

ANNUAL REPORT 2013-2014



**VETERINARY RESEARCH INSTITUTE
LAHORE - PAKISTAN**

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FOREWORD

Veterinary Research Institute playing a vital role in preventing and controlling of livestock and poultry diseases by providing quality biologics to ensuring food safety and security.

During the year the institute came up with its commitment to help the farmers in disease diagnosis at their doorsteps. Newly 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 executed for the development of effective vaccines against various Livestock and Poultry diseases.

Schemes have been launched for the production and quality assurance of various biologicals 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. 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 and biological production activities along with quality control, new laboratories established with state of the art equipment and machinery.

The laboratories have been established with all types of advanced diagnostic and biological production equipments for instant diagnosis and 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 was established on 7th June 1962. The institute is administratively controlled by the Punjab Government. The institute has an area of 200 kanals consisted of highly equipped laboratories and animal houses.

The institute undertakes research on existing and newly emerging animal diseases as well as production of vaccines against Livestock diseases. The institute is producing about 26 quality biologics covering a significantly large population of livestock and poultry. The primary aim of this institute is to sustain and uplift 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 for the nation.

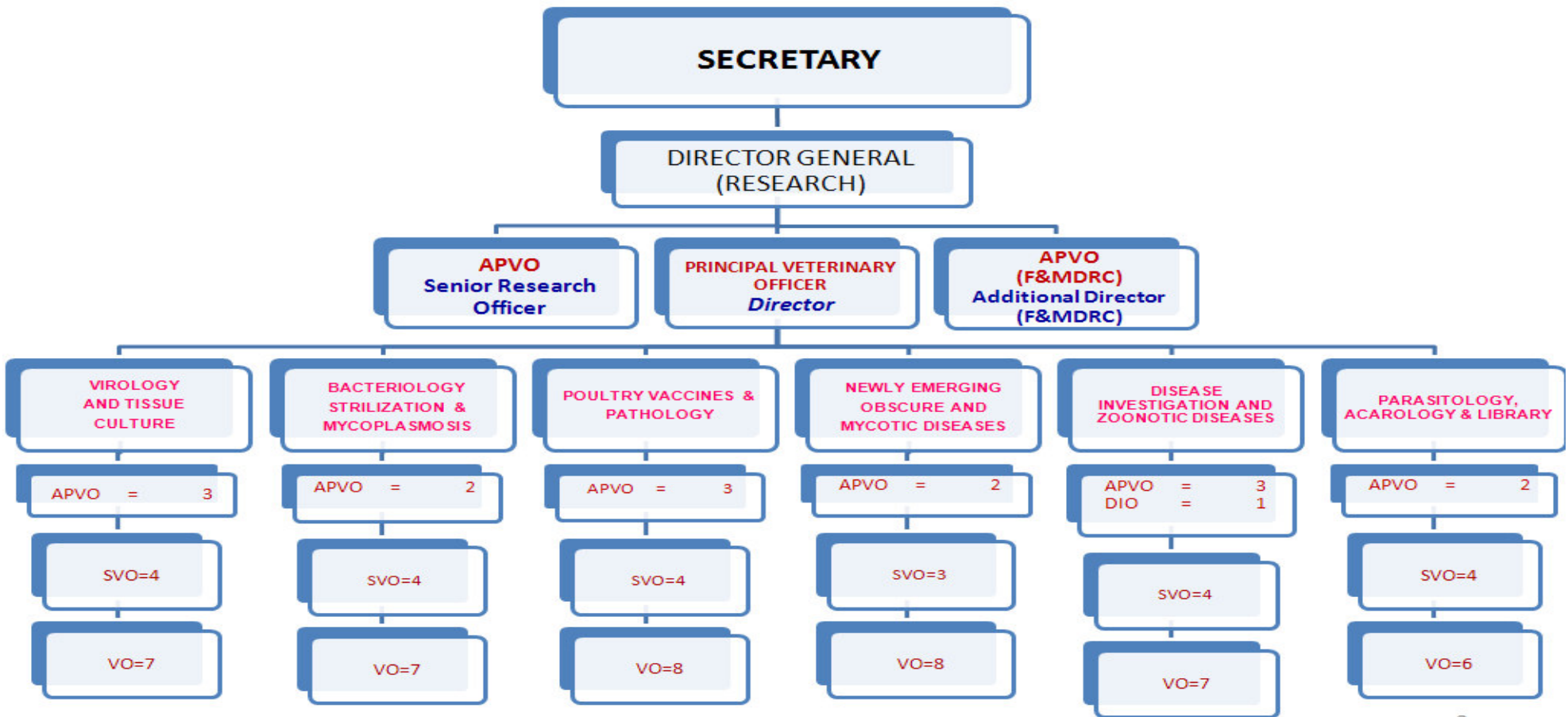
OBJECTIVES

- Large scale production of quality biologics for the control of infectious diseases of livestock and poultry.
- Research studies in related disciplines of animal health.
- Studies related to obscure diseases of livestock and newly emerging diseases of poultry.
- Development and standardization of modern techniques for vaccine production and disease diagnosis.
- Development of disease surveillance and investigation system.
- Trainings for in-service veterinary professionals in advanced production and diagnostic techniques.
- Training of the farmers, to enable them to help themselves in easy operations like dosing and vaccination etc.

