ANNUAL REPORT
2015-2016

VETERINARY RESEARCH INSTITUTE
ZARAR SHAHEED ROAD,
LAHORE CANTT.


VETERINARY RESEARCH INSTITUTE
LAHORE

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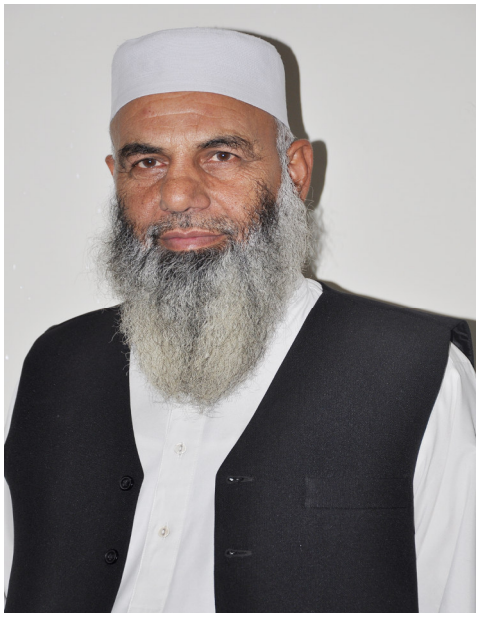

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Veterinary Research Institute, Lahore is the premier research organization in the country administratively controlled by Government of the Punjab. The institute was established in 1963 and is located on Zarar Shaheed Road, Lahore Cantt with a complex of hi-tech laboratories and animal houses extended over a vast area.

The nature of the work in Veterinary Research Institute, Lahore is basically a combination of biologics production and applied research. The institute has contributed significantly towards prevention and control of prevailing, newly emerging and reemerging diseases of livestock & poultry. Thus it has created conditions conducive for the expansion of poultry and livestock industries in the country. The activities during the year 2015-16 also remained significant in every aspect of function and development.

MOTTO

"Prevention is better than cure"

OBJECTIVES

a. Large scale production of quality biologics for control of infectious diseases of livestock and poultry.
b. Research studies in the related disciplines of animal health and biologics being produced in VRI.
c. Development of modern techniques for production of new biologics and to improve the quality and quantity of vaccines being produced at VRI.
d. Training of field in-service veterinarians, post-graduate students, graduate internees from Veterinary / other universities of all over the Punjab.

Achievements for 2015-16

- Production of vaccines enhanced multiple times to provide prophylaxis against major infectious diseases in Livestock and rural Poultry.
- Lyophilizer installed to enhance the production of PPR vaccine.
- Enhanced the production of diluents for reconstitution of lyophilized vaccines in field formation.
- Production of ND Lasota Vaccine initiated for field supply.
- Molecular Laboratory started working.
- Poultry unit at VRI revived to provide embryonated eggs for production of Newcastle Disease and Bird Flu vaccines.
- Filling and labeling of *Haemorrhagic septicemia* Vaccine (HSV), Black Quarter Vaccine (BQV) and Enterotoxaemia Vaccine (ETV) shifted from manual to automation.
- ISO-9001-2008 certification of Quality Control Laboratory of VRI.

STAFF

Sr. No.	Designation	BPS	Nos.
a. NON-DEVELOPMENT STAFF			
1.	Principal Veterinary Officer (Director)	20	1
2.	Additional Principal Veterinary Officer (SRO/RO)	19	12
3.	Senior Veterinary Officer	18	27
4.	Veterinary Officer (O/I Stores / curator)	17	42
5.	Bio-Chemist	18	1
6.	Assistant Research Officer (Bio-Chemist)	17	1
7.	Statistical Officer	17	1
8.	Ministerial Staff	1-16	54
9.	Para-technical staff	1-16	260
b. SNE STAFF			
1	Ministerial Staff	1-16	07

STAFF STRENGTH

Sr. No.	BS	Nos.
1.	17 to above	57
2.	01 to 16	257

RECRUITMENT MADE DURING THE PERIOD FROM 01.07.2015 TO 30.06.2016

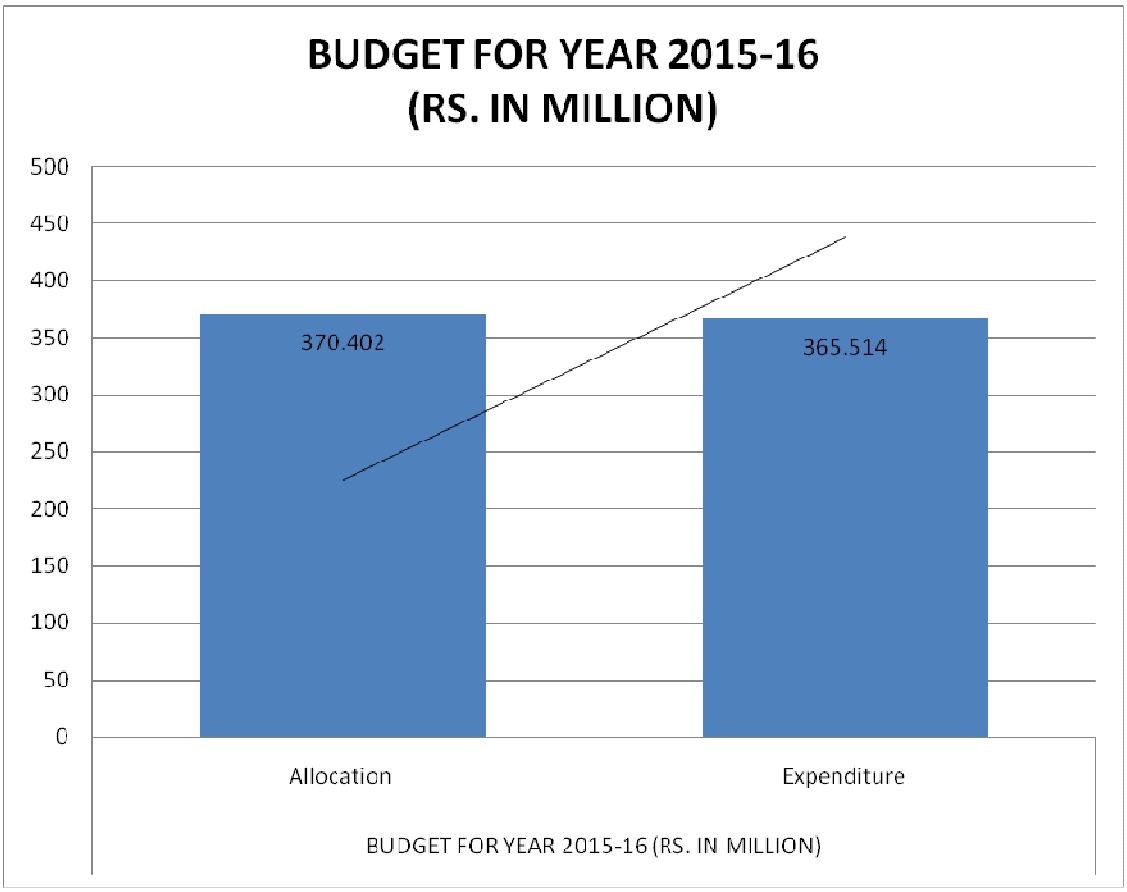
Sr. No.	BS	No. of Recruitment
1.	01 to 04	07
2.	05 to 16	13
3.	17 to Above	--

OFFICERS/OFFICIAL RETIRED FROM 01.07.2015 TO 30.06.2016

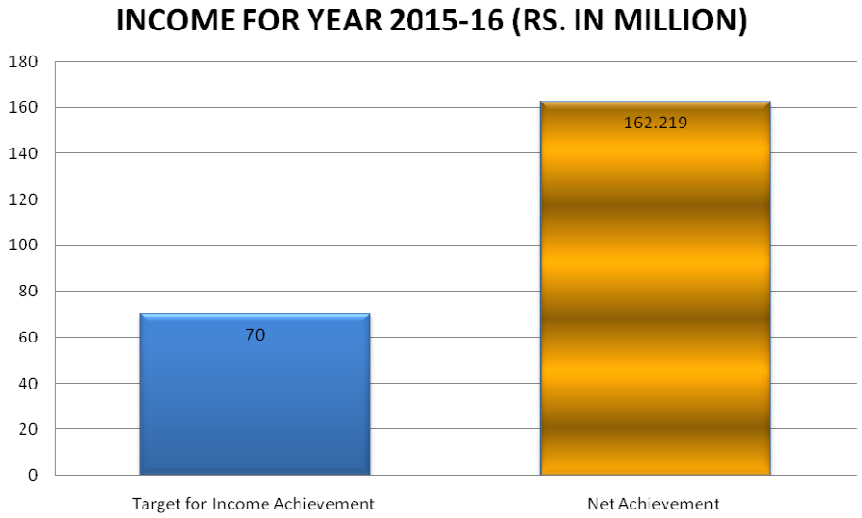
Sr. No.	BS	No. of Retirement
1.	01 to 04	03
2.	05 to 16	04
3.	17 to Above	04

BUDGET FOR YEAR 2015-16 (RS. IN MILLION)

Allocation	Expenditure
370.402	365.514



INCOME FOR YEAR 2015-16 (RS. IN MILLION)



Biologics Production Division

Biological production division is a complex of 13 sections, poultry unit and mice colony producing 20 vaccines and 06 diagnostics for livestock & poultry.

