ANNUAL REPORT
2011-2012

VETERINARY RESEARCH INSTITUTE
LAHORE - PAKISTAN

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C O N T E N T S

FOREWORD

Veterinary Research Institute is playing a vital role in preventing and controlling the diseases of livestock and poultry by providing quality biologics for ensuring the provision of protein of animal origin (milk, meat and eggs) to the nation by reducing the gap between supply and demand for the increasing human population.

During the year the institute came up with its commitment to help the farmers in disease diagnosis at their doorsteps. New emerging diseases of poultry and livestock were also investigated by providing prompt diagnostic help to the in service field veterinarian and Para Veterinary Staff. Various research projects were exploited for the development of effective vaccines against various diseases.

Schemes have been launched for the production and quality assurance of various biological used for the livestock, poultry and wildlife. The activities during the year were significant in every aspect of function and development. The biological production was enhanced to combat the increased demand with the use of refined technical protocols and investigation methods. In order to maintain standards, series of in vivo and in vitro tests were performed to evaluate the biological products.

In order to modernize and strengthen the research activities along with quality control, new laboratories were constructed including cold rooms and renovation of different laboratories.

The laboratories have been equipped with all type of advanced diagnostic and biological production equipments for the instant diagnosis and speedy production of biologics to meet the challenging demands.

DIRECTOR

PUNJAB GOVERNMENT

"Relentless pursuit of modernization, innovation, confidence and tolerance leading to fully literate, fully employed, highly educated, skilled, talented, tolerant, culturally sophisticated, internationally connected and reasonably well off healthy society."

LIVESTOCK AND DAIRY DEVELOPMENT DEPARTMENT

"To create environment for raising livestock production and use it as a vehicle for social security, poverty alleviation and rural development ultimately leading towards domestic food security and creation of exportable surplus."

VETERINARY RESEARCH INSTITUTE LAHORE

"To improve the health and productivity of livestock & poultry through quality vaccines and disease control."

MOTTO

“Prevention is better than cure”

INTRODUCTION

The Veterinary Research Institute Lahore started working on 7th June 1962. The institute is controlled administratively by the Punjab Government. The institute is spread on an area of 200 kanals consisting of laboratories and animal housed.

The institute is producing about 24 quality biologics covering a significant number of livestock and poultry. The institute undertakes research on prevailing problems of animals in country. The aim of this institute is always towards to the benefit of the livestock industry through prompt disease diagnosis and control by quality vaccines.

MISSION

Quality Biologics for livestock and poultry through research, development and innovation along with disease investigation on obscure and emerging problems to make livestock and poultry healthy and prosperous.

OBJECTIVES

- Large scale production of standard biologics for control of infectious diseases of livestock and poultry.
- Research studies in the related disciplines of animal health.
- Studies related to obscure diseases of livestock and newly emerging diseases of poultry.
- Development of modern techniques for vaccine production and disease diagnosis.
- Development of disease monitoring and disease investigation system.
- In service training `for the members of Veterinary profession in advance techniques.
- Training of the farmers to enable them to help themselves in easy operations like dosing and vaccination etc.

ORGANOGRAM

SECRETARY
Livestock & Dairy Development Department
↓
DIRECTOR GENERAL (RESEARCH)
↓
DIRECTOR
Veterinary Research Institute, Lahore Cantt
↓

Quality Control Section	Poultry Pathology Section	Bacteriology Section	Avian Leukosis Section	Cell Culture Section	H.S. Section	Anaerobe Section	D.I. Section	Mycoplasma Section	Flury/ Sheep Pox Section	Antigen Section	T.B. Section	Hydropericardium Section	Bio-Chemistry Section	Poultry Vaccine Section	Parasitology Section	Avian Influenza Section
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
SRO	PP	RO	RO	RO	RO	RO	DIO	RO	RO	RO	RO	RO	Bio-Chemist	RO	Helmen-thologist	RO
↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
RO=1	ARO=1	ARO=1	ARO=1	ARO=1	ARO=1	ARO=1	ADIO=2	ARO=1	ARO=1	ARO=1	ARO=1	ARO=1	ARO(Bio)=1	ARO=1	RO(T)=1	ARO=1
↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
ARO=1	VO=3	BPO=1	VO=1	BPO=1	VO=4	VO=2	ARO=1	VO=2	VO=3	VO=2	VO=2	VO=3	VO=1	BPO=1	ARO=1	VO=2
↓		↓		↓			↓							↓	↓	
VO=5		VO=2		VO=2			VO=2							VO=3	VO=1	
				↓			↓							↓		
				OIC Store=1			Curator=1							SO=1		

SRO = Senior Research Officer
RO = Research Officer
ARO = Assistant Research Officer
PP = Poultry Pathologist
DIO = Disease Investigation Officer
ADIO = Assistant Disease Investigation Officer
BPO = Biological Production Officer
VO = Veterinary Officer
SO = Statistical Officer

Technical Staff = 85
Para-Technical Staff = 314
Total: = 399

DIRECTOR

Dr. Saeed Ahmad	D.V.M; M.Sc (Hons)

RESEARCH OFFICERS/DISEASE INVESTIGATION OFFICERS

1.	Dr. Abdus Sattar	D.V.M; M.Sc. (Hons.)
2.	Dr. Shahida Afzaal	D.V.M; M.Sc. (Hons.)
3.	Dr. Zahid Saeed	D.V.M; M.Sc. (Hons.)
4.	Dr. Azhar Hussain	D.V.M; B.Com; M.Sc. (Hons.) D.F.L.; P.G.T. (U.K.)
5.	Dr. Muhammad Arshad	D.V.M; M.Sc. (Hons.); Ph.D.
6.	Dr. Kausar Tasneem Jaffery	D.V.M; M.Sc. (Hons.)
7.	Dr. Shabbir Ahmed	D.V.M; M.Sc. (Hons.); Ph.D.
8.	Dr. Waheeda Raana	D.V.M; M.Sc. (Hons.); Ph.D.
9.	Dr. Zafar-ul-Ahsan Qureshi	D.V.M; M.Sc. (Hons.); M.S.(U.S.A)
10.	Dr. Afzal Sajid	D.V.M; M.Sc. (Hons.); Ph.D.
11.	Dr. Azam Ali Nasir	D.V.M; M.Sc. (Hons.)
12.	Dr. Sarwat Naz	D.V.M; M.Sc. (Hons.)
13.	Dr. Rashid Munir Khawaja	D.V.M; M.Sc. (Hons.); Ph.D.
14.	Dr. Khawar Mehboob	D.V.M; M.Sc. (Hons.)
15.	Dr. Ghazala Nawaz	D.V.M; M.Sc. (Hons.)

BIOCHEMIST

1.	Mrs. Asma Aziz	M.Sc. (Bio-Chemistry)

ASSISTANT RESEARCH OFFICERS/ BIOLOGICAL PRODUCTION OFFICERS/ASSISTANT DISEASE INVESTIGATION OFFICERS

1.	Dr. Mehboob Alam Qureshi	D.V.M; M.Sc. (Hons.); Ph.D.
2.	Dr. Manzoor Ahmad Ghumman	D.V.M; M.Sc. (Hons)
3.	Dr. Sajjad Hussain Shah	D.V.M; M.Sc. (Hons.)
4.	Dr. Azmat Sultana	D.V.M; M.Sc. (Hons.)
5.	Dr. Jamshed Iqbal	D.V.M; M.Sc. (Hons.)
6.	Dr. Muhammad Asad Raza	D.V.M; M.Sc. (Hons.) ; P.G.T.(China)
7.	Dr. Asifa Rasool Bhatti	D.V.M; M.Sc. (Hons.)
8.	Dr. Muhammad Nauman	D.V.M; M.Sc. (Hons.)
9.	Dr. Muhammad Asim	D.V.M; M.Sc. (Hons.) ; P.G.T.(France)
10.	Dr. Shahida Parveen	D.V.M; M.Sc. (Hons.)
11.	Dr. Sajjad Hussain	D.V.M; M.Sc. (Hons.)
12.	Dr. Saeed A Khan	D.V.M; M.Sc. (Hons.)
13.	Dr. Bushra Zameer	D.V.M; M.Sc. (Hons.)
14.	Dr. Tariqu Butt	D.V.M
15.	Dr. Muhammad Ikram-ul-Haq	B.Sc. M.Sc. (Hons.); Ph.D.
16.	Dr. Shahbaz Ali	D.V.M; M.Sc.