ORGANOGRAM

ORGANIZATIONAL CHART

VETERINARY RESEARCH INSTITUTE, LAHORE CANTT



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

DIRECTOR

Dr. Rashid Ahmad

D.V.M; M.Sc (Hons); Ph.D

ADDITIONAL PRINCIPAL VETERINARY OFFICER

1. Dr. Azhar Hussain D.V.M; B.Com; M.Sc. (Hons.)
D.F.L.; P.G.T. (U.K.)
2. Dr. Kausar Tasneem Jaffery D.V.M; M.Sc. (Hons.)
3. Dr. Shabbir Ahmed D.V.M; M.Sc. (Hons.); Ph.D.
4. Dr. Zafar-ul-Ahsan Qureshi D.V.M; M.Sc. (Hons.);
M.S.(U.S.A)

SENIOR VETERINARY OFFICER / RESEARCH OFFICER / ASSISTANT RESEARCH OFFICERS/ BIOLOGICAL PRODUCTION OFFICERS/ASSISTANT 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. Muhammad Arshad D.V.M; M.Sc. (Hons.); Ph.D.
5. Dr. Waheeda Raana D.V.M; M.Sc. (Hons.); Ph.D.
6. Dr. Muhammad Afzal Sajid D.V.M; M.Sc. (Hons.); Ph.D.
7. Dr. Azam Ali Nasir D.V.M; M.Sc. (Hons.); Ph.D.
8. Dr. Sarwat Naz D.V.M; M.Sc. (Hons.); Ph.D.
9. Dr. Khawar Mehboob D.V.M; M.Sc. (Hons.)
10. Dr. Ghazala Nawaz D.V.M; M.Sc. (Hons.)
11. Dr. Mehboob Alam Qureshi D.V.M; M.Sc. (Hons.); Ph.D.
12. Dr. Manzoor Ahmad Ghumman D.V.M; M.Sc. (Hons)

- | | | |
|------|---------------------------|--|
| 13. | Dr. Sajjad Hussain Shah | D.V.M; M.Sc. (Hons.) |
| 14. | Dr. Azmat Sultana | D.V.M; M.Sc. (Hons.) |
| 15. | Dr. Muhammad Asad Raza | D.V.M; M.Sc. (Hons.) ;
P.G.T.(China) |
| 16. | Dr. Asifa Rasool Bhatti | D.V.M; M.Sc. (Hons.) |
| 17. | Dr. Muhammad Nauman | D.V.M; M.Sc. (Hons.) |
| 18. | Dr. Muhammad Asim | D.V.M; M.Sc. (Hons.) ;
P.G.T.(France) |
| 19. | Dr. Shahida Parveen | D.V.M; M.Sc. (Hons.) |
| 20.. | Dr. Sajjad Hussain | D.V.M; M.Sc. (Hons.) |
| 21. | Dr. Saeed A Khan | D.V.M; M.Sc. (Hons.) |
| 22. | Dr. Bushra Zameer | D.V.M; M.Sc. (Hons.) |
| 23. | Dr. Tariq Butt | D.V.M |
| 24. | Dr. Muhammad Ikram-ul-Haq | B.Sc. M.Sc. (Hons.); Ph.D. |

BIOCHEMIST

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

VETERINARY OFFICERS/CURATOR/ OFFICER INCHARGE STORE

- | | | |
|----|----------------------|---|
| 1. | Dr. Yasmeen Abbass | D.V.M |
| 2. | Dr. Shehzada Parveen | D.V.M. |
| 3. | Dr. UMBER RAUF | D.V.M; M.Sc. (Hons.) |
| 4. | Dr. Rizwan Qayyum | D.V.M; M.Sc. (Hons.) |
| 5. | Dr. Asif Rashid | D.V.M; M.Phil, M. Med. Sci
(Infection Biology); M. Med. Sci.
(Medical Science) Sweden |
| 6. | Dr. Muhammad Abbas | D.V.M.MBA, MS (M.B) Sweden |

7.	Dr. Syed Abdul Khaliq	D.V.M. MS/M.Phil, TQM
8.	Dr. Muhammad Naji Ullah	D.V.M; M.Sc. (Hons.)
9.	Dr. Abdul Razzak	D.V.M; M.Sc. (Hons.)
10.	Dr. Sajjad Ali	D.V.M.
11.	Dr. Iffat Huma	D.V.M
12.	Dr. Sobia Aamir Chughtai	D.V.M. M.Sc. (Hons)
13.	Dr. Rasha	D.V.M. M. Phil
14.	Dr. Nadeem Akram	D.V.M; M.Sc (Hons)
15.	Dr. Sumayya Sattar	D.V.M
16.	Dr. Zain-ul-Abidin	D.V.M. M. Phil
17.	Dr. Naila Maqsood	D.V.M
18.	Dr. Abdul Wahab Manzoor	D.V.M; M. Phil
19.	Dr. Ashi A Morris	D.V.M
20.	Dr. Hina Afroz	D.V.M
21.	Dr. Aqsa Mushtaq	D.V.M, M. Phil
22.	Dr. Nida Arooj	D.V.M
23.	Dr. Rehan Rafique	D.V.M, M.Phil
24.	Dr. Shiraz Shahid	D.V.M. M.Sc. (Hons)

BUDGET

ANNUAL BUDGET GRANT (2013-2014)

1.	<u>Allocation</u>	<u>Rupees</u>
	C-1 LO-4208	9,94,14,000
	C-2 LO-4209	6,01,07,000
	E-3 LO-4212	92,84,000
	Total:	168805000
2.	<u>EXPENDITURE</u>	
	C-1 LO-4208	9,69,40,023
	C-2 LO-4209	5,85,23,394
	E-3 LO-4212	75,09,000
	Total:	162972417

BIOLOGICAL PRODUCTION

PRODUCTS

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

During the year, institute produced 26 different products including 7 bacterial Vaccine, 13 viral vaccines and 6 diagnostic agents.