Functions

- Production of biologics.
- Maintenance and characterization of seeds for production of biologics.
- Preparation and sterilization of different types of media and solutions for production of biologics, propagation & characterization of seeds and quality testing of biologics.
- Maintenance and propagation of cell lines (For cell culture vaccines).
- To maintain poultry flocks to supply embryonated eggs for production of NDV & influenza vaccines.
- In- house quality control testing of each and every batch of biologics
- Training of undergraduate internees on microbiological techniques and production of biologics.
- To provide assistances.
- Assist in diagnosis of diseases like pox, rabies, mycoplasmosis, PPR, brucellosis, clostridial diseases.
- Provision of assistance and supervision to post graduate students in their research
- Dissemination of knowledge on prevention and control of infectious diseases of livestock and poultry through radio talks and print media
- Allied Research

Activities of Biologics Production Division

1	Media, Reagents & solutions produced	2, 08,417Liters
2.	Diluent Produced	1,46,40,000 Doses
3	No. of lab animals maintained & produced	3470
4	No. of poultry birds maintained	949
5	No. of Vaccine Doses Lyophilized	81509200
6	No. of Seed Vials Lyophilized	1220
7	Biologics produced*	172409820 doses

Types of Biologics Produced* for Livestock & Poultry

Types of Biologics	Livestock	Poultry	Total
Bacterial Vaccines	6	0	6
Viral Vaccines	3	11	14
Diagnostic Agents	6	0	6
Total	**15**	**11**	**26**

Quality Control Section

Functions

- To test the biologics (Vaccine/sera/antigen) being produced in VRI.
- To check nutritive value of feed and level of aflatoxin in them.
- To implement the standard of ISO-9001-2008 and ISO-17025 in lab management system and lab testing.

Services provided by the section

- Quality control section is testing biologics (Vaccines, diagnostic antigens and anti sera) prepared at VRI.
- Feed testing for nutritional value as well as toxins level.
- To conduct training of students and in service personals.

Activities of Quality Control Lab

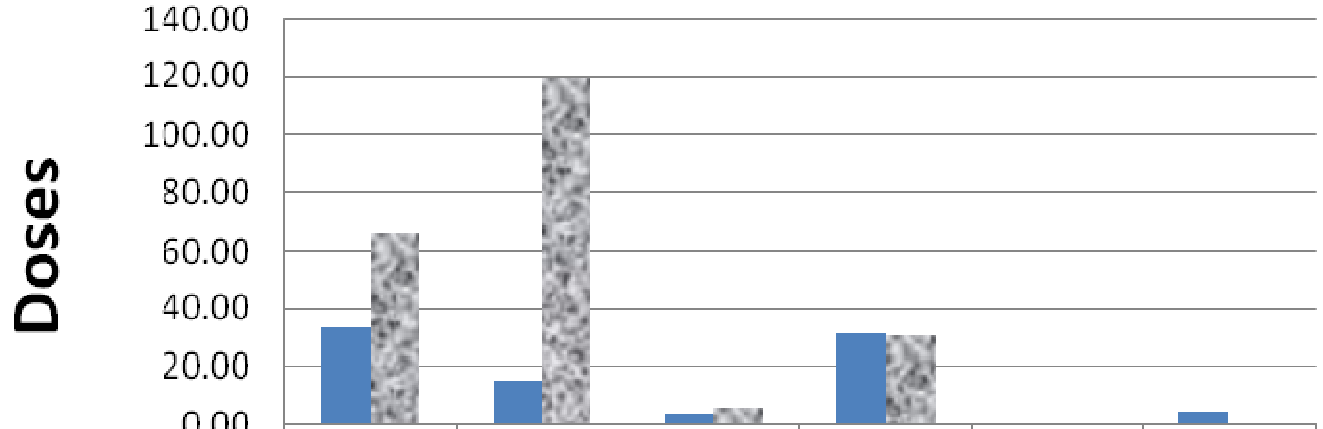
Biologics, Diluent and NDV Anti-bodies Titer Tested for Quality Control

Sr. No.	Vaccine	No. of Batch tested	Test performed
1	H.S Vaccine	229	Sterility test, safety test
2	B.Q.V	19	Sterility test, safety test
3	E.T.V	50	Sterility test, safety test
4	Anthrax Vaccine	04	Spore count, Sterility test, safety test
5	C.C.P.P Vaccine	12	Sterility test, safety test
6	Sheep pox	08	Sterility test, safety test
7	Goat pox	02	Sterility test, safety test
8	P.P.R Vaccine	36	Sterility test, safety test
9	Avian Influenza+ND	29	Sterility test, safety test
10	N.D.V	130	Sterility test, safety test, EID_{50}/ELD_{50}
11	FMD Serum	29	Sterility test, safety test
12	Mallien	01	Sterility test, safety test
13	Tuberculin	05	Sterility test, safety test
14	Brucella Antigens	05	Spot Test
15	Diluent	99	Sterility test
16	NDV Antibodies titer	1660	HIT

TESTS REPORT OF ANIMAL NUTRITION LABORATORY OF QCL VRI LAHORE FOR THE YEAR 2015-16

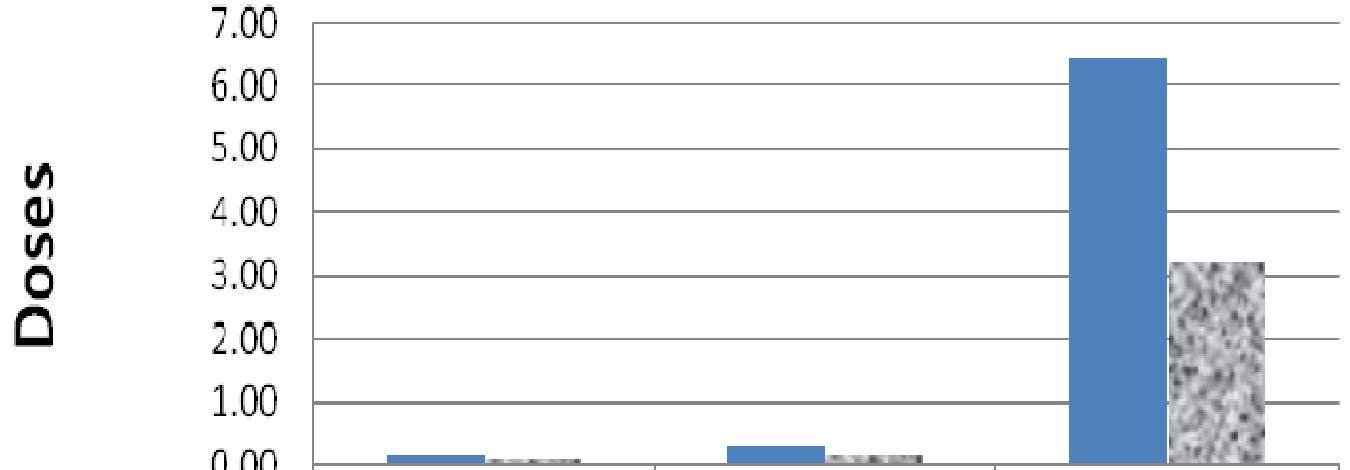
Kind of test performed and other Number	**No. of feed/fodder & miscellaneous samples tested**
Milk Power Adulteration	102
Whey Powder Adulteration	102
Total Aflatoxin level	459
Aflatoxin –MI	9
C.P%	717
Moisture	377
pH	173
Aroma	53
Colour	53
Na	316
K	316
Ca	316
P	316
Nittates	16
Purity Test	8
Grand Total of Test	3333

Bacterial Vaccine Production in 2015-16 and their worth (Rs in Millions)