VETERINARY OFFICERS/Curator/Officer Incharge Store

1.	Dr. Yasmeen Abbass	D.V.M; M.Sc. (Hons.)
2.	Dr. Rubina Anjum	D.V.M; M.Sc. (Hons.)
3.	Dr. Shehzada Parveen	D.V.M.
4.	Dr. Umber Rauf	D.V.M; M.Sc. (Hons.)
5.	Dr. Rashid Manzoor	D.V.M; M.Sc. (Hons.); Ph.D.
6.	Dr. Munazza Shaukat	D.V.M; M.Sc. (Hons.)
7.	Dr. Rizwan Qayyum	D.V.M; M.Sc. (Hons.)
8.	Dr. Asif Rashid	D.V.M; M.Phil, M. Med. Sci (Infection Biology); M. Med Sci. (Medical Science) Sweden
9.	Dr. Muhammad Abbas	D.V.M.MBA, MS (M.B) Sweden
10.	Dr. Syed Abdul Khaliq	D.V.M. MS/M.Phil, TQM
11.	Dr. Muhammad Naji Ullah	D.V.M; M.Sc. (Hons.)
12.	Dr. Afshan Jan	D.V.M; M.Sc. (Hons.)
13.	Dr. Abdul Razzak	D.V.M; M.Sc. (Hons.)
14.	Dr. Sajjad Ali	D.V.M.
15.	Dr. Iffat Huma	D.V.M
16.	Dr. Sobia Aamir Chughtai	D.V.M. M.Sc. (Hons)
17.	Dr. Rasha	D.V.M. M. Phil
18.	Dr. Nadeem Akram	D.V.M
19.	Dr. Sumayya Sattar	D.V.M
20.	Dr. Zain-ul-Abidin	D.V.M. M. Phil

21.	Dr. Naila Maqsood	D.V.M
22.	Dr. Abdul Wahab Manzoor	D.V.M
23.	Dr. Ashi A Morris	D.V.M
24.	Dr. Hina Afroz	D.V.M
25.	Dr. Aqsa Mushtaq	D.V.M, M.Phil
26.	Dr. Nida Arooj	D.V.M
27.	Dr. Rehan Rafique	D.V.M, M.Phil
28.	Dr. Elina Ali	D.V.M
29.	Dr. Shiraz Shahid	D.V.M. M.Sc. (Hons)

BUDGET

ANNUAL BUDGET GRANT (2011-2012)

1.	**Allocation**		**Rupees**
	C-1 LO-4208		9, 96, 39,000
	C-2 LO-4209		6, 16, 30,000
	E-3 LO-4212		1, 02, 42,000

		Total:	**17, 15, 11,000**

2.	**EXPENDITURE**		
	C-1 LO-4208		9, 40, 47,000
	C-2 LO-4209		5, 78, 99,000
	E-3 LO-4212		93, 03,000

		Total:	**16, 12, 49,000**

BIOLOGICAL PRODUCTION

PRODUCTS

Veterinary Research Institute is engaged in the production of vaccines and diagnostic agents for different livestock diseases of bacterial and viral origin and thus saves the livestock industry from heavy financial losses which may run upto billions of rupees. We adopt the policy of preventing major infectious and contagious diseases by preparation and use of effective vaccines.

During the year institute produced 24 different products including 16 bacterial & viral vaccines and 6 diagnostic agents.

PRODUCTS	LIVESTOCK	POULTRY	TOTAL
1. Bacterial Vaccines	7	0	7
2. Viral Vaccines	4	8	12
3. Diagnostic agents	5	0	5
Total:	16	8	24

In fact these prophylactic and diagnostic agents have led to the development of the livestock and poultry industry in Pakistan and have helped in its survival against major infectious epizootics. The livestock health and production will be adversely affected, if these infectious diseases are not properly controlled.

The biological production at VRI, Lahore confirms to the international standard of vaccine production. The use of these vaccines not only saves huge losses likely to be caused due to contagious diseases in the field but is also a profitable activity of this organization. During the year, the institute earned Rs.83.20 million as detailed in the following page.

INCOME

TARGET AND RECEIPT

(2011-2012)

Particulars	Target (Rs)	Earned (Rs)
Vaccine	**8,40,29,000**	**8,18,99,639**
Book Adjustment	**--**	**13,00,620**
Achievement	**99%**	
Total:-	**8,40,29,000**	**8,32,00,259**

ASSUMPTIVE BENEFITS ESTIMATED ON THE BASIS OF VACCINE PRODUCED (2011-2012)

1. Annual capital values

ANIMAL SPECIES	RUPEES (IN MILLION)
Cattle & Buffaloes	123360
Sheep & Goat	32750
Poultry	862
Total: -	**156972**

2. Saving in terms of prevention of clinical sickness & losses in production

Cattle & Buffaloes	5150
Sheep & Goat	3270
Poultry	690
Total: -	**9110**
GROSS TOTAL: -	**166082**

VACCINES

Two types of vaccines are being produced:

A. Bacterial Vaccines
B. Viral Vaccines

A. BACTERIAL VACCINES

I. Livestock Vaccines
II. Poultry Vaccines

I. LIVESTOCK VACCINES

1. HAEMORRHAGIC SEPTICAEMIA (alum precipitated) VACCINE

The vaccine is prepared in large quantity for the field control of HS disease. It is a formalinized, alum precipitated vaccine produced from a local selected strain of *Pasteurella multocida* Carter type 6-B. The vaccine is used twice a year preferably in May/June and November/ December.

2. HAEMORRHAGIC SEPTICAEMIA (oil adjuvant) VACCINE

It is formalized killed oil adjuvant vaccine prepared from local strain of *Pasteurella multocida*, carter type 6-B. The vaccine is used once a year.

3. BLACK QUARTER DISEASE VACCINE

Clostridium chauvoei infection in cattle is a problem in certain hilly & sandy areas. The vaccine manufactured is an alum precipitated formalinized culture of *Cl. chauvoei*. A single injection provides adequate prophylactic cover for one year. April & May are the months of choice for vaccination.

4. LISTERIOSIS VACCINE

The vaccine is prepared on special demand. It is a killed vaccine prepared from local strain of *Listeria monocytogenes*. The vaccine may be used in March & September, twice in a year.

5. CONTAGIOUS CAPRINE PLEUROPNEUMONIA VACCINE

It is formalinized culture of *Mycoplasma mycoides var capri* in 20% Serum PPLO broth. It gives immunity for one year.

6. ENTEROTOXAEMIA CUM LAMB DYSENTERY VACCINE

It is an alum precipitated formalinised whole culture vaccine prepared by incorporating equal amount of *Cl. perfringens* type B & D (ana-culture). Its immunity is for over six months.

7. ANTHRAX SPORE VACCINE

This vaccine is used against a highly fatal disease of sheep, goat & large animals and has zoonotic importance. A spore suspension of live attenuated non-capsulated *Bacillus anthracis* strain in Glycerin saline confers solid immunity for one year.

II. POULTRY VACCINES

1. SPIROCHAETOSIS VACCINE

It is a killed vaccine prepared from *Borrelia anserina.* This organism is transmitted through soft tick *Argas persicus*. A good immunity is produced through this vaccine.

B. VIRAL VACCINES

I. Livestock Vaccines
II. Poultry Vaccines

I. LIVESTOCK VACCINES

1. ANTIRABIES - FLURY (LEP) VACCINE

Rabies is an enzootic disease of all mammals including man. The disease assumes an epizootic form during breeding seasons of carnivora which are the chief vectors of the disease. Flury (LEP) vaccine is single shot live viral vaccine prepared on developing chicken embryo. It is used to protect dogs. It provides immunity at least for one year.

2. ANTI RABIES VACCINE - SEMPLES

It is used as post exposure prophylactic in animals bitten by rabid animals. It is a phenolized 6% suspension of sheep brain infected with Pasteur's rabies virus.

3. SHEEP POX VACCINE

It is an attenuated live viral vaccine (Virus Strain RM65) prepared on primary lamb kidney cells and gives immunity for one year.

4. GOAT POX VACCINE

It is an attenuated virus vaccine (Virus Strain Gorgan 56) cultivated in primary lamb kidney cells and provides immunity for one year.

II. POULTRY VACCINES

1. NEWCASTLE DISEASE VACCINE

Newcastle disease is an enzootic problem in Pakistan and possess a serious threat to poultry industry. Vaccination at proper time and at regular intervals provides solid protection against the disease. Egg adopted Mukteswar strain is being used for production of this very potent vaccine.

2. FOWL POX VACCINE

It is a live viral vaccine prepared in embryonated eggs. It affords lifelong immunity against the highly infectious fowl pox disease of poultry.