PRODUCTS	LIVESTOCK	POULTRY	TOTAL
1. Bacterial Vaccines	7	0	7
2. Viral Vaccines	5	8	13
3. Diagnostic agents	6	0	6
Total:	18	8	26

In fact, these prophylactic and diagnostic agents have played vital role for the development of the livestock and poultry industry in Pakistan and contributed 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 confirm 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 but 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

(2013-2014)

<u>Particulars</u>	<u>Target (Rs)</u>	<u>Earned (Rs)</u>
Vaccine	9,60,00,000	12,27,19,026
Book Adjustment	--	--
Achievement	128%	
Total:-	9,60,00,000	12,27,19,026

ASSUMPTIVE BENEFITS ESTIMATED ON
THE BASIS OF VACCINE PRODUCED
(2013-2014)

1.	Annual capital values	
	ANIMAL SPECIES	RUPEES (IN MILLION)
	Cattle & Buffaloes	123720
	Sheep & Goat	20600
	Poultry	1014
	Total: -	145334
2.	Saving in terms of prevention of clinical sickness & losses in production	
	Cattle & Buffaloes	5155
	Sheep & Goat	1973
	Poultry	676
	Total: -	7804
	GROSS TOTAL: -	153138

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 (HS) VACCINE (ALUM PRECIPITATED)

The vaccine is prepared in large quantity for the control of HS disease in Cattle and Buffalo. It is a formalinized, alum precipitated vaccine produced from a local selected strain of *Pasteurella multocida* Carter type B-2. The vaccine is used twice a year preferably in May/June and November/December.

- 2. HAEMORRHAGIC SEPTICAEMIA (HS) VACCINE (OIL ADJUVANT)

It is formalized killed oil adjuvant vaccine prepared from local strain of *Pasteurella multocida*, carter type B-2. The vaccine is used once a year.

- 3. BLACK QUARTER (BQ) VACCINE

Clostridium chauvoei infection in cattle is a problem in certain hilly & sandy areas. The vaccine prepared is 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

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

- 5. CONTAGIOUS CAPRINE PLEUROPNEUMONIA VACCINE (CCPP)

It is live attenuated 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 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 which has zoonotic importance. A spore suspension of live attenuated non-capsulated *Bacillus anthracis Sterne* strain in Glycerin saline confers solid immunity for one year.

II. POULTRY VACCINES

1. SPIROCHAETOSIS VACCINE

It is a killed vaccine prepared on demand 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
(ON DEMAND)

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 Vero Cell Line and gives immunity for one year.

4. GOAT POX VACCINE

It is an attenuated virus vaccine (Virus Strain Gorgan 56) cultivated in Vero Cell Line and provides immunity for one year.

5. PPR VACCINE

It is an emerging disease of small ruminants. Live attenuated cell have been culture vaccine prepared on Vero cell line using viral strain Nigeria 75/I.

II. POULTRY VACCINES

1. NEWCASTLE DISEASE VACCINE

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

2. NEWCASTLE DISEASE VACCINE KILLED (OIL BASED)

Newcastle Disease oil based killed vaccine Mukteswar strain is also being produced on demand.

3. FOWL POX VACCINE

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

4. HYDROPERICARDIUM SYNDROME VACCINE

Hydropericardium disease was first reported emerged in 1988 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.

5. AVIAN INFLUENZA VACCINE
(BIRD FLU AQUEOUS)

In 2006 an outbreak of avian influenza serotype H₅N₁ (Bird Flu) was reported 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 H₅N₁ (A / Chicken / Pakistan / NARC – 2238 / 06) seed virus received from Ministry of Food and Agriculture (MINFA).

6. AVIAN INFLUENZA (Bird Flu) VACCINE (Oil Based)

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

7. AI (BIRD FLU) + ND (Oil Based) VACCINE
Avian Influenza Strain H₉ and Newcastle Disease Mukteswar strain oil based killed vaccine is being produced on demand.

8. INFECTIOUS BURSAL DISEASE VACCINE

It is live attenuated cell culture viral vaccine prepared from local strain of IBV virus. It is an economically important disease.

DIAGNOSTIC ANTIGENS

1. MALLEIN

a. *Mallein (Concentrated)*

The concentrated mallein is used for intradermal palpaberal test (IDP) for the diagnosis of Glanders. This is the most sensitive, reliable and specific screening test for glanders.

b. *Mallein (ORD)*

The ordinary Mallein is injected subcutaneously for the diagnosis of Glanders and produced on demand.

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 antigen detects as well as specific antibodies from *Br. abortus* infection.

c. *Milk Ring Antigen*

It is also killed phenolised suspension of *Brucella abortus*, strain 99. This test is an efficient means of screening dairy herds by testing milk from the bulk tank.

PRODUCTION

VACCINES AND ANTIGENS (2013-2014)

BACTERIAL VACCINES

1.	Anthrax spore vaccine	502400	Doses
2.	Black Quarter vaccine	4724400	Doses
3.	Contagious Caprine Pleuropneumonia Vaccine	10200000	Doses
4.	Enterotoxaemia Vaccine	9987100	Doses
5.	Haemorrhagic Septicaemia Vaccine	17369100	Doses
6.	Haemorrhagic Septicaemia (Oil Base)	18000	Doses
7.	Listeriosis Vaccine	Nil	Doses
Total: -		42801000	Doses