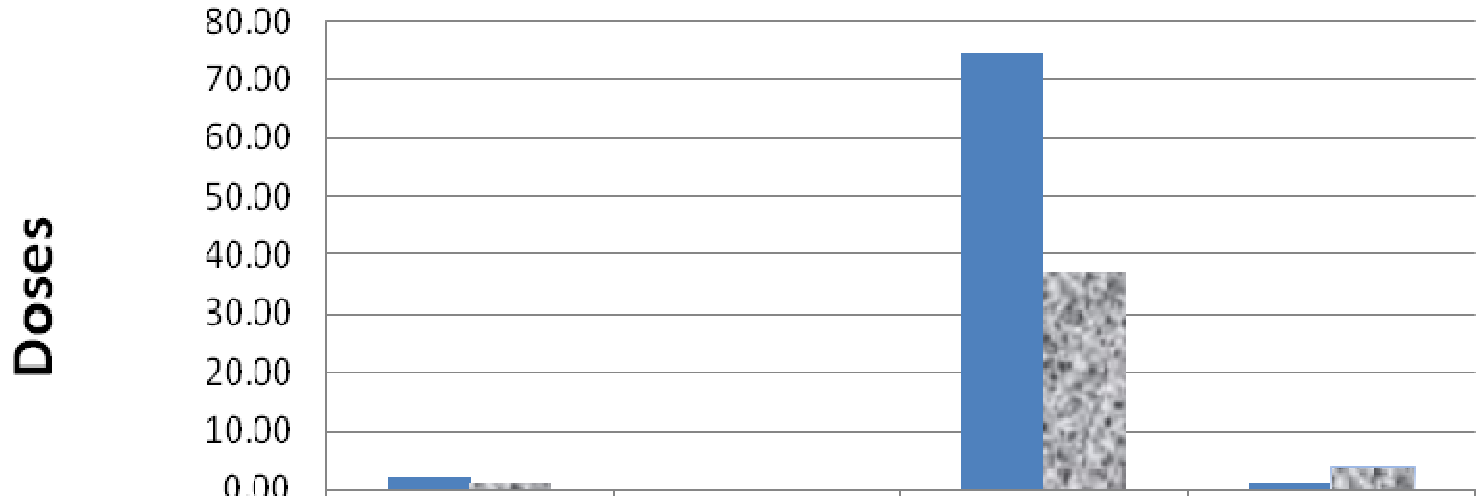
	H.S Vaccine	H.S Vaccine (O.B)	B.Q.V	E.T.V	ASV	C.C.P.V
Production (doses in million)	33.17	14.95	3.86	31.03	0.31	4.50
Values (Rs. In million)	66.33	119.59	5.79	31.03	0.94	0.68

Viral Vaccines for Livestock Produced in 2015-16 and Values (Rs. In Million)

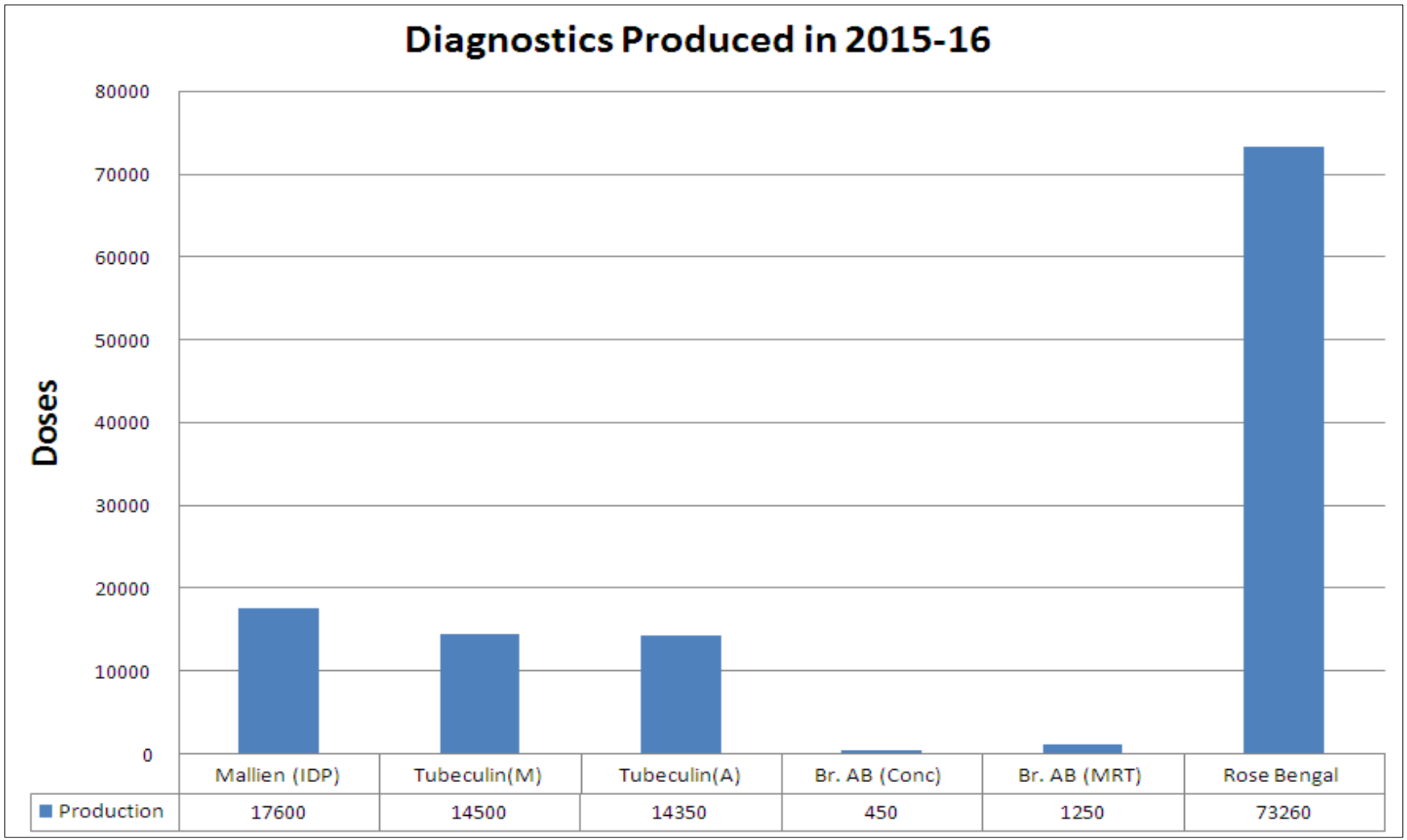


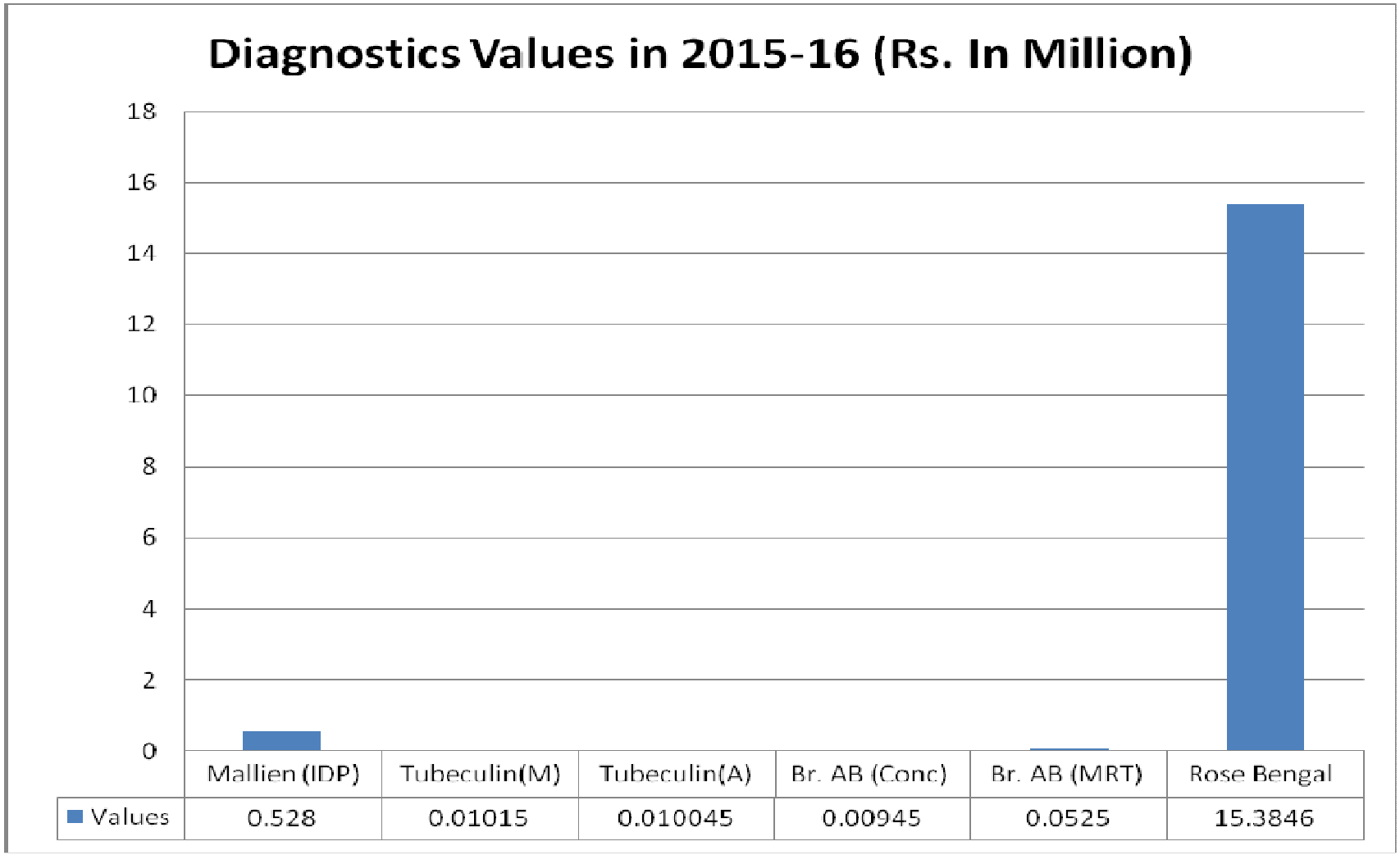
	Sheep Pox	Goat Pox	P.P.R.V
Production (doses in million)	0.19	0.33	6.43
Values (Rs. In million)	0.10	0.16	3.22