3. HYDROPERICARDIUM SYNDROME VACCINE

Hydropericardium disease emerged in 1988-89 in broiler chickens in Pakistan and caused heavy losses. An effective vaccine has been prepared against this disease and is being used in the field successfully. It gives immunity for 7-8 weeks.

4. AVIAN INFLUENZA (BIRD FLU) VACCINE (AQUEOUS)

In 2006 an outbreak of avian influenza serotype H_5N_1 (Bird Flu) was noticed in the capital and northern areas of the country which not only caused heavy losses to the poultry industry but also posed a serious threat to human health. The institute developed an effective vaccine from subtype H_5N_1 (A / Chicken / Pakistan / NARC – 2238 / 06) seed virus received from MINFAL.

5. AVIAN INFLUENZA (BIRD FLU) VACCINE (OIL BASED)

An oil based Avian Influenza Vaccine is also being produced for breeder flocks.

DIAGNOSTIC ANTIGENS

1. MALLEIN (CONCENTRATED)

The concentrated mallein is used for intradermal palpaberal test (IDP).This is the most sensitive, reliable and specific clinical test for glanders. Mallein, a protein fraction of the organism, is injected intradermally into the lower eyelid of equids.

2. TUBERCULIN P.P.D. (MAMMALIAN & AVIAN)

Purified protein derivative (PPD) obtained from pure culture of *Mycobacterium tuberculosis human C, DT, and PN & Mycobacterium avian D4 strain.* It is used for the diagnosis of Tuberculosis in animals.

3. BRUCELLA ABORTUS AGGLUTINATION CONCENTRATE

a. Rose Bengal Plate Test Antigen

It is a killed phenolised suspension of *Brucella abortus*, strain 99. It is used for rose Bengal plate test (RBPT) for the diagnosis of Brucellosis which is a simple spot agglutination test.

b. Brucella Abortus Agglutination Concentration Antigen

It is a killed phenolised suspension of Brucella abortus, strain 99. This test detects non-specific antibodies as well as specific antibodies from *Br. abortus* infection.

c. Milk Ring Antigen

It is also killed phenolised suspension. This test is an efficient means of screening dairy herds by testing milk from the bulk tank. When a positive test result is obtained, all animals contributing milk should be blood tested.

PRODUCTION

VACCINES AND ANTIGENS
(2011-2012)

BACTERIAL VACCINES

1.	Anthrax spore vaccine	260000	Doses
2.	Black Quarter vaccine	3049560	Doses
3.	Contagious caprine pleuropneumonia vaccine	10075700	Doses
4.	Enterotoxaemia cum-Lamb Dysentery vaccine	9032100	Doses
5.	Haemorrhagic septicaemia allum	15958380	Doses
6.	Haemorrhagic septicaemia (Oil Base)	24000	Doses
7.	Listeriosis Vaccine	Nil	Doses
	Total: -	**38399740**	**Doses**

VIRAL VACCINES

1.	Fowl Pox vaccine	Nil	Doses
2.	Goat Pox vaccine	497900	Doses
3.	Sheep Pox Vaccine	975100	Doses
4.	Hydropericardium vaccine	1503500	Doses
5.	Newcastle disease vaccine	79805400	Doses
6.	Flury	950	Doses
7.	Anti Rabies Vaccine (Samples)	7480	ML
8.	Avian Influenza	3675000	Doses
9.	Avain Influenza (Oil Base)	326000	Doses
10.	Peste Des Petits Ruminants (PPR) Vaccine	1097000	Doses
11.	I.B.D Vaccine	117000	Doses
12.	ND + H_9	2134000	Doses
13.	N.D vaccine (Oil Base)	115000	Doses
	Total: -	**90246850**	**Doses**

14.	Anti Rabies Vaccine (Samples)	7480	ML

DIAGNOSTIC ANTIGENS

1.	Mallein IDP	9250	Doses
2.	Mallein ORD	Nil	Doses
3.	Tuberculin Antigen (Mamm)	17550	Doses
4.	Rose Bengal, plate tests Antigen.	1540	ML
5.	Brucella abortus Antigen (M.R.T.)	1650	ML
6.	Brucella abortus Antigen (Conc:)	2000	Ml

TOTAL PRODUCTION

Vaccines	**=**	**128646590**	**Doses**
	+	**7480**	**Ml**
Diagnostic antigens:	**=**	**26800**	**Doses**
	+	**5190**	**Ml**

SERVICES

DISEASE INVESTIGATION

In the field of disease investigation, the institute caters for the identification of specimens and confirmation of diagnosis on the basis of suspected materials received from extension workers. In addition to the diagnostic service, the institute also helps in the epizootological investigation of disease outbreaks in order to define the problem of communicable diseases, appraise their importance on local or regional basis and contribute to improve methods of control.

Brief details of the morbid materials received, postmortem examination performed and the diseases diagnosed at VRI during the year, 2010-2011 are as follows :-

- Postmortems conducted including wildlife. = 71
- Specimen tested for Parasitic infestation = 470
- Specimens tested for Haemoparasites Infestation = 544
- Specimens tested for Zoonotic diseases = 89
- Milk samples tested for Mastitis = 112
- Morbid material processed for Bacteriological examination = 116
- Animals treated at section and in field = 4777
- Animals vaccinated at section and in field = 1310
- Disease Out breaks Attended = 29
- Drug sensitivity tests performed = 121

PERCENTAGE OF DISEASES DIAGNOSED (2011-12)

No. of Animals	**Bacterial**	**Viral**	**Parasitic**	**Protozoan**	**Miscellaneous**
Specimen/Carcasses/ Postmortem = **909**	73	04	714	112	06
	8.03%	0.44%	78.54%	12.32%	0.66%

DISEASES DIAGNOSED

(A) (Livestock & Zoo animals)

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/ Camel	Wild life	Other Animals	Total
BACTERIAL DISEASES						
Staphylococosis	1	2	--	1	--	4
Pasterurellosis	3	--	--	--	--	3
E.Coli	1	--	--	--	1	2
Tuberculosis	6	--	--	--	--	6
Brucellosis	51	--	--	--	--	51
Black Quarter	2	--	--	--	--	2
Diplococci	--	5	--	--	--	5
(B) VIRAL DISEASES						
Diseases	**Cattle/ Buff.**	**Sheep/ Goat**	**Equine/ Camel**	**Wild life**	**Other Animals**	**Total**
FMD	4	--	--	--	--	4

(C) PROTOZOAN DISEASES

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/ Camel	Wild life	Other Animals	Total
Theileriasis	26	26	--	--	--	52
Babesiasis	08	09	05	--	--	22
Coccidiosis	01	30	--	--	07	38
(D) PARASITIC DISEASES						
Diseases	**Cattle/ Buff.**	**Sheep/ Goat**	**Equine/ Camel**	**Wild life**	**Other Animals**	**Total**
Roundworm Infestation	02	164	02	03	--	171
Tapeworm Infestation	43	12	11	01	--	67
Liverfluke Infestation	56	--	--	--	--	56
Mange	49	181	--	--	69	299
Tick Infestation	46	37	--	03	35	121

(E) MISCELLANEOUS MALADIES						
Diseases	**Cattle/ Buff.**	**Sheep/ Goat**	**Equine/ Camel**	**Wild life**	**Other Animals**	**Total**
Accidental	--	--	--	01	--	01
Snake Bite	--	--	--	01	--	01
Pneumonia	--	--	--	02	02	04

POULTRY PATHOLOGY

Apart from diagnosis and treatment of poultry diseases, Poultry Pathology section plays an important role of analyzing various samples and performing various microbiological tests. The detail of these activities is as under:

Serum samples for HI test	54
Feed samples	38
Water samples	28
Litter Samples	--
Cultural tests	48
Treatment advice given	4615450
Vaccination of Chickens	5935
Tours/Poultry farms visited	--
Post Mortems Conducted.	55

PARASITOLOGY
DIAGNOSTIC WORK

Sr. No.	Species	No. of samples	No. of positive samples	% age of positive samples
1.	Cattle & Buffalo	1733	1097	63.03%
2.	Sheep & Goat	1833	1309	71.4%
3.	Others	872	487	55.85%
	Total: -	**4438**	**2893**	65.18%

BRUCELLOSIS

Serological surveys for brucellosis in farm animals were carried out regularly. Stained antigens for Rose Bengal Ring test and tube agglutination test against the disease are produced. During the year under report 654 samples were tested for Brucellosis.