VIRAL VACCINES

1.	Fowl Pox vaccine	Nil	Doses
2.	Goat Pox vaccine	800200	Doses
3.	Sheep Pox Vaccine	875700	Doses
4.	Hydropericardium vaccine	105000	Doses
5.	Newcastle Disease vaccine	57110100	Doses
6.	Flury	Nil	Doses
7.	N.D vaccine (Oil Base)	1000	Doses
8.	Avian Influenza	1510800	Doses
9.	Avian Influenza (Oil Base)	4000	Doses
10.	Peste Des Petits Ruminants (PPR) Vaccine	1805300	Doses
11.	I.B.D Vaccine	77500	Doses
12.	ND + H ₉	1635000	Doses
13.	Anti Rabies Vaccine (Samples)	Nil	ML
Total: -		64098600	Doses

DIAGNOSTIC ANTIGENS

1.	Mallein IDP	7875	Doses
2.	Mallein ORD	Nil	Doses
3.	Tuberculin Antigen (Mamm)	3000	Doses
4.	Rose Bengal, plate tests Antigen.	2065	ML
5.	Brucella abortus Antigen (M.R.T.)	1225	ML
6.	Brucella abortus Antigen (Conc:)	850	MI

TOTAL PRODUCTION

Vaccines	=	106910475	Doses
Diagnostic antigens:	=	10875	Doses
	+	4140	MI

SERVICES

DISEASE INVESTIGATION

For disease investigation, the institute gives services for outbreak investigation in order to define the problem of communicable diseases, appraise their importance on local or regional basis and contribute to improve methods of control. In addition to the disease investigation, the institute also provides services for laboratory diagnosis of various livestock and poultry diseases.

Brief details of the samples received, postmortem examination performed and the diseases diagnosed at VRI during the year, 2013-2014 are as follows :-

Diagnostic Services (2013-14)

Sr. No.	Diagnostic Services	No. of Cases
1.	Postmortems conducted including wildlife	215
2.	Specimen tested for Parasitic infestation	366
3.	Specimens tested for Haemoparasites Infestation	608
4.	Specimens tested for Zoonotic diseases	101
5.	Milk samples tested for Mastitis	200
6.	Morbid material processed for Bacteriological examination	116
7.	Animals treated at section and in field	4047
8.	Animals vaccinated at section and in field	4655
9.	Disease Out breaks Attended	26
10.	Drug sensitivity tests performed	200
11.	Farmer's Education	146
12.	House Job Training	30

Percentage of Diseases Diagnosed (2013-14)

No. of Animals	Bacterial	Viral	Parasitic	Protozoan	Miscellaneous
Specimen/Carcasses/ Postmortem = 1606	40	4	542	245	18
	2.49%	0.249%	33.75%	15.25%	1.12%

(A) BACTERIAL DISEASES

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/Camel	Wild life	Other Animals	Total
Staphylococosis	--	--	--	--	--	--
Pasterurellosis	14	--	--	--	--	14
E.Coli	--	--	--	--	--	--
Tuberculosis	--	--	--	1	--	1
Brucellosis	24	--	--	--	--	24
Black Quarter	--	--	--	--	--	--
Diplococci	1	--	--	--	--	1

(B) VIRAL DISEASES

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/Camel	Wild life	Other Animals	Total
FMD	2	840	--	--	--	840
Rabies	--	2	--	--	--	2

(C) PARASITIC DISEASES

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/Camel	Wild life	Other Animals	Total
Theileriasis	61	18	--	1	--	80
Babesiasis	--	1	6	--	2	9
Coccidiosis	21	116	--	9	9	155
Trypanosomiasis	1	--	--	--	--	1

(D) PARASITIC DISEASES

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/Camel	Wild life	Other Animals	Total
Roundworm Infestation	38	93	7	14	2	154
Tapeworm Infestation	48	62	--	15	4	129
Liverfluke Infestation	44	37	--	3	8	93
Mange	20	30	--	6	47	103
Tick Infestation	14	22	--	--	27	63

(E) MISCELLANEOUS MALADIES

Diseases	Cattle/ Buff.	Sheep/ Goat	Equine/Camel	Wild life	Other Animals	Total
Accidental	2	1	--	--	--	3
Snake Bite	5	1	1	--	--	7
Pneumonia	2	5	1	--	--	8

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:

Sr. No.	Diagnostic Services	Current year 2013-2014
1.	Treatment advice given to poultry flock	2564221
2.	Total Number of Cell Culture	5202
3.	Live Birds examined	3021
4.	Culture test	135
5.	Serology done (H.A, H.I test)	196
6.	Vaccination of birds in field and section	3022
7.	Tour done (on request)	100
8.	Radio talks Delivered	04
9.	Number of samples sent to other sections of VRI for confirmation are as under	
10.	Histopathology	95
11.	IBD	09
12.	Mycoplasma	10
13.	Parasitology	07

PARASITOLOGY **DIAGNOSTIC WORK**

Total No. of samples	=	2915
Total No. of positive samples	=	2048
Percentage of Positive samples	=	70.25%

RABIES

Rabies disease is caused by lyssavirus 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. The concern Section is involved in preparation of rabies vaccines both prophylactic (Flury-LEP) and post-exposure (Sample's type). Section in also process 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 caused huge economic losses 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 high as compare to goat pox. 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 Vero 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, 167 samples of morbid tissues were processed for histopathological examination for aid in diagnosis of different diseases.