Viral Vaccines for Poultry Produced in 2015-16 and Values (Rs. In Million)



	ND+H9	A.I.V (aq)	N.D.V	ND Flue(O.B)
Production (doses in million)	1.95	0.08	74.56	1.00
Values (Rs. In million)	1.23	0.14	37.28	3.76


Diagnostics Produced in 2015-16
Doses
80000
70000
60000
50000
40000
30000
20000
10000
0
Mallien (IDP)
Tubeculin(M)
Tubeculin(A)
Br. AB (Conc)
Br. AB (MRT)
Rose Bengal
Production
17600
14500
14350
450
1250
73260


Diagnostics Values in 2015-16 (Rs. In Million)
18
16
14
12
10
8
6
4
2
0
Mallien (IDP)
Tubeculin(M)
Tubeculin(A)
Br. AB (Conc)
Br. AB (MRT)
Rose Bengal
Values
0.528
0.01015
0.010045
0.00945
0.0525
15.3846

TRAINING

Capacity building of internee / House Job doctors of different universities / veterinary colleges from Punjab.

Sr. No.	University / Institute	Prog.	No. trainees	Date	
				To	From
1.	The University of Agriculture Faisalabad (UAF)	DVM	16	26.01.2016	18.02.2016
2.	University of Veterinary and Animal Sciences, Lahore	DVM	02	25.01.2016	24.05.2016
3.	Bahauddin Zakariya University, Multan	DVM	08	08.02.2016	06.06.2016
4.	Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi	DVM	06	15.02.2016	14.04.2016
5.	Bahauddin Zakariya University, Multan	DVM	04	14.03.2016	23.03.2016
6.	Gomal University Dera Ismail Khan	DVM	01	15.05.2016	15.06.2016
7.	The University of Agriculture Faisalabad (UAF)	DVM	04	04.04.2016	28.05.2016
8.	Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi	DVM	04	15.04.2016	18.06.2016
9.	Gomal University Dera Ismail Khan	DVM	02	16.06.2016	15.07.2016

EXECUTIVE SUMMARIES OF RESEARCH ACTIVITIES UNDERTAKEN DURING YEAR 2014-15 AND 2015-16

ANTHRAX

GENETIC CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF BACILLUS ANTHRACIS STERNE STRAIN BY 16S rRNA GENE SEQUENCING

Anthrax, caused by Bacillus anthracis, is a fatal disease and all the warm blooded animals are susceptible to this disease. Many livestock populated areas in Pakistan are endemic for Anthrax (Thappa and Karthikeyan 2001), therefore the livestock population is annually vaccinated against Anthrax. Veterinary Research Institute (VRI) has been producing Anthrax Spore Vaccine (ASV) for a couple of decades. The current study was designed for molecular characterization of the microorganism used for vaccine production (Bacillus anthracis Sterne strain) and its phylogenetic relationship with related strains and Bacillus species. For this purpose, 16S rRNA gene was amplified and sequenced and was found 100% genetically similar to the reference sequence with respect to 16S rRNA gene. Phylogenetic analysis revealed that sequenced strain showed a close phylogenecity with Bacillus anthracis strain 1144 and Bacillus anthracis strain V77-NP-1R. Moreover, the organism was found genetically closer to Bacillus cereus than to Bacillus mycoides and Bacillus thuringiensis with respect to 16S rRNA gene sequence.

BIRD FLU

EMERGING THREAT OF H9N2 VIRUSES IN POULTRY OF PAKISTAN AND VACCINATION STRATEGY

In Pakistan, H9N2 avian influenza viruses were first reported in 1998, and since then, they have been prevalent in chickens and have continuously evolved through reassortment in live bird markets. Poultry carrying H9N2 viruses act as incubators for the evolution of influenza viruses in wild birds. Recent reports have shown that the Pakistani H9N2 viruses have undergone antigenic drift and evolved into novel genotypes posing a potential threat to poultry and human health having genome segments similar to H5N1 and H7N3 viruses. Recent evidence of interspecies transmission suggested that the H9N2 avian virus could be the next human

pandemic strain. Continuous monitoring of viral evolution and updates on vaccines are warranted to achieve efficient control and eradication of H9N2 viruses in Pakistan. The following review covers the emergence and evolution of H9N2 viruses and vaccination strategy in Pakistan.

BRUCELLA ABORTUS

EPIDEMIOLOGY OF BOVINE BRUCELLOSIS- A REVIEW OF LITERATURE

Brucellosis is mainly caused by *Brucella abortus* in bovines which results in great effect on economy, reduced milk production, abortions in last trimester and long calving interval. In Pakistan incidence is increasing day by day due to unawareness. Brucellosis is also a greater Zoonotic risk for human being, especially for veterinarians. It is diagnosed by different tests e.g. Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and ELISA. Brucella is also considered a strong bioterrorist. Brucellosis is controlled by medication and vaccination. RB51 vaccine is used. Now a day's DNA vaccines are used. Brucellosis eradication program is needed in Pakistan with the help of government, international organizations like OIE, FAO to prevent the spreading of the disease.

BUFFALO POX

ISOLATION OF BUFFALO POXVIRUS FROM CLINICAL CASE AND VARIATIONS IN THE GENETICS OF THE B5R GENE OVER FIFTY PASSAGES

Outbreaks of buffalopox affect udder and teats, which may ultimately lead to mastitis in dairy buffalo and can significantly compromise the production. In this study, we report isolation of buffalo poxvirus and sequence analysis of the B5R gene collected from the buffalo clinically suspected to be poxvirus infected. The virus was isolated on BHK-21 cell line and was passaged for 50 times, B5R gene was amplified and sequenced using gene-specific primers, and analyzed at both nucleotide and amino acid levels. Phylogenetically, the isolate can be classified close to the previously reported Pakistani and Indian isolates with certain level of differential clustering patterns. Three significant putative mutations (I2K, N64D, and K111E) were observed in the B5R protein. The K111E was common with previous human isolate from Karachi, Pakistan in 2005. These mutations differed from poxviruses reported from the neighboring countries. Some deletion mutations were observed which were

recovered in upcoming passages. The K111E mutation suggests potential to cause zoonotic infection in human all over the country.

FOOT AND MOUTH DISEASE

RAISING OF HYPER IMMUNE SERUM AGAINST FOOT AND MOUTH DISEASE VIRUS TYPE "O" PREVAILING IN PUNJAB, PAKISTAN.

Foot and mouth disease (FMD) is supposed to be an imperative disease of domestic and wild ruminants which is a vast reason of high mortality in young animals and production losses in adults. The supreme prevailing stains of FMD in Asia are "O", "A "and "Asia-I", which are supposed to be a big threat to economy and commonly not properly diagnosed. For appropriate diagnoses, hyper-immune serum is required. A study was conducted to produce hyper-immune serum in rabbits which were divided into three groups including Group-I, Group-II and a control group. First two groups were weekly inoculated with FMD virus Serotype "O" for six weeks and confirmation of the infection was done with the help of compliment fixation test (CFT), while antibody titer was measured by using ager gel precipitation test (AGPT). Group-II consisting of female rabbits showed earlier and higher titer ($Log2^7$) than group-I (Male rabbits) having lower titer ($Log2^5$).The study recommended the use of female rabbits to raise hyper-immune serum to attain higher titer.