Species	Nature of Specimen	Results			
		No. of samples (Serum)	Positive	Doubtful	Negative
Brucellosis	Cattle	393	55	--	338
	Buffalo	252	9	--	243
	Mare	2	--	--	2
		Fetus =7	--	--	7
	Total: -	654	64	--	590

BOVINE TUBERCULOSIS

The disease is caused by acid fast organisms. Allergic skin tests are carried out to assess the incidence of Bovine tuberculosis and Johne's diseases (Paratuberculosis). Regular monitoring through tuberculin testing of animals in farms and culling of positive animals has brought the incidence of disease to a significantly low level.

During the year under report scheduled tuberculin testing at various livestock farms was conducted using Comparative Intradermal Tuberculin Test. (CIDT). A total of 475 animals were tested, Out of these 29 animals were found reactive to the test.

SPECIMEN TESTED FOR TUBERCULOSIS

- Milk 141
- Fecal 196
- Morbid material 4

MASTITIS

A total of 232 milk samples of clinical and sub-clinical mastitis affected animal were received in the section from Lahore and other areas near Lahore. These samples were processed for the diagnosis and antimicrobial susceptibility tests.

RABIES

Rabies disease is caused by rhabodovirus and characterized by nervous signs. Its alarming manifestation in man and dogs ensure continued public attention. The disease is enzootic and occurs throughout the country, particularly during breeding season of carnivorous animals which are the chief vector of disease in these areas. Section is involved in preparation of rabies vaccines both prophylactic (Flury-LEP) and post-exposure (Sample's type). Section in also handling the suspected samples for rabies diagnosis.

SHEEP POX AND GOAT POX

Sheep pox and goat pox are infectious diseases caused by capripox group. In Pakistan the disease exists in enzootic form and result in heavy losses in animals. In endemic areas these diseases give economic burden to the farmers in the form of mortality and reduced productivity. Many outbreaks have been recorded in the past and disease was prevalent throughout the country. Intensity of sheep pox disease is more out of these two diseases. Mortality rate in lamb is high i.e. up to 90% and more than 30-50% in adult sheep breeds, in which disease runs in generalized form. Presently, sheep pox and goat pox vaccines are produced on ovine fetal muscle cells and kidney cells. Availability of fetal cells is easy and is also more economical, moreover growth of these cells is much better.

HISTOPATHOLOGICAL EXAMINATION

During the year under report, 261 samples of morbid tissues were processed for histopathological examination for aid in diagnosis of different diseases.

- Livestock = 158
- Poultry = 58
- Wildlife = 01
- Miscellaneous = 44

Total = 261

DISEASES/CONDITIONS DIAGNOSED

- Jaundice = 01
- Mycotoxicosis = 06
- Pneumonia = 05
- Spirochetosis = 10
- Infectious Bursal Disease = 16
- Fatty Change = 11
- Miscellaneous = 212

BIOCHEMISTRY

Serum samples of normal and infected animals were analyzed for albumin, Calcium, Glucose Cholesterol, Magnesium, Triglyceride, total protein uric acid etc.

Total 1480 serum samples were analyzed for different parameters for different sections.

BLOOD SERUM SAMPLES ANALYSIS

H.S Section

Total 324 of cattle serum samples were tested for

TEST	TEST NUMBER
TP	54
Al	54
Ch	54
Glu	54
Tri	54
Uri	54

Race Club Horses

Total 158 of serum samples of horses were analyzed for

TEST	TEST NUMBER
TP	20
AL	16
Ca	20
Ch	20
Glu	20
Tri	19
Uri	17
Mg	14
Na	12

Disease Investigation Section

Total 505 of serum samples were analyzed.

TEST	TEST NUMBER
TP	52
Al	52
Ca	52
Ch	52
Glu	52
Tri	52
Uri	52
Mg	52
Na	52
Bill	37

Antigen Section

Total 333 serum samples were analyzed for

TEST	TEST NUMBER
TP	37
Al	37
Ca	37
Ch	37
Glu	37
Tri	37
Uri	37
Mg	37
Na	37

FMD

Total 160 of serum samples were analyzed

TEST	TEST NUMBER
TP	32
Al	16
Ca	16
Mg	16
Na	16
Ch	16
Glu	16
Tri	16
Uri	16

COMMUNICATIONS AND TRAININGS

Radio Talks

Radio talks in regional languages are an effective method of communication of scientific knowledge and approved techniques to the common man. Efforts were therefore made to educate the farmers on day to day problems and control measures regarding important infectious, contagious and seasonal maladies.

Trainings Provided

The Institute arranges In-service training programs and offers a series of courses. These courses emphasize laboratory procedures in virology, bacteriology, parasitology and poultry with special concern to the diagnosis, treatment and control of diseases. These trainings help field workers in handling disease problems efficiently.

- House Job Trainings = 40 Persons
- In-Service Trainings = 08 Persons
- Internship = 47 Persons
- Farmer Training and Education (Informal) = 55 Persons

ACTIVITIES

To carry out the activities i.e. production of biologics, effective and efficient disease diagnosis including disease out break management, field and laboratory aided work systematically, the institute is divided into ten disciplines, which are as under:

1. Virology, Fluorescent Microscopy and Tissue Culture.
2. Aerobic and Anaerobic.
3. Poultry vaccines.
4. Biochemistry, Media & Sterilization.
5. Quality Control.
6. Disease Investigation and Epizootology.
7. Poultry Pathology and Histopathology
8. Newly emerging and obscure diseases
9. Parasitology.
10. Auxiliary services.

VIROLOGY, FLUORESCENT MICROSCOPY AND TISSUE CULTURE

The major diseases being worked on in this division are

1. PESTE DES PETITS RUMINANTS DISEASE

Peste des petits ruminants (PPR) are an acute febrile, viral disease of small ruminants with great economic importance. The causative agent of PPR disease is closely related to the Rinderpest virus. In the past, PPR disease was controlled by using the heterologous rinderpest vaccine. But due to rinderpest eradication programs, use of rinderpest vaccine for the control of PPR disease in small ruminants has been banned. The only way to control PPR disease is the use of homologous vaccine. PPR cell culture vaccine (live) has been developed at Veterinary Research Institute, Lahore, Pakistan from a Nigerian strain (75/I) adopted on vero cell line. The vaccine has completed the stage of field trial. Good quality lyophilyzers are awaited for commercial scale vaccine production to be used in the field.

2. POX DISEASES

Pox diseases causes considerable economic losses to the farmers in the form of morbidity and reduced production potential. Pox diseases are being studied in sheep, goat, buffalo, cattle and camel. Goat pox and sheep pox are diseases mainly affect caprine & ovine respectively. However due to mass vaccination reported cases to the institute have been reduced many folds.

The institute is preparing two types of vaccines:

i. Live Goat Pox cell culture vaccine

ii. Live Sheep Pox cell culture vaccine

These vaccines are prepared on primary lamb kidney or muscle cell culture/lines and give one year immunity.

3. RABIES DISEASE

Rabies is an enzootic viral disease of all mammals including man. The disease assumes an epizootic farm during breeding seasons of carnivores which are the chief vectors of the disease.

Section is preparing two types of rabies vaccines;

i. Antirabies – Flury(LEP) vaccine (Pre-exposure)

ii. Antirabies - Semple's vaccine (Post-exposure)

FREEZE DRYING/LYPHOLIZATION

This is an important section of virology division which is responsible for lyophilization of various viral vaccines and seed cultures. The Institute produces lyophilized vaccines and maintains bank of pure cultures. The freeze drying units are kept pressed in service round-the-clock in order to meet lyophilization requirements.

During the year under report 90,93,38,880 doses of various vaccines and 121 vials of various seed cultures were freeze dried. The detail is as under:-

New Castle Disease Vaccine (N.D.V)	78262700	Doses
Goat Pox Vaccine	501100	Doses
Sheep Pox	982900	Doses
Contagious Caprine Pleuropneumonia Vaccine (CCPV)	10076700	Doses
P.P.R VACCINE	988500	Doses
Flury (LEP) Vaccine	980	Doses
IBD Vaccine	121000	Doses
Total	909338880	Doses

BACTERIOLOGY

This division is further sub-divided into various sections which deal with diseases caused by aerobic, anaerobic and mycoplasma bacteria. Empirical studies were made to reach optimum conclusions relevant to pathogenesis, diagnosis and control of these diseases.