Total Morbid Materials Processed During the Year	167
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Poultry	99
Animal	41
Zoo	01
Miscellaneous	26

Specimens Processed

Liver	44
Heart	14
Lung	21
Kidney	15
Spleen	23
Bursa	2
Proventriculous	6
Trachea	6
Brain	3
Sciatic Nerve	3
Intestine	7
Gizzard	13
Adrenal Gland	1
Testis	1
Tongue	1
Lymph Node	5
Duodenm	1
Jejunm	1

Diseases Conditions Diagnosed

Aflatoxicosis
Spirochetosis
Infectious Bursal Disease
Fatty change
New Castle Disease (NDV)
Tuberculosis
Avian leukosis
Infectious Laryngo Tracheaetis (ILT)
Inflammation
Hypertrophy

BIOCHEMISTRY, MEDIA AND STERILIZATION

Bio-Chemistry is the concern with all the sections as it is considered as a fundamental basis for all biological sciences. In this way, it is helpful to know the different changes taking place in living body that is hormonal enzymatic and bio-chemical.

In veterinary research and development bio-chemistry is highly relevant to the metabolism and process of health and disease conditions of animals.

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, indicator and stain were also prepared.

Sr. No.	Solution/Buffer/Reagents/Media	Quantity
1.	Phosphate Buffer	30 L
2.	Borate buffer	2 L
3.	Normal Saline	5 L
4.	NaoH Solution	1 L
5.	HCL Solution	3 L
6.	LJ Media	4 L
7.	BIA Media	30 L
8.	BBA Diluents	2500 ml
9.	Phenol Phthaline Solution	100 ml
10.	H ₂ SO ₄ 12% Solution	6 L
11.	3 M KCL	1 L
12.	Congored	100 ml
13.	Bacl ₂	100 ml

Serum and Nutrient Broth Analysis

Serum samples of normal and infected animals were analyzed for AI, Ca, Glu, Chol, Mg, Triglyrand, TP, Na, Uric acid etc.

Total 2651 serum samples were analyzed for different parameters for different sections.

Blood serum samples analysis for HS Section

Total 2036 of serum samples were analyzed

Test	Test Number
Albumin	163
Calcium	163
Cholestrol	163
Glucose	163
Creatinine	173
Magnesium	173
Potassium	173
Phosphorus	173
Sodium	173
Triglycide	173
Total Protein	173
Uric Acid	173

Central Reference Laboratory

Total 135 serum samples were analysed

Test	Test Number
Albumin	9
Calcium	9
Cholestrol	9
Creatinine	9
GOT	9
GPT	9
Bilirobin	9
ALP	9
Magnesium	9
Glucose	9
Phosphorus	9
Potassium	9
Total Protein	9
Triglycide	9
Uric Acid	9

Avian Leukosis

Total 267 serum samples were analyzed

Test	Test Number
Calcium	24
Phosphorus	24
Total Protein	20
Creatinine	20
Bilirubin	20
Magnesium	26
Calcium	26
Glucose	59
Sodium	26
Phosphorus	26

Poultry Pathology

Total 150 serum samples were analysed

Test	Test Number
Albumin	15
Calcium	15
Cholesterol	15
Glucose	15
Magnesium	15
Potassium	15
Triglyceride	15
Total Protein	15
Uric Acid	15
Creatinine	15

DISEASE INVESTIGATION

Total 27 serum samples were analysed

Test	Test Number
Albumin	3
Calcium	3
Cholesterol	3
Glucose	3
Magnesium	3
Sodium	3
Total Protein	3
Uric Acid	3
Creatinine	3

Race Club Horse

Total 36 serum samples were analysed

Test	Test Number
Albumin	3
Cholestrol	3
Calcium	3
Creatinine	3
Glucose	3
Magnesium	3
Potassium	3
Phosphorus	3
Sodium	3
Total Protein	3
Triglycide	3
Uric Acid	3

Nutrient Broth Samples

Total 337 Nutrient Broth Samples were tested for optical density and 100 were tested for total protein for different section.

Section	No. of Samples for OD	No. of Samples for total Protein
Media Section	294	98
HS	26	--
Anaerobe	7	--
Antigen	--	2

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 veterinarians and field Para-Veterinary Staff in handling disease problems efficiently.

- Internship / House Training 12
No. of Participants 93
- In-service Training 02
No. of Participants 09

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 division is working of following viral diseases of poultry and livestock

1. PESTE DES PETITS RUMINANTS DISEASE

Peste des Petits Ruminants (PPR) is a highly acute, febrile, viral disease of small ruminants with huge 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) attenuated has been developed at Veterinary Research Institute, Lahore, Pakistan from a Nigerian strain (75/I) adopted on vero cell line. Sufficient amount of PPR vaccine is being produced in the section.

2. POX DISEASE

Pox disease cause considerable economic losses to the livestock 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. Goat Pox cell culture live attenuated vaccine
- ii. Live Sheep Pox cell live attenuated culture vaccine

These vaccines are prepared on Vero cell lines and give one year immunity.

3. **RABIES DISEASE**

Rabies is an enzootic viral disease of all mammals including humans. The disease assumes an epizootic form 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 section is regularly working round-the-clock in order to meet lyophilization requirements.

During the year under report 7,35,31,800 vials of various vaccines have been lyophilized.

The detail is as under:-

New Castle Disease Vaccine (N.D.V) 200 Doses	21007400	Vials
New Castle Disease Vaccine (N.D.V) 500 Doses	38916500	Vials
Goat Pox Vaccine	819300	Vials
Sheep Pox	899300	Vials
Contagious Caprine Pleuropneumonia Vaccine (CCPV)	10206500	Vials
P.P.R Vaccine	1682800	Vials
Total	7,35,31,800	Vials

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 caused by clostridium perfringens (B&D) 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 assessing the immune response against the disease.

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 which has zoonotic importance. A spore vaccine from a non pathogenic F2 stern strain of *Bacillus anthracis* is being produced against this lethal disease. This vaccine is safe, 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 major industry 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 production 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 family paramyxoviridae causes great losses in poultry. It is cosmopolitan in nature. It is controlled by prophylactic immunization of the poultry flocks. Egg adapted 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. 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 reported during the year 1988. 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 is able 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 pericicus* 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.

