Cloacal swab collection for AI diagnosis



Mice inoculation for rabies diagnosis



Salmonella culture bottle in an incubator for antigen preparation.