1. HAEMORRHAGIC SEPTICAEMIA

Haemorrhagic Septicaemia (HS) is a highly fatal infectious disease of Cattle & Buffaloes caused by *Pasteurella multocida*. The vaccine presently used is a broth culture alum-precipitated bacterin, which although being satisfactory, yet does not confer immunity for very long duration. Large quantities of this vaccine are used to protect animals against haemorrhagic septicaemia. Oil based vaccine is also being produced on limited scale.

2. CLOSTRIDIAL DISEASES (ANAEROBES)

Diseases like black quarter, enterotoxemia and lamb dysentery are caused by clostridial group of organisms. *Cl. chauvoei* infection in cattle and buffaloes (Black quarter) is a problem in certain areas of sub-hilly regions and the bacterin confers adequate prophylactic protection.

Enterotoxemia and lamb dysentery in sheep is being effectively controlled with the help of a combined vaccine. Identification of clostridial toxins in the intestinal contents by mouse inoculation test has been found to be a quick and the most reliable method for rapid diagnosis of enterotoxemia and lamb dysentery. Another test indirect haemagglutination (IHA) has also been developed for checking the immune response.

3. BOVINE TUBERCULOSIS

Bovine tuberculosis is an important infectious disease worldwide that threatens the lives and livelihood of those people associated with livestock industry that causes respiratory problems in both livestock & humans. The disease is caused by *Mycobacterium bovis*. The bacteria are acid fast,

filamentous curved rods, the organism can be transmitted to humans through infected/ contaminated un-pasteurized milk, the inhalation of organism at the time of slaughter.

Planned tuberculin testing drastically reduced the burden of bovine tuberculosis in livestock, especially in large ruminants in Punjab during the last year. Tuberculin (PPD) allergic test is presently used to screen out the animals for bovine tuberculosis. Tuberculin which is purified protein derivative (PPD) is prepared from *Mycobacterium tuberculosis* and Mycobacterium para-tuberculosis (Avian strain).

4. JOHNE'S DISEASE

Johne's disease is a chronic infectious disease of ruminants involving the small & large intestine. None of the available diagnostic methods can give a reasonable assurance that the animal is not carrying latent infection. Thus apparently healthy animals with latent infection are likely to get introduced in to the herds and upset the whole control program. The incidence of sub clinical cases shedding organisms intermittently may be as high as 15%. Control measures to check the spread of infection should be undertaken.

5. MASTITIS

Mastitis occurs in all species of livestock in Pakistan. However, it is of major economic importance only in dairy cattle and buffaloes. The main assignment of the section dealing with this disease is to study the incidence, economic losses, development of diagnostic tests of the disease and effectiveness of various antibiotics against this milk depriving menace. Possibility of producing prophylactic agent is being studied.

6. ANTHRAX

Anthrax is a highly fatal disease of sheep, goat & large animals and has zoonotic importance. A spore vaccine from a non pathogenic F2 stern strain of Bacillus anthracis is being produced aginst this lethal disease. This vaccine is safe and potent and confers immunity for one year.

7. MYCOPLASMOSIS

Contagious Caprine pleuropneumonia (CCPP) caused by *M. mycoides var Capri* is a highly contagious disease of goats which is enzootic in certain areas of Pakistan and results in major losses. Along with the production of caprine pleuropneumonia vaccine, isolation and identification of different strains of mycoplasma is being carried out.

POULTRY VACCINES

Poultry industry is one of rapidly developing areas in livestock sector in Pakistan. This industry has recorded a steady growth during the last two decades. Increase in egg

Production and poultry meat in commercial poultry farming sector is being achieved at a steady rate. In fact increasing poultry population demands a judicious combination of poultry husbandry, disease diagnosis and prevention program. In the industry, the main losses occur due to the infectious, non-infectious, parasitic and metabolic diseases.

The poultry vaccines being prepared include Newcastle disease vaccine, Avian Influenza (Bird flu) vaccine, Hydropericardium syndrome vaccine, Fowl pox vaccine, Spirochetosis vaccine and Infectious Bursal Disease vaccine.

1. NEWCASTLE DISEASE

Newcastle disease is an important highly contagious viral disease caused by virus that belongs to paramyxoviridae cause great losses in poultry. It is cosmopolitan in nature. It is controlled by prophylactic immunization of the poultry flocks. Egg adopted strain (Mukteswar) is used for the production of live vaccine.

2. AVIAN INFLUENZA (BIRD FLU)

Avian influenza (AI) is a viral disease infects poultry and wild birds. The disease may range from sub-clinical infections of respiratory tract or drops in eggs production to severe systemic infection with high mortality. During the recent years, outbreaks of avian influenza were reported throughout the country which not only ruined the poultry industry but also posed threat to human health. Veterinary Research institute participated actively in the diagnosis (field surveys and sero-surveillance) and took up the challenge of vaccine production against this havoc. The institute successfully prepared an effective vaccine from the local isolate which consequently lead to its control.

3. HYDROPERICARDIUM SYNDROME

A mysterious disease named as Hydropericardium Syndrome (Angara) in broiler chicks appeared during the year 1988-89. The disease was diagnosed at Veterinary Research Institute Lahore and an effective vaccine was prepared. The vaccine provides 95-97% protection to the vaccinated flock. The vaccine has helped to control the disease and saved heavy losses to poultry industry. During the last few years under report the useful work of production of this vaccine on large scale is continued.

4. FOWL POX

It is a contagious, slow spreading viral disease of chicken characterized by proliferative lesions on the skin, gastrointestinal and respiratory tracts. The institute is producing an effective vaccine against this disease which confers life long immunity.

5. AVIAN SPIROCHETOSIS

Avian Spirochetosis is an acute, febrile, septicemic, bacterial disease of poultry which causes considerable losses. The disease is caused by *Borriellia ancerina* transmitted by tick *Argus percicus* specie. An effective vaccine is being prepared by the institute against this disease.

6. INFECTIOUS BURSAL DISEASE.

Infectious Bursal Disease (IBD) is highly contagious viral disease of poultry and causes heavy mortality and immunosuppression in young chicks. Cell culture adapted IBD vaccine has been developed and has completed the stage of field trial. Lypholizers are awaited for commercial scale vaccine production to be used in the field.

BIOCHEMISTRY, MEDIA & STERILIZATION

In Veterinary Research and education biochemistry is highly relevant to the metabolism and function of animals in health and disease and forms the basis for an intelligent understanding of major aspects of the veterinary science and animal husbandry.

The determination of blood constituents is helpful in diagnosing various diseases and abnormalities in the body. Sera, vaccines and standardized with biochemical techniques.

PREPARATION OF BUFFERS, REAGENTS, STAINS AND SOLUTIONS

Different types of buffers, stains and solutions were prepared according to the requirement of various sections. The buffers used in laboratory work like phosphate buffer, EDTA buffer, borate buffer, buffer of different PH for calibration and standardization of PH meter were prepared.

Reagents solutions, indicators and stains of methylorange, phenol red, phenolphthaline, congo red, Potassium chromate, biruet reagent, normal saline, acid and alkali solutions of various normalities, molarities and concentrations were prepared.

Sr. No.	Solutions / Buffers / Reagents / media	Quantity
1	Phosphate Buffer	37550 ml
2	Borate Buffer	2 litre
3	Normal Saline	5 litre
4	KCL solution	1500 ml
5	N/10 NaOH	1 litre
6	N/1 NaOH	2100 ml
7	N/1 HCL	1110 ml
8	Biuret Reagent	2 litre
9	LJ media	600 ml
10	BIA Media	8 litre
11	BBA Diluents	1800 ml
12	phenolphthaline Solution	50 ml
13	0.5% Acetic Acid	1 litre
14	H_2SO_4 12% Solution	7 litre
15	Silver nitrate	2 litre
16	Methyl orange	20 ml

Standardization of Solutions

Biological evaluation of various solutions was carried out in the section i.e. Nutrient broth being prepared in bulk for use in the preparation of major vaccines, like Haemorrhagic septicemia, Enterotoxemia, and Black quarter disease, was analyzed and standardized for its pH and protein contents. Protein contents of avian and mammalian tuberculins were also analyzed for their standardization.

Nutrient Broth Sample

Total 87 Nutrient Broth Samples were tested for optical density and 31 were tested for total protein for H.S Section.