CENTRAL REFERENCE LABORATORY (CRL)

OBJECTIVES

1. Establishment of Central Reference Laboratory to maintain the seed bank of different biologics.
2. Initiating and carrying out Research and Development
3. Local and foreign training of technical staff in various field of specialization to equip with latest and modern techniques for capacity building.

Service Provided by Section

Sr. No.	Diseases	No. of tests
1.	Salmonella	15
2.	T.B	1
3.	E.Coli	20
4.	Pseudomonas	2
5.	Sheep Pox	20
6.	Goat Pox	20
7.	PPR	4
Grand Total:-		82

QUALITY CONTROL LABORATORY

Aims and Objectives

1. The major objective of the quality control section is to test the quality of biologics prepared at VRI.
2. To perform allied research
3. To conduct training of students and in services personals.
4. Additional assignments

Quality Testing of Biologics / Feed and Fodder

Following are the biologics / Feed and Fodder samples which were tested during the year 2013-14 at different time intervals for quality assurance.

Sr. No.	Biologics Name	No. of Samples
1.	Haemorrhagic Septicaemia	82
2	Black Quarter vaccine	24
3	Enterotoxaemia Vaccine	18
4	Newcastle Disease vaccine	17
5	Peste des Petits Ruminants (PPR) Vaccine	3
6	Contagious Caprine Pleuropneumonia Vacc	10
7	Sheep Pox Vaccine	8
8	Goat Pox vaccine	6
9	Hydropericardium Syndrome Vaccine	1
10	Anthrax spore vaccine	1
11	Infectious Bursal Disease Vaccine	1
12	Mallien Antigen	2
13	RBPT Antigen	3
14	Brucella Abortus Conc.	4
15	Feed and Fodder Samples	101
	Total	281

Training / Research Activities

Fifty Students from Different universities got training in this laboratory during the year. They were taught and practically trained about different techniques and quality tests in the laboratory.

Research and Development Activities

- Cell line is maintained in the section for different Cell Culture Vaccines test
- Quality testing of newly developed vaccine that is IBD and ND+H₉
- Production of thermostable ND Vaccine and its field trials.

DISEASE INVESTIGATION AND EPIZOOTOLOGY

Disease Investigation division is actively involved in disease out-break investigation 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 samples 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 carried out 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 morbid 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 laryngotracheitis (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 infestation 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 and goat pox vaccines are produced on vero cell line 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 and 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 detected 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 separated 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.

➤ **SPECTROPHOTOMETRY**

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 outbreaks 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 are 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 officers and scientists working in the field of animal science and biological production. Valuable literary/ current affairs book are also available. However, it is dire need of the time that the library may be modernized on digital basic. 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	5,958
➤ Number of books added during the year under report	24
➤ Number of Volumes of Journals available in the Library	13,576
➤ Number of Volumes of Journals added.	95

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 2504 mice, 08 rabbits, 26 guinea pigs were provided for various experiments in the institute as well as to other organizations.

3. CARPENTRY

Carpentry gives the services of preparation and repair of different structures in the institute. The section also involved in repairs/preparation of culture rooms and installment of different equipments 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 in existed which 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 3885ML and 100539888 doses 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

Pathotyping of Newcastle Disease Virus using Multiplex Reverse Transcription Polymerase Chain Reaction and Pathological Studies in Naturally Infected Broiler Chicks.

Abdul Whab Manzoor, Farzana Rizvi, Mohsan Javed, Muhammad Numan, Ahrar Khan and Sajjad Ur Rehman

Multiplex RT-PCR was standardized to diagnose Newcastle disease (ND) in field survey of 50 poultry (Broiler) farms suspected for ND, under local laboratory conditions. Clinical signs and gross lesions of infected broilers were recorded. Respiratory signs were observed at 50 (100%) farms visited. Enteric signs were seen at 45 (90%) farms. Nervous signs including torticollis, paralysis of wings and legs, blindness and depression were found at 15 (30%) farms. Among these 15 farms, 10 (20%) farms were found to be infected with velogenic NDV through multiplex RTPCR. Congestion and mucosal haemorrhages were seen throughout the mucosa of trachea at 35 (70%) farms visited along with mucoid and catarrhal secretions trapped inside the tracheal tract. Congested lungs were found at 30 (60%) farms. Marked splenomegaly was observed at 15 (30%) farms. Lymphoid hyperplasia with haemorrhages and necrosis was seen in spleen. Visceral organs including trachea, lungs and spleen were collected for histopathological studies and virus isolation. Virus isolation was carried out into 9-day- old chicken embryonated eggs. Multiplex RT-PCR was conducted for the identification of different pathotypes of ND virus, using a set of three primers (P1, P2 and P3). A total of 10 (20%) isolates having HA titre of 1:1024 (3 isolates), 1:512 (5 isolates), 1:256 (2 isolates) and showing amplicons of 204 bp were pathotyped as velogenic NDV and other 20 (40%) samples which resulted the amplification of 204bp and 364bp amplicons simultaneously, were categorized as mesogenic NDV. The conditions of multiplex RT-PCR optimized in this study can be used for rapid identification and differentiation of NDV pathotypes from field outbreaks or experimental pathogenesis of ND.