HAEMORRHAGIC SEPTICEMIA

COMPARISON OF IMMUNOGENIC EFFECT OF THREE OIL ADJUVANT VACCINES AGAINST HAEMORRHAGIC SEPTICAEMIA IN CATTLE AND BUFFALO

Haemorrhagic septicaemia caused by *Pasteurella multocida* is one of the most common, fatal and acute bacterial disease of livestock with more than 70% mortality. The only satisfactory and practical method to control and prevention is vaccination of all the healthy and in contact animals. Different types of vaccine are being used for the immunity against this disease. In this project three oil adjuvant vaccines were produced. Two single emulsion vaccines were prepared by utilizing Montanide ISA-50 and liquid paraffin with lanolin were as one double emulsion with the help of Montanide ISA-206 was prepared. In house quality control testing and safety testing were performed on Swiss albino mice. To check immune titer IHA was performed by collecting serum from each and every animal including control animals.

The comparison of IHA was done via statistical analysis using GMT, Single emulsion vaccine prepared from liquid paraffin with lanolin gave maximum immune titer out of all the three vaccines in large animals and in young claves ISA 206 gave a significant titer.

INFECTIOUS BURSAL DISEASE

IDENTIFICATION OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) THROUGH AGAR GEL IMMUNODIFFUSION (AGID) TEST AND ITS PURIFICATION BY ULTRACENTRIFUGATION

Infectious bursal disease (IBD) is a highly significant immunosuppressive disease of young chickens caused by infectious bursal disease virus (IBDV). Infected bursae were collected from field outbreaks of IBD at Lahore, Pakistan. A 10% W/V suspension was made in the phosphate buffer saline and centrifuged at 5000 rpm for 20 min. The presence of IBDV in the supernatants of suspect homogenates were checked through agar gel immuno-diffusion test. The collected material was given serial passage in 10 days old embryonated eggs at an inoculation rate of 0.2 ml/egg through chorio-allantoic membrane (CAM) route. The material collected at different passages was centrifuged twice at 2000 rpm for 45 min to remove tissue debris. The clear supernatant was subjected to ultracentrifugation at 49500 x 9 for 6 h at *4°C.* The purified ISO virus was obtained in pellet form and these pellets were disrupted in TNE buffer to be stored at -70°C till further use. The study suggested that the bursa of fabricius is the best tissue source for the isolation of virus.

MILD FORM OF PESTE DES PETITIS RUMINANTS VIRUS (PPRV) IN PAKISTAN

An outbreak of Peste Des Petitis Ruminants (PPR) in suburban area of Rawalpindi District of Punjab province Pakistan has been investigated. A total of 38 clinically affected animals out of 140 goats and sheep 10 - 18 months old with no history of PPR vaccination were found harboring PPR virus. Nasal and ocular swabs were analyzed by RT-PCR for the presence of PPRV specific genome and their sera were analyzed for PPR antibodies by competitive ELISA. Eight out of 10 swab samples were found positive for PPRV and all sera were positive for PPRV specific antibodies. It is thus speculated that a comparatively mild strain of PPR virus exists in the population.

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

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 diarrhea, 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.

MYCOPLASMA

SERO-PREVALENCE OF *MYCOPLASMA CAPRICOLUM* SUBSP. *CAPRIPNEUMONIAE* IN GOATS THROUGH cELISA IN DIFFERENT DISTRICTS OF PUNJAB, PAKISTAN

A cross-sectional study was conducted to determine the sero-prevalence of CCPP, in five districts of Punjab that are Okara, Faisalabad, Lahore, Bahawalpur and Pakpatan and three Govt. livestock research institutes which are Research and Development Center, Rakh Khare Wala (District Layyah), Barani Livestock Production Research Institute, Kherimorat (District Attock) and Livestock Production Research Institute, Bahadurnagar, Okara. A total of 364 serum samples were collected from July, 2012 to July, 2013, from clinically respiratory distressed and unvaccinated goats of different breeds, age and sex. Samples were subjected to monoclonal antibody-based competitive enzyme-linked immunosorbent assay (cELISA) for the specific measurement of antibodies to *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) bacterium. Thirty one out of 364 samples were positive by cELISA indicating overall sero-prevalence of CCPP as 8.52 %. Statistically the proportional prevalence of CCPP in male and female Beetal goats was significantly higher in Faisalabad district and also at Research and Development Center, Rakh Khare Wala (District Layyah) rather than other districts and research centers. The findings of this survey revealed the evidence of goat exposure to Mccp in different districts and research centers in Punjab province, although at a low prevalence. This sero-survey was conducted for the first time in Pakistan by applying the latest cELISA technique, urging the need of control of this economically important disease for resource poor livestock keepers in Pakistan.

NEW CASTLE DISEASE

IMMUNE STATUS AGAINST NEWCASTLE DISEASE VIRUS IN BACKYARD CHICKENS OF PUNJAB PROVINCE, PAKISTAN

Newcastle disease, one of the most important health problems of commercial and backyard poultry. It is producing significant economic lose via severe fatalities globally. A serum base investigation regards the anti-NDV antibody occurrence in different seasons of a year was carried out to document the current immune status of

unvaccinated backyard poultry. A sum of 1468 sera samples were collected from 32 various districts of Punjab, Pakistan. Using Haemagglutination inhibition (HI) test, high sero prevalence (92%) has been discovered in the Sargodha district, whereas Mianwali showed low sero prevalence (15.25%). Overall sero positivity (58.78%) has been observed between $4\log^2$ and $9\log^2$ antibody titer with 16.59 GMT. Based on different age groups, >65 weeks of age birds showed previous natural exposure to ND virus with higher geometric mean titer value of 20.89 as compared to other groups. Furthermore, the presence of serum based antibodies of NDV were found higher in summer season (64.50% by 21.38 GMT) but lost in winter season at 50% by 15.13 GMT. Hence, the overall high existence of antibodies against NDV were observed with 16.59 GMT. So, need of regular surveillance is of utmost important to explore the genetic nature of said virus because of the significant roles of backyard poultry in viral transmission. But in Pakistan, the epizootiological information of NDV in backyard poultry is scarce.

EFFECT OF LENTOGENIC NEWCASTLE DISEASE VIRUS (LASOTA) ON LOW PATHOGENIC AVIAN INFLUENZA VIRUS (H9N2) INFECTION IN FAYOUMI CHICKEN

Low pathogenicity avian influenza virus (LPAIV) and lentogenic Newcastle disease virus (NDV) are two of the most economically important viruses affecting poultry worldwide. Co-infections usually occur but cannot be easily diagnosed due to confusing similar clinical signs. Fayoumi is indigenous chicken of Pakistan on which the impact of co-infections is still unknown. The objective of this study was to investigate the effect of *LaNDV* on the infectivity and excretion of LPAIV in fayoumi chicken. Four week old fayoumi chicks were inoculated intranasal with 106 median embryo infectious of *LaNDV* vaccine strain (LaSota) and a H9N2 LPAIV (A/Chicken/Pakistan/UDL/08 H9N2) simultaneously. No clinical signs were observed in chickens infected with the *LaNDV*. All chicken showed mild to moderate respiratory distress with LPAIV alone or in combination with *LaNDV*. Clinical and necropsy findings revealed non synergistic behaviour of two viruses for the development of clinical signs and lesions. However, the pattern of virus shedding was different with co-infected chickens, which excreted lower titers of *LaNDV* and LPAIV at first three days post inoculation (dpi) as compared to singly inoculated chicken but after 3 dpi co-infection resulted in significantly higher number of

oropharyngeal and cloacal swabs detected positive for LPAIV and lower number for *LaNDV*. The knowledge obtained from the study serves the dual purpose of shedding light on the different replication behaviors of LPAIV in early days of experiment which may be due to competition for receptor binding with *LaNDV*, as well as the more pathogenic behavior of LPAIV (H9N2) in fayoumi chickens of Pakistan.

EFFECT OF MULTISTRAIN PROBIOTIC ON IMMUNE RESPONSE AND GROWTH OF BROILERS VACCINATED AGAINST NEWCASTLE DISEASE

The objective of the study was to investigate the effect of a probiotic on growth and immune response of the broiler chicks vaccinated against Newcastle disease. Parameters of investigation were weight gain, feed conversion ratio (FCR), live to dressed body weight ratio; weight of various lymphoid organs i.e. Bursa of Fabricius, thymus and spleen; immune response of the broilers. The findings were compared with the cyclophosphamide (cyc.) treated Newcastle disease virus (NDV)-vaccinated; untreated and NDV-vaccinated; and the unvaccinated untreated control chicks. The probiotic treated chicks showed higher mean body weights, better FCR, higher NDV HI antibody titers, lesser overall mortality, no NDV post challenge mortality and no detrimental effects on their lymphoid organs, compared to the cyclophosphamide treated and untreated chicks. Probiotic had good effects on growth and immune response of the broilers.