Section	Sample of DD	Total Proteins
H.S	45	19
Anaerobe	42	03
T.B	--	08
Flurry	--	01

MEDIA & STERILIZATION

Media and sterilization section serves as central facility for various laboratories of the institute. During the period under report, the section produced the following media.

a. Media for the production of different vaccines and antigens

1. Nutrient Broth 123136liters
   For Haemorrhagic septicaemia (alum ppt) 79975 liters
   Enterotoxemia & Black Quarter 43025 liters
   Others Diseases 136
2. Brain Heart Infusion Broth: 135 liters
3. Tryptose Phosphate Broth. 21 liters
4. Dorset Synthetic Media: 15 liters
5. Potato infusion Agar 40 liters

b. Media for research and diagnostic purposes

The section prepared 27 different types of media and 19 different sugars for Biochemical characterization of different microbes in varying quantities and supplied to the various laboratories in the institute for research and diagnostic purposes.

Apart from the routine sterilization and preparation of media, the section was also engaged in research work for improvement in techniques and nutrients for attaining maximum growth of organism for biologics production.

CENTRAL REFERENCE LABORATORY (CRL)

CRL section is an important section of the institute as far as the veterinary research is concerned. Section received samples suspected for Hemorrhagic septicaemia on the basis of which a project was designed for Biochemical identification and molecular characterization of *Pasteurella multocida* field isolates from the suspected samples and comparison was made with that of the *Pastuerella multocida* vaccinal strain. Suspected samples were streaked onto different selective and differential medias and then microscopic identification was done by observing slide under microscope stained with gram's stain. After that biochemical identification was done in which different types of tests were performed i.e. Spot oxidase test (Kovac's method), catalase test, indole test (Kovac's method), rapid urease test, carbohydrate fermentation test (for six sugars). This was further confirmed with API20E test kit. After that, molecular identification was performed by PCR for molecular characterization following TA cloning and gene elution (the abstract is attached herewith).

Apart from this project, a lot many other samples were processed for HI test (for ND and H9), plate agglutination test for *Mycoplasma gallisepticum* (MG). These tests were performed using blood and serum samples received from different poultry farms and sheds. Other samples were also received for general viral and bacterial isolations. A brief description of samples has been shown under:

Blood samples for plate agglutination test for MG	19 (Positive=11, Negative=8)
Serum samples for HI test:	19 (Variable results)
Samples for bacterial isolation:	21

QUALITY CONTROL

Quality control is one the most important division of the institute as it is concerned with the quality assurance of the biologics produced at Institute.

The division procured latest laboratory equipments and is establishing latest techniques for quality testing of different biologics. At present this division is engaged in cell culture techniques, identification and purification of different vaccinal antigens and different biologics through column chromatography, SDS-PAGE, gel documentation, PCR and Western blot. Apart from this, animal feed testing laboratory is being established to develop the facility for feed analysis. For this purpose HPLC, ELISA Kits + Instruments, spectrophotometer and other related equipment have been procured.

1. Testing of Vaccines

Following vaccines were tested during the year for their quality.

Sr. No.	Vaccines
1.	H. S. Vaccine
2.	B.Q. Vaccine
3.	Anthrax spore Vaccine
4.	Goat Pox Vaccine
5.	Sheep Pox Vaccine

2. Testing of Antigens

Following antigens were tested during the year for this quality.

Sr. No.	Antigen
1.	Rose Bengal Plate Test Antigen
2.	*Brucella abortus* Concentrated Antigen
3.	*Brucella abortus* Milk Ring Test Antigen

3. ENZYME LINKED IMMUNOSORBENT ASSAY

The ELISA test of following serum samples was performed during the year at different time intervals for the following diseases.

- Serum samples of breeding bulls of SPU Qadirabad for *Brucella abortus*.
- Serum samples of breeding bulls of SPU Kherimurat for Bovine Viral Diarrohea Virus.
- Serum samples of breeding bulls of SPU Qadirabad and Kherimurat for paratuberculosis (Indirect ELISA).
- Serum samples of breeding bulls of SPU Qadirabad and Kherimurat for *Mycoplasma bovis*.

4. POULTRY

Apart from above, ELISA was carried for Antibodies against Avian Influenza.

DISEASE INVESTIGATION AND EPIZOOTOLOGY

Disease Investigation division is actively involved in disease out break management in livestock, wildlife and pets, throughout the province. The aims and objectives of the division is to help the livestock farmers and concerned departments to control the disease and enhance the productivity through in time and accurate disease diagnosis and improved husbandry practices. The services rendered by the division includes postmortem examination and laboratory investigations of the material received from the field i.e, morbid material, blood/serum/plasma, milk body fluids etc. These services are provided free of cost.

The division is striving hard to uplift the economy of farmer specially the livestock sector by managing the disease problems effectively and efficiently. The division remained busy in diagnosis of various livestock, pets and wildlife diseases. Bacterial, viral, parasitic and metabolic diseases have been effectively controlled through prompt actions and suggestive control measures.

POULTRY PATHOLOGY

This division has an important task of providing diagnostic services to the poultry farmers and suggesting disease control measures. In addition to the identification and confirmation of poultry diseases, long term studies on the epidemiology and pathogenesis of major diseases are also being carried out. Sick as well as, dead birds and pathological materials are received daily from all over Punjab for diagnosis of poultry diseases and isolation of causative agents. The control of infectious diseases through effective vaccines and prompt efficient diagnostic facilities has encouraged and created confidence in private enterprises to invest in the poultry industry.

HISTOPATHOLOGY

Histopathology is an important tool for the diagnosis and confirmation of different diseases of livestock & poultry. Morbid material and tissue specimens are processed for histopathological examination. In routine, paraffin section technique, haematoxylin & eosin staining method are mainly

used for this purpose. This section also helps some other laboratories like disease investigation, poultry pathology, antigen and flury for preparation of slides to observe the microscopic changes in different types of diseased tissues in comparison of healthy ones.

NEWLY EMERGING AND OBSCURE DISEASES

This important division is planned to meet the future challenges regarding animal health aspect of livestock and poultry industry. It includes studies on several diseases which remained obscure, studies on newly emerging problems of poultry and livestock.

An independent laboratory for diagnosis of newly emerging poultry diseases had been established, this division has worked on different diseases such as Hydropericardium syndrome (HPS), Infectious bursal disease (IBD), Infectious bronchitis (IB), Infectious laryngeotracheitis (ILT), viral arthritis, Avian Influenza (AI) and Femur head necrosis in the near past. Consequently HPS, Avian influenza (Bird Flu) and IBD vaccines have been developed in the institute to over come these problems.

PARASITOLOGY

This division is playing an important role through diagnosis of parasitic diseases, which cause great economical losses to the livestock and poultry industry. Leather industry which is earning a handsome amount of foreign exchange for the country is also affected by this problem. The parasitic infestatation not only itself produces specific diseases but also predisposes the animals to a large number of other diseases. That's why the diagnosis and the treatment of parasitic problems are of great importance. Pakistan is very rich in tick fauna, which along with other arthropods are mainly responsible for the transmission of protozoan diseases of livestock. Parasitology division is carrying-out surveys of helminths infestations with a view to have an accurate data on incidence and distribution of these parasites.

PRINCIPAL TECHNIQUES APPLIED

- **CELL CULTURE TECHNOLOGY**

Veterinary Research Institute is pioneer of this work in Pakistan. The development of Cell Culture has revolutionized the production and standardization of viral vaccines. The Cell Culture vaccines have proved very efficacious as well as very economical in terms of cost of production. Cell Culture FMD Vaccine is prepared on baby hamster kidney cell line while the sheep pox vaccine is produced on monolayers of primary cells obtained from lamb kidney. The lamb kidney and testis cells are being used for preparation of goat pox vaccine. Cell Culture technology is being used in other areas like diagnosis of viral diseases and in diagnosis of newly emerging diseases of poultry like IBD (gumboro) etc.

- **GEL ELECTROPHORESIS**

It is an advance technique for protein analysis on the basis of molecular weights. This technique can be used for diagnostic as well as research purpose. Proteins of different molecular weights can be isolated by western blot method.

- **LOW PRESSURE COLUMN CHROMATOGRAPHY**

This technique is used for fractionation of different proteins according to their sizes or charges. These fractions can be further processed in different diagnostic techniques. Further more different types of immune-globulins and enzymes can also be isolated and purified by this technique.

- **MICROSCOPY**

Quality Control Section is equipped with advanced techniques of microscopy like Fluorescent Microscopy, available with both Inverted and Research microscopes apart from light microscopy. This is used for identification of different bacteria, and fungi and also for histopathological purposes.

- **FLUORESCENT MICROSCOPY**

Applications of Fluorescent antibody technique to various basic microbiological studies is numerous. The technology is an aid for quick and accurate diagnosis of various disease conditions. This technique is being given due consideration for its development. Various conjugates which were imported in the past are planned to be produced in this organization.