**Prevalence of Tuberculosis in Cattle and Buffalo at
Various Livestock Farms in Punjab**

Manzoor Ahmed Ghumman, Abdul Whab Manzoor, Sarwat Naz,
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Tuberculosis is an infectious diseases of animals which causes heavy economic losses in livestock. A total of 17601 animals including 9021cattle and 8580 buffalo were screened for tuberculosis at five government and fifty private livestock farms in Punjab, Pakistan from 2006 to 2010. Out of these, 1057 (11.71%) cattle and 1027(11.96%) buffalo were found to be positive for tuberculosis in intradermal tuberculin test while 499 (5.53%) cattle and 567 (6.62%) buffalo were found suspected for tuberculosis. Statistical analysis showed insignificant difference in both species for prevalence of TB..

Ante-Mortem Diagnosis of Rabies in Cows and Buffaloes

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The current study was carried out to compare the conventional diagnostics for the diagnosis of rabies with advanced diagnostic technique. Ante-mortem diagnosis of rabies in cows and buffaloes is described using newer molecular technique and conventional methods for the detection of rabies virus RNA from saliva samples. Saliva samples from animals at different time intervals were collected and tested for rabies virus using reverse transcriptase polymerase chain reaction (RT-PCR) and mouse inoculation test (MIT). Results obtained by RT-PCR (1-2 days required) were easy, non-laborious, satisfactory and can be applied in replacement of routine laboratory test i.e. Mouse inoculation test (21 days required) for ante-mortem diagnosis of rabies virus from saliva.

Estimation of Aflatoxin B1 in Poultry Feed Ingredients Collected from Different Poultry Farms and Markets of Lahore, Pakistan

Zain ul Abidin, Tariq Mahmood Butt, Aisha Khatoon
and Mahboob Alam Qureshi

Aflatoxins when present in feed pose a great threat to poultry birds. The present study was designed to estimate the levels of aflatoxin B1 (AFB1) in different poultry feed ingredients (canola meal, corn, corn gluten 30%, corn gluten 60%, soya bean meal, sunflower meal, cotton seed cake and cotton seed meal) collected from different poultry farms and markets of Lahore, Pakistan. The study was conducted over a period of three years (2009-12) and each year was further divided into 3 periods according to environmental conditions of Lahore i.e. Jul-Oct (hot and humid), Nov-Feb (winter/cold) and Mar-Jun (dry and hot) and in each period 90 samples were analyzed for each ingredient constituting a total of 810 samples for each ingredient and analysis was done using Enzyme Linked Immuno-sorbent assay (ELISA). It was noticed that the incidence of AFB1 was highest in all ingredients during rainy season (Jul-Oct) followed by that found in Mar-Jun. However, the mean levels of AFB1 were minimum during Nov-Feb (winter) in all ingredients except sun flower meal and cotton seed cake which showed high prevalence of AFB1 throughout the study. Similarly, minimum numbers of samples were below maximum tolerable level (MTL) as recommended by FDA i.e. 20 ppb during Jul-Oct while maximum numbers of samples were below MTL during Nov-Feb resulting in lower mean levels of AFB1 during these periods as compared to rainy and dry hot seasons. This is the first most elaborative study regarding the levels of AFB1 in poultry feed ingredients collected from Lahore, Pakistan showing a huge number of samples for each ingredient.

Isolation and Molecular Identification of Infectious Bursal Disease (IBD) Virus from Commercial Poultry: Effects of Field Isolate on Cell Mediated Immune Response and Serum Biochemical Parameters in Broilers

Zain ul Abidin, Aisha Khatoon, Tariq Mahmood Butt, Sajjad Hussain, Ayesha Kanwal, Sajjad Ali and Asma Aziz

Belonging to genus *Avibirnavirus* and family *Birnaviridae* infectious bursal disease virus (IBDV) is a double stranded RNA virus and it causes an acute highly infectious disease in poultry resulting in watery diarrhoea, anorexia, high morbidity and mortality and hemorrhagic lesions on breast and leg muscles leading to down grading of poultry meat. The present study was designed to isolate and molecularly identify the causative agent (IBDV) from a clinically suspected flock of infectious bursal disease and to check the effects of isolated virus on cell mediated immune response and serum biochemical parameters in broilers along with reference strain (IBDV-2512). Bursae were collected and subjected to trituration and supernatant when inoculated in 9-day old embryonated chicken eggs resulted in the death of all the embryos during first three blind passages. Every triturate produced a clear and distinct line of precipitation with IBDV-known antisera in agar gel precipitation test (AGPT). Serum samples collected at the time of occurrence of disease presented a low anti-IBDV titer which was between 1:2 and 1:8 as elucidated by indirect haemagglutination inhibition (IHA) test while serum samples of same flock collected 14 days after first sampling presented a drastic increase in anti-IHA-IBDV antibodies that was between 1:64 and 1:512. Reverse transcriptase polymerase chain reaction (RT-PCR) revealed a product of approximately 743bp of VP2 gene of IBDV for all three suspected samples along with the reference strain. Broiler birds of 3 weeks of age when injected with field isolated resulted in decreased lymphoproliferative response as elucidated by tuberculin test and serum biochemical parameters were also altered in field isolated injected birds and these alterations were more or less similar to that of birds injected with reference strain (IBDV-2512) suggesting high pathogenicity of isolated virus.