EFFICACY OF EXPERIMENTALLY PREPARED OIL-BASED NEWCASTLE DISEASE (ND) VACCINE (MUKTESWAR STRAIN) AGAINST PREVAILING VIRULENT ND VIRUS IN PUNJAB, PAKISTAN

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.

PESTE DES PETITIS RUMINANTS (PPR)

DETECTION OF PESTE DES PETITIS RUMINANTS VIRAL RNA IN FECAL SAMPLES OF GOATS AFTER AN OUTBREAK IN PUNJAB PROVINCE OF PAKISTAN: A LONGITUDINAL STUDY

Peste des petitis ruminants (PPR) is a highly contagious viral disease of domestic and wild small ruminants and thus has serious socioeconomic implications. In Pakistan, during the year 2012-2013, estimated losses due to PPR were worth Rs. 31.51 billions. Close contact between infected and susceptible animals is an important route of transmission of PPR. Therefore, carrier animals play an important role in unnoticed transmission of PPR. The objective of the study was to investigate the detection of PPR virus in goats recovered from PPR. A suspected PPR outbreak was investigated and confirmed as PPR after analyzing appropriate samples collected from infected animals using rRT-PCR. A longitudinal study was conducted over the period of 16 weeks to ascertain the detection of PPR virus (PPRV) in fecal samples of recovered goats. Ninety-six (96) fecal samples from each sampling were collected at 4, 8, 12, and 16 weeks after the outbreak. Fecal samples were analysed using rRT-PCR. Of 96 from each sampling a total of 46, 37, 29, and 25 samples were positive for PPR viral genome at 4, 8, 12, and 16 weeks, respectively, after recovery. Attempts were made for the isolation of PPR virus on Vero cells, but results were negative. These results indicated the detection of PPR viral RNA up to 16 weeks after infection. Therefore, these results may help in the future epidemiology of PPR virus shedding and possible role as source of silent infection for healthy animals especially when there is no history of any outbreak in nearby flock or area.

TIMELINE FOR THE DEVELOPMENT OF COMPARATIVE CLINICAL DISEASE FOLLOWING EXPERIMENTAL INFECTION WITH LINEAGE-IV PESTE DES PETITIS RUMINANTS VIRUS IN GOATS

The virulence of a local isolate of Peste Des Petitis Ruminants (PPR) virus belonging to lineage 4 was studied in susceptible goats following experimental challenge by subcutaneous, intra-nasal and direct contact with experimentally infected animals. All experimentally infected animals developed disease and died in 12 to 22 days post challenge. Timeline for the development of various clinical signs is reported. The RT-PCR detected the PPR virus genome from the nasal and ocular swabs during the febrile phase of the disease from day 4 to day 9 of experimental challenge. The PPR virus was also isolated from the lymph nodes of one animal challenged subcutaneously after a single blind passage on Vero cells. The virus was not isolated from lungs or spleen of these animals. Though the swab samples from all the exposed animals tested positive by RT-PCR, PPR virus was not isolated on Vero cells from any tissue sample of exposed animals collected at necropsy. PPR virus exposed goats started developing specific antibodies 2 to 3 days post inoculation. The antibody titers continued to rise and were diagnostically positive (> 50% Percent Inhibition values) by day 7 post challenge. These findings can be used to develop effective PPR control strategies during the outbreaks.

EPIDEMIOLOGICAL ANALYSIS OF PESTE DES PETITIS RUMINANTS (PPR) OUTBREAKS IN PAKISTAN

The current study reports the outbreaks of Peste des Petitis Ruminants (PPR) in the small ruminant population of Pakistan. The objectives were to understand the clinical picture of disease under field conditions, estimate the basic epidemiological parameters for the local population of small ruminants and to determine the spatial and temporal distribution of PPR during 2005 to 2007 in Pakistan. A total of 62 outbreaks were investigated among sheep and goat flocks in the five provinces of Pakistan and Azad Jammu & Kashmir (AJK). The PPR virus activity in these outbreaks was demonstrated by clinical picture and presence of PPR virus specific antibodies by employing cELISA. The combined estimates of mean cumulative morbidity and mortality for sheep and goats were estimated 65.37% and 26.51% respectively with a case fatality of 40.40%. The species specific mean cumulative

morbidity, mortality and case fatality for goats were 68.80%, 29.45% and 42.75% respectively, while these estimates for sheep were 48.77%, 14.98% and of 26.16% respectively. These estimates for goats were significantly higher ($P < 0.001$ to $P = 0.001$) than those for sheep. It was concluded that PPR is wide spread throughout the country and epidemiological picture suggest that disease has established as an endemic infection in the country.

PESTE DES PETITIS RUMINANTS VACCINE (NIGERIAN STRAIN 75/1) CONFERS PROTECTION FOR AT LEAST 3 YEARS IN SHEEP AND GOATS

The present study reports the duration of immunity and protective efficacy of Peste des Petitis Ruminants (PPR) vaccine (Nigerian strain 75/1) in sheep and goats. A total of 105 sheep and goats were divided into three groups A, B and C. Group A received normal recommended dose (1.0 ml) of PPR vaccine, group B received half dose (0.5 ml) of PPR vaccine and group C was kept as unvaccinated control group in contact with vaccinated animals. The post vaccination dynamics of antibodies against PPR virus was studied. It was found that significant antibody titers persisted for 3 years post vaccination in sheep and goats vaccinated with either full dose or half dose of PPR vaccine. The challenge protection studies were carried out in experimental animals at 24 and 36 month post vaccination. The vaccinates withstood challenge and were found completely resistant clinically and virologically to virulent PPR virus for 24 and 36 months post vaccination. The unvaccinated control animals developed typical clinical signs of PPR and the challenged virus was detected in ocular, nasal and oral secretions of these animals. This study demonstrated that a single immunization with PPR vaccine conferred solid protection in sheep and goats for 3 years.

CLINICAL INVESTIGATION OF PESTE DES PETITIS RUMINANTS OUTBREAK IN SHEEP AND GOATS AT ISLAMABAD, PAKISTAN

Clinical and laboratory investigations were carried out during an outbreak of Peste des Petitis Ruminants (PPR) in sheep and goats in Islamabad Capital Territory (ICT), Pakistan. The overall morbidity in goats (27.95%) was higher as compared to sheep (10%). Goats experienced severe clinical disease while mild form of disease was observed in sheep. Eleven swab samples (ocular/nasal) from live animals and eight tissue samples (lung, liver, spleen, lymph nodes) from dead animals

were collected and analyzed by RT-PCR in the laboratory. All tissue samples while 5 of 11 swab samples were positive for PPR. History of the flock revealed that mix grazing and introduction of new animals might be important factors in introduction of disease in the flock

EVALUATION OF HAEMAGGLUTINATION ASSAY (HA) FOR THE DETECTION OF PESTE DES PETITIS RUMINANTS VIRUS (PPRV) IN FAECAL SAMPLES OF RECOVERED GOATS

This paper reports the findings of evaluation of Haemagglutination Assay (HA) for detection of Peste des Petitis Ruminants (PPR) in fecal samples of sheep and goats persistently infected with PPR. Fecal samples (n=100) collected during an outbreak of PPR were subjected to HA and RT–PCR (gold standard). HA produced more positive results (77/100; 77%) as compared to RT–PCR (29/100; 29%). Kappa analysis indicated no agreement between HA and RT–PCR (kappa = –1.5159). In this study, we found that HA is a non–specific test for detection of PPR Virus (PPRV) in fecal samples of small ruminants, infected with PPRV. Therefore, other sensitive and specific laboratory test should be used for detection of PPRV in fecal samples of persistently infected animals.

ISOLATION AND CHARACTERIZATION OF LINEAGE-IV *PESTE DES PETITIS RUMINANTS* (PPR) VIRUS STRAINS FROM PAKISTAN

A total of 62 Peste des Petitis ruminants (PPR) outbreaks in sheep and goat flocks were investigated in Pakistan during 2005-2007. The presence of PPR virus (PPRV) was confirmed by clinical picture, necropsy examination, Ic-ELISA, virus isolation and RT-PCR. Of 397 tissue samples, 65% tested positive by Ic-ELISA. Six PPR virus isolates were obtained through cell culture on VERO or GKC cell from 61Ic-ELISA positive samples identified by characteristic CPEs and confirmed by testing the cell culture supernatant by Ic-ELISA and RT-PCR using PPRV specific F gene based primers. The sequence data of F gene from 6 isolates was analyzed for identities and a phylogenetic tree was generated based on 372bp F gene sequences of PPRV. The isolates were clustered into lineage 4 along with other Asian isolates. The recent isolates and a previous isolate from Pakistan (PAK-2004) were found to be monophyletic having close relationship with an Indian isolate (IND-PON).