- **ULTRA CENTRIFUGATION**

For the isolation of different viruses Quality Control Section is well equipped with latest equipments of Ultracentrifugation.

- **ULTRASONIC CELL DISRUPTION**

This section has also latest technique for release of viruses from the cell by Ultra sonic Cell Disruption.

- **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

For minor quantitative analysis of mycotoxins, amino acids, sugars, drugs and proteins of different types this instrument is very much useful and authenticated.

- **NITROGEN AND PROTEIN ESTIMATION**

For Nitrogen and Crude Protein estimation, this section has an Automatic Kjeldhal Digestion and Distillation system along-with automatic Titration Unit.

- **CRUDE FIBER ESTIMATION**

For Crude Fiber contents estimation in food and feed, this section has an instrument for this purpose.

- **CRUDE FAT ESTIMATION**

For Fats and Oils estimation, Soxhlet Extration Unit is available in this section.

- **SPECTROPHOTOMETERY**

Latest Double Beam Spectrophotometer is available in this section for the serum analysis and other biological assays.

- **FLAME PHOTOMETRY**

For the estimation Macro minerals in feed samples, the section has the facility of Flame Photometer instrument.

- **ELISA TECHNOLOGY**

ELISA technique is more sensitive, reliable and quick to perform. The disease out-breaks can be diagnosed more rapidly and efficiently. Moreover, the immunological response in various infectious diseases can be determined by employing this technique in herds/flocks.

The addition of advanced technology is a step forward in efforts to use the latest technology to effectively control infectious diseases of animals/poultry.

AUXILIARY SERVICES

1. LIBRARY

Library is a facility that is most important for maintaining steady flow of latest information among research workers. Biological sciences are developing at fantastic pace and is gaining excellence in standards. It is very essential to keep abreast with what is going on in the world of science. The library is also serving as a good source of knowledge for the post graduate students, house job officer and scientists working in the field of animal science and biological production. Valuable literary/ current affairs book are also available. However then it is dire need of the time that the library may be modernized on digital bases. Efforts are therefore, continued to organize library by obtaining latest books and journals of which report during the year is as under:-

- Number of Books available in the Library 5,909
- Number of books added during the year under report 30
- Number of Volumes of Journals available in the Library 13,391
- Number of Volumes of Journals added. 110

2. EXPERIMENTAL ANIMALS

Experimental animals like rabbits, guinea pigs, albino mice and chicks are maintained to fulfill the requirements of various laboratories in the institute. These experimental animals play an important role for research and testing of different biologics. Guinea pigs, albino rabbits and Swiss mice are bred at the Institute while ordinary rabbits and chicks are procured from local market to replenish the stock.

During the year under report 4785 mice, 07 rabbits, 88 guinea pigs and 29 chinchala rabbits were provided for various experiments in the institute as well as to other organizations.

3. CARPENTRY

This serves the basic need of preparation and repair of wooden boxes which are used for packing of vaccines and sera supplied to the field. This section also caters for petty repairs/preparation of culture rooms and fixing up of equipment in various laboratories of the Institute.

4. ELECTRICITY

A wide variety of electric machines and equipments are used in different laboratories of the Institute. A separate electricity and mechanical section takes care of various machines, refrigerators, air-conditioners and other laboratory fittings and ensures smooth and uninterrupted functioning of equipments and fittings. The processing of delicate materials and the storage of different bacteria and virus demand continuous supply of power, and this is being ensured by installation of two generators. The installation of generator has helped in overcoming the problem of frequent break-down in electric power.

5. ESTATE

The section looks after the maintenance of buildings, roads, grassy plots, flower-beds and trees. A small nursery has been established in order to maintain a regular supply of flowering plants. High standard of cleanliness and landscaping is maintained to achieve conditions conducive to better output.

6. SUPPLY SECTION

This section acts as a liaison between the laboratories preparing the various biologics and the extension workers who use these biologics.

Indents for these biologics received from all over the country and extension wing of livestock are dealt with, and the biologics are delivered under proper conditions to all parts of the country. During the year under report a total of 99162195doses were supplied to various parts of the country.

7. STATISTICS SECTION

Statistics Section is one of the important sections of Veterinary Research Institute. The key role of this section is to collect the data, and analysis of vaccine production data. The section prepares the weekly, fortnightly, monthly, quarterly, six monthly, yearly, progress and efficiency, monthly disease incidence, monthly activities performed reports regarding biological productions. The section holds the records of all biological vaccines production.

RESEARCH ACTIVITIES

Efficacy of experimentally prepared oil-based Newcastle disease (ND) vaccine (Mukteswar strain) against prevailing virulent ND virus in Pakistan

Ali, Zahid Hussain, Sajjad Hussain, Muhammad Arshad, Sobia Aamir, Iffat Huma and Nida Arooj

Oil-based inactivated Newcastle disease (ND) vaccine was prepared and its efficacy against the prevailing velogenic ND virus was determined. Oil-based vaccine was prepared by mixing one part of the inactivated antigen with three parts of the montanide oil. The vaccine was evaluated for its safety, stability and immunogenicity. One hundred and twenty five day old birds were divided in 5 groups designated as A to E. The birds of different groups were treated with experimentally prepared vaccine alone and in combination with live ND vaccine (Mukteswar) at different age by using different dose rate and routes of administration. The anti-NDV- HI- antibody response of all the four groups was determined on day 14, 21, 28, 35 and 42 post-vaccination. On 28^{th} day post vaccination, the birds were challenged with velogenic field isolated virus. The birds that survived from challenge were also bled at day 42 of age to determine vaccine response. High antibody titers and 100% protection was observed in birds of group B which suggested that simultaneous use of both live and killed oil-based vaccines at day 7^{th} of age is helpful in the prevention against disease challenge. In A, C and D groups 90 % protection was seen. Oil-base ND vaccine containing Mukteswar strain gave remarkable antibody titers to resist the field virus. So it was concluded that oil based vaccine can give better immune response and protection against disease when used in early age in broiler chicks.

Pathotyping of field isolates of new castle disease virus from different outbreaks and determination of anti- NDV antibody titer during convalescence using different NDV antigens

Sajjad Hussain, Muhammad Arshad, Zain ul Abidin and Sajjad Ali

New castle disease is highly contagious disease of poultry causing high morbidity and mortality. A total of 120 samples were used for isolation and identification of new castle disease virus (NDV). Out of these, 106 samples caused embryo death while 103 samples were confirmed positive for NDV by haemagglutination inhibition (HI) test using three different strains (field isolate, lasota and muktaswar). Anti-NDV antibody titer was very high (1:256 to 1:1024) with all three strains used. Pathotyping of positive samples was done by calculating mean death time (MDT) and intracerebral pathogenecity index (ICPI). Results obtained from these experiments confirmed the presence of velogenic strain which was obviously responsible for severe outbreaks in those farms.

Preparation of hyper immune serum against infectious bursal disease virus in Rabbits and standardization of ACID test for confirmation of IBD in poultry

Tariq Mahmood Butt, Zahid Saeed and Umber Rauf

A batch of rabbits were hyperimmned against infectious bursal disease virus. The IBD virus was replicated on chicken embryo fibroblast and cytopathic changes were noticed. The material thus collected was used as IBD antigen. Six serial inoculation of IBD antigen were given to rabbits at one week intervals. The sera collected from rabbits was evaluated through agar gel precipitation test against serial ten fold dilutions of IBD antigen. PPT lines were noticed against hyperimmune sera up to 10^{-3} dilutions. Which indicated positive reaction. Hence the hyperimmune sera was positive for IBD antibody.