Ameliorative Effects of L-Carnitine and Vitamin E (A-Tocopherol) on Haematological and Serum Biochemical Parameters in White Leghorn Cockerels Given Ochratoxin a Contaminated Feed

Zain ul Abidin, Muhammad Zargham Khan, Aisha Khatoon,
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1. L-carnitine is a quaternary ammonium compound biologically synthesised from the amino acids methionine and lysine while vitamin E (α -tocopherol) is an important antioxidant. The objective of the present study was to evaluate the ameliorative effects of L-carnitine and vitamin E upon haematological and serum biochemical parameters in ochratoxin A intoxicated birds. **2.** Day-old White Leghorn cockerels were acclimatised for 2 d, divided in 12 groups with 20 birds in each group. From d 3 of age, they were given different combinations of ochratoxin A (1.0 and 2.0 mg/kg), L-carnitine (1 g/kg) and vitamin E (200 mg/kg) in feed. Haematological (erythrocyte count, leucocyte count, haemoglobin concentration and haematocrit percentage) and serum biochemical parameters (serum urea, creatinine, albumin, total proteins and alanine aminotransferase) were evaluated. **3.** Results confirmed that L-carnitine and vitamin E given alone or combined with 1.0 mg/kg ochratoxin A ameliorated toxin induced alterations in haematological and serum biochemical parameters. This amelioration, however, did not occur when ochratoxin of 2.0 mg/kg was given. **4.** L-carnitine and vitamin E in combination have the ability to ameliorate ochratoxin altered haematological and serum biochemical parameters. However, the optimum ratio of L-carnitine + vitamin E, to be used to assure such mitigation of ochratoxin A altered changes in haematological and serum biochemical parameters in cockerels, has yet to be determined. The combination used in this study was indeed sufficient to ameliorate the alterations induced by ochratoxin A up to 1.0 mg/kg feed.

Efficacy of Experimentally Prepared Oil-Based Newcastle Disease (ND) Vaccine (Mukteswar Strain) against Prevailing Virulent ND Virus in Punjab, Pakistan

Sajjad Ali, Zahid Hussain, Sajjad Hussain, Aisha Khatoon, Zain ul Abidin, Sobia Aamir and Abdul Whab Manzoor

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 28th 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 7th 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.

Heat Stress in Poultry and the Beneficial Effects of Ascorbic Acid (Vitamin C) Supplementation during Periods of Heat Stress: A Review

Zain ul Abidin and Aisha Khatoon

Pakistan is an agro-livestock based economy with a poultry share of 55% of the total agricultural GDP (21%). The environment in Pakistan remains hot and humid during July to August, which renders the survival of poultry critical during these months, as birds are more susceptible to changing environments than other domestic animals. Heat stress is a combination of high environmental temperature and humidity, hindering proper thermoregulatory processes. It diminishes immunity, feed intake, weight gain, egg production, number of chicks per hen, hatchability of fertile eggs, egg and carcass quality, mineral balance, and increases panting and mortality and affects semen quality and fertility in male birds. Ascorbic acid (vitamin C), a white crystalline compound (also known as L-ascorbic acid) is primarily synthesised in the chicken by the kidneys, however, during heat stress, endogenous ascorbic acid becomes insufficient to meet the bird's requirements. Vitamin C ameliorates heat stress induced problems such as poor immunity, feed intake, weight gain, oxidative stress, rectal and body temperature, fertility and semen quality, carcass weight and mortality in birds.

Controlling of Mycotoxicosis in Poultry Feed and Industry

Zain ul Abidin, Aisha Khatoon and Mahboob Alam Qureshi

Mycotoxins, the secondary metabolites of toxigenic fungi, are a group of chemically toxic compounds and up till now, about 300 different mycotoxins have been identified like aflatoxins, ochratoxins, zearalenone, trichothecenes, patulin, fumonisins, deoxynivalenol, nivalenol etc and among all these ochratoxins and aflatoxins are considered to be highly toxic in the poultry industry. According to World Health Organization (WHO) 25% of world's food crops while in Asia pacific region only, one third of the grains are significantly contaminated with these toxic and deleterious compounds. Due to high quantity of different grains being used in manufacturing poultry feed, this industry is at a high risk of adverse effects of mycotoxins.

**Determination of Aflatoxin B1 in Finished Poultry Feed Samples
Collected From Different Poultry Farms and
Markets of Lahore, Pakistan**

Zain ul Abidin, Aisha Khatoon, Mahboob Alam Qureshi
and Tariq Mahmood Butt

The present study was designed to determine the levels of aflatoxin B1 (AFB1) in the poultry finished feed samples collected from different poultry farms and local markets of Lahore, Pakistan. This study was conducted from July 2009 to June 2012 with each year divided into three periods i.e. July-October (hot and humid), November-February (winter) and March-June (moderate). During each period 80 samples were analyzed by competitive direct-Enzyme Linked Immuno-sorbent assay (CD-ELISA) constituting a total of 720 samples throughout the study. The levels of AFB1 in poultry feed samples were highest during rainy seasons (48.2 ± 20.0 , 51.6 ± 22.6 and 46.0 ± 19.8 $\mu\text{g}/\text{kg}$) followed by Mar-Jun (29.9 ± 10.4 , 27.2 ± 9.72 and 28.8 ± 13.1 $\mu\text{g}/\text{kg}$) and Nov-Feb (19.7 ± 6.30 , 16.3 ± 6.76 and 17.1 ± 6.20 $\mu\text{g}/\text{kg}$). The levels were below maximum tolerable levels (MTL) for poultry as recommended by US-Food and Drug Administration (FDA) i.e. $20 \mu\text{g}/\text{kg}$ during winter seasons only. The highest level during this study was $119.2 \mu\text{g}/\text{kg}$ in Jul-Oct (2010-11). Percentage of samples below MTL was minimum during rainy season and at the peak during winter season confirming a high production of AFB1 in stored feed during rainy season compared to other seasons. Poultry feed becomes highly contaminated with AFB1 during rainy season due to high humidity and hot atmosphere which gives best favorable conditions for the growth of different storage fungi. This is the first most extensive study of levels of AFB1 from poultry finished feed samples collected from different areas of Lahore (Pakistan)

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