ISOLATION OF PPR VIRUS FROM THE BLOOD SAMPLES COLLECTED DURING ACUTE PHASE OF INFECTION

Peste des Petitis Ruminants (PPR) is an acute and highly contagious viral disease of small ruminants mainly affecting goats and sheep. Early diagnosis of PPR is a prerequisite for controlling the mortality in an infected herd or scattered population of sheep or goats. During the study under report, two outbreaks of PPR occurring in sub-mountainous regions of Taxila village of Pakistan were investigated and a technique for PPRV isolation from blood samples collected during acute febrile phase of infection was standardized. From the suspected animals, samples of oculo - nasal swabs and blood were collected. In addition, tissue specimens including lymph node (LN), spleen and lungs were also collected upon postmortem examination of the dead animals. Using RT-PCR primers, F and N genes of PPR virus were visualized. During this study the PPR virus isolation was attempted from blood and tissue samples using Vero cells. This technique for isolation of PPR virus from blood of infected goats and sheep was standardized by inoculating sub-confluent monolayers of Vero cells with peripheral blood mononuclear cells (PBMC's) obtained after Ficoll Histopac density gradient fractionation of heparinized blood. The pelleted PBMC's were processed by re-suspending in various concentrations of Hanks' buffered saline solution (HBSS) before inoculation onto Vero cells. The inoculated cells showing no CPE's were blind passaged seven days post-inoculation (PI). A total of 03 PPR isolates were obtained from blood of three affected goats. Of these, 02 isolates were obtained after seven days PI on Vero cells and 01 isolate was obtained after a single blind passage in Vero cells. However, only 01 isolate, after three blind passages on cells, was obtained from the LN tissue samples. The isolates were identified using identical primers from PPR virus and compared in RT-PCR. This study reveals that chances of PPR virus recovery are higher from the blood collected during the febrile phase of PPR virus infection compared to virus isolation from the tissues collected upon postmortem examination. This is the first report on isolation of PPR virus from the goats kept in sub-mountainous region of Islamabad at the NARC Animal Health labs.

EVALUATION OF THERMAL STABILITY AND INACTIVATION OF PESTE DES PETITIS RUMINANTS VIRUS CULTIVATED IN CELL CULTURE

Peste des Petitis Ruminant (PPR) is a very contagious viral disease affecting large number of small ruminants predominantly goats and sheep. Vaccines are available for controlling this fatal disease but the efficiency of these vaccines depends upon the maintenance of cold chain in areas of extreme weather conditions. Uptil now, little is known about the stability of PPRV and its inactivation at different environmental conditions. In the current study, thermal stability of seven cell culture derived PPRV isolates under different environmental temperatures (370C, Room temperature and 40C) and the ability of heat and UVC light irradiation to inactivate PPRV were evaluated. To assess the thermal stability, aliquots of PPRV isolates incubated at 37^{o}C were removed every 3 hours while those incubated at RT or 4^{o}C were removed every day and every 2 days respectively. To evaluate the sensitivity of PPRV to extreme heat, aliquots were subjected to four increasing temperatures (56^{o}C, 60^{o}C, 62^{o}C and 65^{o}C) for 6 different time intervals. To investigate the effect of UVC light on PPRV infectivity, aliquots were subjected to UVC irradiation for 03 different time points. Tissue culture infective dose (TCID50) of all treated samples was calculated to determine the infectious titers using VERO cells. PPR in cell culture medium was found to survive 37°C for 2 days but due to hot climate of most countries, RT reach approx. 40-45°C in summer, all isolates inactivated within 2-3 days and the infectious titer was close to the detection limit. PPRV was relatively stable at 4^{o}C with no radical loss of infectivity for 3 weeks. PPR in cell culture medium was sensitive to heat and could be inactivated in 7 and 5 min when incubated at 600C and 650C respectively. However at 560C, 20 to 25 min was required to eliminate PPRV infectivity. UVC light irradiation effectively inactivated PPRV within 2-3 min. This study reveals that thermal stability of PPRV may be influenced by high temperatures, extreme heat and UVC irradiation and selecting the suitable heat resistant PPRV isolates may be a good approach to develop a thermostable vaccine for PPR

PERSISTENCE OF *PESTE DES PETITIS RUMINANTS VIRUS* (PPRV) IN GOATS AFTER AN OUTBREAK IN PUNJAB PROVINCE OF PAKISTAN; A LONGITUDINAL STUDY

SL-CARP International Research Symposium Colombo Sri Lanka 11-12 August 2014. Peste des Petitis Ruminants (PPR) is a highly contagious viral disease of domestic and wild small ruminants causing high morbidity and mortality (up to 100%) and thus have serious socioeconomic implications. In Pakistan, during the year 2012-13 estimated losses due to PPR were worth Rs. 31.51 billion. Close contact between infected and susceptible animal is an important route of transmission of PPR. Therefore, carrier animals play an important role in unnoticed transmission of PPR. However, limited information is available about the persistence of PPR virus in goats. The objective of the study was to investigate the persistence of PPR virus in goats recovered from PPR. A suspected PPR outbreak in sub urban area of Lahore district of Punjab Province was investigated. The outbreak was confirmed as PPR after analyzing appropriate samples (nasal/ocular swabs, fecal and tissue samples) collected from infected animals using RT-PCR. A longitudinal study was conducted over the period of 16 weeks to ascertain the persistence of PPRV in fecal samples of recovered goats (n=96) collected at 4, 8, 12 and 16 weeks after the outbreak. Samples were analyzed using real time-PCR. Of 96 goat's fecal samples from 46, 37, 29 and 7 goats remained positive for PPR viral genome at 4, 8, 12 and 16 weeks respectively after recovery. These results indicated the persistence of PPR virus in goats 16 weeks after recovery. Therefore, it can be concluded that PPR virus has a carrier potential and goats recovered from PPR infection may act as source of silent infection for healthy animals.

RABIES

SURVEILLANCE FOR PROBABLE DETECTION OF RABIES VIRUS IN WILD AND DOMESTIC ANIMALS

Rabies (lyssavirus) virus is an avertable viral disease caused by the rabid animals to the warm blooded animals. Presence and prevalence of rabies virus threatens population hygiene since spread cases often knock out in villages and cause substantial losses to human populations. Lyssavirus surveillance in wild animals including bats, dogs, cattle and mules was performed. Twenty Brain samples from

the suspected wild animals were processed by means of the fluorescent antibody test (FAT) and mouse inoculation test (MIT). The brain samples were retested through reverse transcriptase polymerase chain reaction (RT-PCR). In the study, suspected brain samples of the dogs, cattle and mules were found positive while all the insectivorous bats representing *Taphozous nudiventris, Scotophilus heathii, Scotoecus pellidus, Pipistrellus pipistrellus* and *Scotophilus kohlil* species were tested negative by all the three assays.

MISCELLANEOUS

SYNTHESIS OF PIROXICAM LOADED NOVEL ELECTROSPUN BIODEGRADABLE NANOCOMPOSITE SCAFFOLDS FOR PERIODONTAL REGENERATION

Development of biodegradable composites having the ability to suppress or eliminate the pathogenic micro-biota or modulate the inflammatory response has attracted great interest in order to limit/repair periodontal tissue destruction. The present report includes the development of non-steroidal anti-inflammatory drug encapsulated novel biodegradable chitosan (CS)/poly(vinyl alcohol) (PVA)/hydroxyapatite (HA) electro-spun (e-spun) composite nanofibrous mats and films and study of the effect of heat treatment on fibers and films morphology. It also describes comparative in-vitro drug release profiles from heat treated and control (non-heat treated) nanofibrous mats and films containing varying concentrations of piroxicam (PX). Electrospinning was used to obtain drug loaded ultrafine fibrous mats. The physical/chemical interactions were evaluated by Fourier Transform Infrared (FT-IR) spectroscopy. The morphology, structure and pore size of the materials were investigated by scanning electron microscopy (SEM). The thermal behavior of the materials was investigated by thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). Control (not heat treated) and heat treated e-spun fibers mats and films were tested for in vitro drug release studies at physiological pH 7.4 and initially, as per requirement burst release patterns were observed from both fibers and films and later sustained release profiles were noted. In vitro cytocompatibility was performed using VERO cell line of epithelial cells and all the synthesized materials were found to be non-cytotoxic. The current observations suggested that these materials are potential candidates for periodontal regeneration.