Characterization of field isolates of pasteurella multocida from cases of haemorrhagic septicaemia

Ayesha Kanwal, Shahida Afzaal, Zafar-ul-Ahsan Qureshi and
Syed Abdul Khaliq

Pakistan is an agro-livestock based economy and livestock sector is very important sector in the agricultural industry. It is the 5th largest milk producing country and the major milk producers in Pakistan are cattle and buffaloes. Various bacterial diseases pose a great threat to the survival and production of these ruminants and Haemorrhagic Septicaemia which caused by serotype B:2*Pasteurella multocida* is one of them. Livestock of Pakistan is at a high risk of HS occurrence due to the sub-tropical climatic conditions of the country and this disease is controlled by timely vaccination with "bacterins" manufactured locally. To have maximum protection against the disease, the field strains should have genetic similarity with the vaccinal strains. In the present study, two bovine field isolates of Pasteurella multocida from HS cases were characterized along with the local vaccinal strain and were compared at biochemical and molecular level. The strains were identified through cultural, biochemical (including Analytical Profile Index), and PCR assays targeting a gene region unique to HS-causing type B Pasteurella multocida. Finally the sequence was determined and field strains were compared with the vaccinal strain through molecular phylogenetic analysis which revealed the maximum similarity between both the field strains and with the vaccinal strain as well. Moreover, all three strains showed homology with the HS-causing type B specific gene of Pasteurella multocida. These results inferred that the field strains could be used to develop vaccine in future against HS in Pakistan and the use of current vaccinal strain could also be continued

Isolation, identification and characterization of pseudomonas aeruginosa from rabbits

Syed Abdul Khaliq

In this study a disease outbreak was investigated in the Chinchillas with respiratory signs and sudden death. Post mortem of the dead rabbits was conducted and lungs, liver and heart blood were collected. The Nasal swabs and the water samples were also collected. The highly virulent *P. aeruginosa* was isolated from the samples. The identification was confirmed by API 20E biochemical profiling. Then different specific tests for *Pseudomonas* were performed. It produced green discoloration of the culture media. It caused complete hemolysis on blood agar. Growth on MacConkey agar and at $42C^0$ temperature was found positive. It was oxidase and catalase positive. The gram negative motile rods were observed on staining. The isolated *P. aeruginosa* broth culture was injected to the mice subcutaneously and intraperitonelly and was found highly virulent as it caused death of the test mice within 18 hours of the injection. The antibiotic sensitivity test showed that the isolate was highly drug resistant to most of the antibiotics. Then the 16S ribotyping was performed for its molecular identification and characterization. The genus specific primers for *Pseudomonas* were used; the PCR product of 618 bp was sequenced, the consensus sequence was matched with the already submitted sequences in NCBI data bank. It was found that the isolated *P. aeruginosa* was matched with the highly antibiotic resistant and highly virulent strains of *P. aeruginosa* isolated from the human clinical samples. The isolated *P. aeruginosa* from the rabbits might have been transmitted from the human or the organism is prevalent in the rabbits also. Therefore, regular and careful monitoring of the rabbits and the lab animals is required to prevent the spread of the pathogen to the lab workers, other animals and the community.

Genomic and biological characterization of a Velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010

Muhammad Munir, Muhammad Abbas, Muhammad Tanveer Khan, Siamak Zohari and Mikael Berg

Background: Newcastle disease virus (NDV) causes severe and economically important disease in poultry around the globe. None of NDV strains in Pakistan have been completely characterized and the role of rural poultry in harbouring NDV is unclear. Since they have a very important role for long-term circulation of the virus, samples were collected from apparently healthy backyard poultry (BYP) flocks. These samples were biologically analysed using mean death time (MDT) and intracerebral pathogenicity index (ICPI), whereas genotypically characterized by the real-time PCRs coupled with sequencing of the complete genome.Despite of being non-pathogenic for BYP, the isolate exhibited MDT of 49.6 h in embryonated chicken eggs and an ICPI value of 1.5. The F gene based real-time PCR was positive, whereas M-gene based was negative due to substantial changes in the probe-binding site. The entire genome of the isolate was found to be 15192 nucleotides long and encodes for six genes with an order of 3'-NP-P-M-F-HN-L-5'. The F protein cleavage site, an indicative of pathogenicity, was 112RRQKRF117. Complete genome comparison indicated that the RNA dependent RNA polymerase gene was the most and the phosphoprotein was least conserved gene, among all the genes. The isolate showed an Y526Q substitution in the HN protein, which determines neuraminidase receptor binding and fusion activity of NDV. Phylogenetic analysis, based on F and HN genes, classified this isolate into genotype VII, a predominant genotype responsible for ND outbreaks in Asian countries. However, it clustered well apart from other isolates in this genotype to be considered a new subgenotype (VII-f). Conclusions: These results revealed that this isolate was similar to virulent strains of NDV and was avirulent in BYP either due to resistance of local breeds or due to other factors such as substantial mutations in the HN protein. Furthermore, we have characterized the first isolate of NDV, which could act as domestic reference strain and could help in development and selection of appropriate strain of NDV for vaccine in the country.

Biological characterization and phylogenetic analysis of a novel genetic group of Newcastle disease virus isolated from outbreaks in commercial poultry and from backyard poultry flocks in Pakistan

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Newcastle disease (ND) is a contagious viral disease of many avian species particularly domestic poultry, and is responsible for devastating outbreaks in the poultry industries around the globe. In spite of its importance and endemicity in Southern Asia, data on the genetic nature of the viruses and epizootiological information of the disease is scarce. In this study, six isolates from an emerging wave of ND outbreaks in the north of Pakistan and two isolates from healthy poultry flocks were biologically and genetically characterized. Based on pathogenicity indices such as intracerebral pathogenicity index (ICPI), mean death time (MDT) and cleavage motifs in the fusion protein, all these isolates were classified as virulent. Phylogenetic analysis of the fusion (F), hemagglutinin-neuraminidase (HN) and matrix (M) genes indicated the emergence of a novel genetic group within lineage 5, distinct from isolates previously reported in the region. Several mutations in the neutralizing epitopes and functionally important motifs of the F and HN genes pose a need for re-evaluation of the currently used vaccine and vaccination practices. The characteristics of Newcastle disease virus (NDV) as virulent (F protein cleavage site, ICPI and MDT) in apparently healthy backyard poultry (BYP) explain that BYP can play crucial role in the epizootiology and spread of the disease. The present investigation provides essential information on the genetic nature of NDV circulating in Pakistan and its implication on disease diagnosis and control. Furthermore, these investigations emphasize the importance of continuous surveillance of ND in developing countries.

Development and comparison of innocuity and potentiating effect of three oil adjuvant vaccines against HS disease caused by *Pasteurella multocida* in buffalo/cattle.

K.T Jaffry, Hina Afroz and Sumiyya Sattar

During the past 2 or 3 decades, HS bacterin was used for vaccination but the period of immunity conferred by this is very short and in many places vaccination have to be repeated two or three times a year as animals were reported to be succumbed to the disease after a short period of vaccination. Therefore, bacterin was switched over to adjuvant vaccine. The adjuvant must be adopted according to several parameters like the targets species, the antigen and the type of immune response, the route of inoculation, or the duration of immunity in order to obtain a good balance between safety and immunogenicity. Montanide ISA-206 and Montanide ISA 50,liquid paraffin and lanolin will be used for the preparation of vaccine. In House Quality Control Testing and potency testing will be performed in Swiss albino mice at different storage temperatures of vaccine. Field trial will be done in large animals on different government farms by constituting five groups of cattle/buffaloes and two subgroups for each group. Experimental vaccines will be compared with NIAB Oil Adjuvant vaccine also. So far two samplings are done in field trials and project will take three years for completion, starting from 15th may 2011.

Study of incidence of haemoparasites in sheep and their effect on biochemical parameters

Shabbir Ahmad, Manzoor Ahmad Ghuman, Saeed A. Khan, Nadeem Akram and Asma Aziz (BIO-Chemist)

The study was conducted to investigate the haemoparasitic incidence in sheep. Out of 50 blood smears of sheep 26 were positive for theileriosis, 9 were positive for babesiosis and 15 were negative for haemoparasites. Serum samples of 26 animals positive for theileriosis and serum samples of 14 animals negative for haemoparasites served as control were studied for serum biochemical parameters. Biochemical analysis of theileriosis infected cases revealed a significant decrease in blood glucose and albumin. In addition to a non-significant decrease in total protein and creatinine concentration when compared to uninfected group. On the other hand significant increase of total bilirubine in addition to a non-significant rise in triglyceride, cholesterol, alkaline phosphotase and uric acid levels were found in the infected group as compared to the control. No change was observed in serum magnesium, calcium concentration. The study concluded that theileriosis in sheep was associated with some alterations in blood parameters which could be useful in the diagnosis of theileriosis.

PREPARATION OF SLIDES OF NORMAL TISSUES OF LUNGS, LIVERS AND HEART OF NORMAL GOATS

Muhammad Afzal Sajid and Shahzada Parveen

50 samples of each tissue (Liver Lung and Heart) were collected from slaughtered healthy animals. This tissues were placed in 10% formaline for fixation. They were processed for histological studies. Slides were prepared and examined under microscope at 10x and 40x.

Lungs revealed respiratory bronchioles, bronchioles, alveolus, cubiodal epithelim and smooth muscle cells. In liver, classical lobules separated from one another by connective tissues were seen along with central vein. Heart showing skeletal muscle and endothelial cell.

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