CHITOSAN-BASED ELECTROSPUN NANOFIBROUS MATS, HYDROGELS AND CAST FILMS: NOVEL ANTI-BACTERIAL WOUND DRESSING MATRICES

The development of highly efficient anti-bacterial wound dressings was carried out. For this purpose nanofibrous mats, hydrogels and films were synthesized from chitosan, poly(vinyl alcohol) and hydroxyapatite. The physical/chemical interactions of the synthesized materials were evaluated by FTIR. The morphology, structure; average diameter and pore size of the materials were investigated by scanning electron microscopy. The hydrogels showed a greater degree of swelling as compared to nanofibrous mats and films in phosphate buffer saline solution of pH 7.4. The in vitro drug release studies showed a burst release during the initial period of 4 h and then a sustained release profile was observed in the next upcoming 20 h. The lyophilized hydrogels showed a more slow release as compared to nanofibrous mats and films. Antibacterial potential of drug released solutions collected after 24 h of time interval was determined and all composite matrices showed good to moderate activity against Gram-positive and Gram-negative bacterial strains respectively. To determine the cytotoxicity, cell culture was performed for various cefixime loaded substrates by using neutral red dye uptake assay and all the matrices were found to be non-toxic.

ROLE OF WHEAT BASED DIET ON THE PATHOLOGY OF NECROTIC ENTERITIS IN TURKEYS

The study was conducted to investigate the effects of wheat based diet on the pathology of necrotic enteritis in turkeys. Turkeys were divided into four groups. Groups A and B were kept as noninoculated and fed normal commercial diet while groups C and D were challenged orally with C. perfringens and fed wheat based diet to promote the development of experimental disease. Infected turkeys showed clinical signs of depression, ruffled feathers, and dark yellowish feces showing the most prominent disease signs in turkeys of group D with 30% mortality. Similarly, turkeys of group D showed more striking gross and histopathologic lesions as compared to turkeys of group C. The most severe gross lesions comprised intestinal distension, small necrotic spots and haemorrhages on intestine, fragile intestinal wall, and gas bubble formation in the small intestine. Histologically, inoculated turkeys showed patchy necrosis, desquamation of intestinal epithelium, and intense leukocyte infiltration in the intestine. Microscopic examination showed significant

decrease in the height of intestinal villi of inoculated birds. Haematological studies showed significant influence of necrotic enteritis on the blood profile of turkeys in group D. The findings revealed that simultaneous feeding of wheat enhanced the pathology of necrotic enteritis in turkeys.

PROTECTIVE EFFECTS OF L-CARNITINE UPON TOXICOPATHOLOGICAL ALTERATIONS INDUCED BY OCHRATOXIN A IN WHITE LEGHORN COCKERELS

This study was designed to investigate the protective effects of L-carnitine (LC) against ochratoxin A (OTA) induced toxicopathological alterations in white Leghorn cockerels. Parameters studied included behavioral parameters, mortality, feed intake, body weight gain, relative organ weights and histopathological alterations. Results suggested that OTA induced suppression in behavioral parameters, feed intake, body weight gain, relative organ weights and histopathological alterations were progressively improved when LC was given with 1 mg/kg OTA; however, this protection subsided when 2 mg/kg OTA was given with it. The optimum level of LC required to produce such mitigation at higher OTA levels is yet to be determined; however, the level used in this study is quite sufficient enough to ameliorate toxicopathological alterations induced by OTA up to 1 mg/kg feed.

STUDY OF FUNGI AND THEIR TOXIGENIC POTENTIAL ISOLATED FROM WHEAT AND WHEAT BRAN

The present study was designed to investigate the fungi and their toxigenic potentials isolated from the wheat and wheat brans. A total of 67 samples of wheat and 17 samples of wheat bran were collected from Faisalabad district of Pakistan. Forty-five (67.16%) samples of wheat yielded fungi. Frequency distribution based on total samples, *Aspergillus* was the highest (44.77%) genus followed by *Penicillium, Fusarium* and *Alternaria. Penicillium verucosum* (30.64%) was the most frequently isolated species followed by *A. niger* aggregates, *A. flavus, A. parasiticus, P. chrysogenum, A. ochraceous, A. carbonarius* and *A. fumigatus.* Among *Aspergilli, A. niger* aggregates (46.67%) were most frequently isolated species. Out of 30 *Aspergilli* isolates from wheat samples, 17 (56.66%) were found toxigenic. AFB1 produced by aflatoxigenic *Aspergilli* varied from 1.44 to 836.3 ng/g, while ochratoxin A levels varied from 0.037 to 15 045 ng/g. Among *Penicillium* species, *P. verrucosum*

(63.15%) were found ochratoxigenic and OTA levels were varied from 7.31 to 8400 ng/g. In wheat bran, 10 (58.82%) samples yielded fungi. Based upon total samples, frequency distribution of *Aspergillus* (35.28%) was the highest followed by *Penicillium* and *Fusarium*. Similar pattern was observed in relative density of isolates. *A. niger* aggregates and *P. verrucosum* were predominant species (23.07%) isolated from wheat bran. Among *Aspergilli, A. niger* aggregates (50%) were the most frequently isolated species followed by *A. flavus, A. fumigatus* and A. *ochraceous* (16.67%) each. The OTA levels of fungi isolated from wheat bran varies from 0.292 to 2500 ng/g. Isolation of toxigenic *A. niger* aggregates from wheat indicates that these species should be considered as possible contributors of OTA contamination in wheat and its by-products in Pakistan.

MICROBIOLOGICAL ANALYSIS OF DIFFERENT SNACK FOODS AS PUBLIC HEALTH SIGNIFICANCE

Food borne illnesses are considered as a major and most important challenge to the public health and significantly contribute to the cost of health. Each year millions of illnesses in the world can be attributed to the contaminated foods. Hence a preliminary study was conducted to estimate the qualitative and microbiological analysis of different snack foods and its evaluation regarding public health significance. Snack food samples (sandwiches, burgers and pizzas) were collected from different retail outlets located at Lahore city and further processed for microbiological quality assays including; total aerobic plate counts, coliform count and enumeration of *Staphylococcus aureus* and detection of *Salmonella.* Results showed that sandwiches had the highest geometric mean of aerobic plate counts followed by pizzas and burgers respectively. In total 73% of the snack foods were contaminated with coliforms. Staphylococcal contamination was higher as compared to coliform contamination. The contamination level was above permissible level in 50% of the sandwiches, 27% of burgers and 45% of the pizza as per guidelines for grading of ready to eat foods in Hong Kong and U.K. However *Salmonella* was not detected in any food sample. Snack foods showed detectable levels of microorganisms of public health significance. These foods are contaminated due to poor hygiene practices. Necessary hygienic measures are recommended to reduce the contamination level.

SNAKE BITE IN JERSEY CATTLE; A CASE REPORT

This clinical article reports a case of snake bite in a five years old female Jersey cow kept at Livestock Research Station, National Agricultural Research Centre Islamabad, Pakistan. The only clinical signs observed in victim were respiratory distress, restlessness and sudden death. Postmortem examination revealed multiple snake bite marks on teats, mammary gland and external genitalia with profuse swelling. The skin of the animal was discolored and appeared bluish in color. The eye pupil of the animal was dilated. Internal examination of the carcass revealed subcutaneous hemorrhages, congested and edematous lungs and trachea filled with frothy discharges. The right chamber of heart was dilated, intestines appeared to be hemorrhagic, liver was discolored and pale and spleen was shrunken. History and necropsy findings revealed that the animal died of snake bite.

CHEMO-PROPHYLACTIC AND HEMATO-SEROLOGICAL EFFECT OF ANTI-DIARRHEAL DRUGS AGAINST NEONATAL CALF DIARRHEA

A very important disease of younger age that usually appears and leads to death of neonate in case of improper diagnosis and treatment is Neonatal calf diarrhea. Disease has its own economic importance as livestock has a major role in gross domestic production (GDP) of Pakistan. A trail was conducted to check chemo-prophylactic effects of different antidiarrheal drugs. Thirty neonates were selected and divided into six equal groups treated respectively with ColimuneOra, Cosumix Plus, Streptomegma, NMK Powder and Biovet by keeping last group as control. Blood sample were collected to check any untoward change in blood just after birth, on 3rd and 28th day of experiment. Mixed results were found in hematology on 28th day with overall increasing pattern in Total leukocyte count (TLC) and Packed cell volume (PCV). IN contras Total erythrocyte count (TEC) shows increase of 16.57%, 96% and 93.47% respectively in case of ColimuneOra, NMK and Cosumix Plus whereas decrease in case of Streptomegma and Bio Vet up-to 5.06%. Mixed results concerning DLC, serum sodium and potassium level were witnessed. Inclusively there was no annoying change was noticed with increase in (TLC) eventually providing protection to animal to avoid the disease. Henceforward use of above

listed drugs prophylactically especially Cosumix Plus, strongly suggested against neonatal calf diarrhea to lessen the mortality due to neonatal calf diarrhea.

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