

ANNUAL REPORT 2014-2015

**VETERINARY RESEARCH INSTITUTE,
ZARAR SHAHEED ROAD,
LAHORE, CANTT**



Dr. Rashid Ahmad, Director
Veterinary Research Institute, Lahore Cantt





AIR VIEW OF VRI

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**DIRECTORATE OF VETERINARY RESEARCH INSTITUTE,
LIVESTOCK & DAIRY DEVELOPMENT
ZARAR SHAHEED ROAD, LAHORE, CANTT.
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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 2014-15 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 Punjab.

Achievements for 2014-15

- Veterinary Research Institute has provided large quantity of quality vaccines like HS, B.Q.V, E.T.V, PPR and NDV for carpet vaccination campaign launched by Punjab Government in the 36 districts of the Punjab to provide coverage against major infectious diseases of livestock and poultry.
- Adhering to VRI motto "Prevention is better than cure" all staff participated enthusiastically to enhance the production of vaccines and to monitor vaccination campaign.
- Controlled disease outbreak in camel in the districts Khushab and Layyah
- Helped to provide coverage against deadly zoonotic Disease in susceptible livestock population in disease prone areas of the Punjab.
- To enhance the efficacy of lyophilized vaccines of livestock & poultry, mass scale production of diluent is started.
- To provide good quality milk to public, facility of milk testing for adulteration at free of cost is initiated in the Quality Control Section.
- Extended full cooperation to control disease problems in livestock and poultry.
- To control the HS disease in livestock three new biologics HS-LA , H-50 and HS-plus are introduced.

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

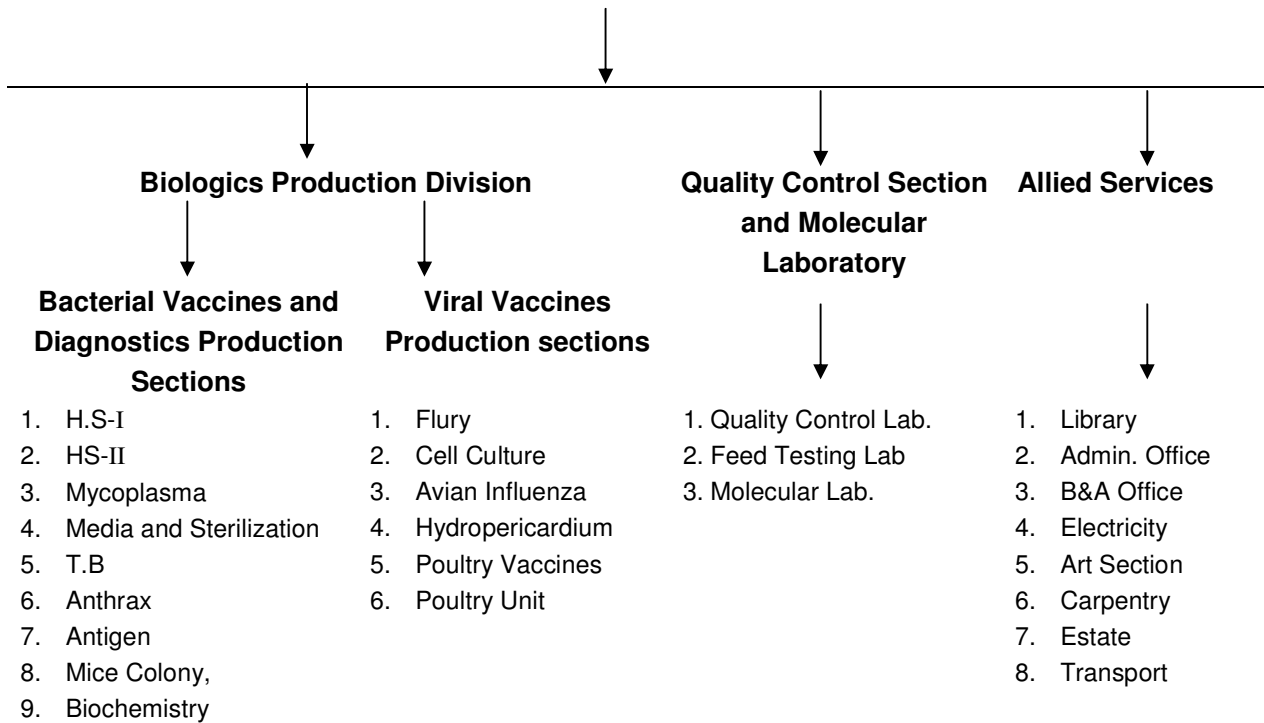
BUDGET

(Rs. In Million)

BUDGET		INCOME	
Allocation	Expenditure	Targets	Achievements
286.184	277.082	130	215

ORGANIZATION

DIRECTORATE OF VRI



Biologics Production Division

Biological production division is a complex of 13 sections, poultry unit and mice colony producing 26 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	243114.25 Liters
2.	Diluent Produced	6,021,000 Doses
3	No. of lab animals maintained & produced	2413
4	No. of poultry birds maintained	1700
5	Lyophilization of vaccines & seed	76,619,100 doses
6	Biologics produced*	147,163,600 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

- The major objective of the quality control section is testing of biologics (Vaccines, diagnostic antigens and anti sera) prepared at VRI.
- Feed testing for nutritional values as well as toxins level.
- To conduct training of students and in service personals.
- Additional assignments as assigned by the authority.
- Preparation of milk samples to test adulteration of whey powder as well as milk powder.
- Attended field problems related to this section.

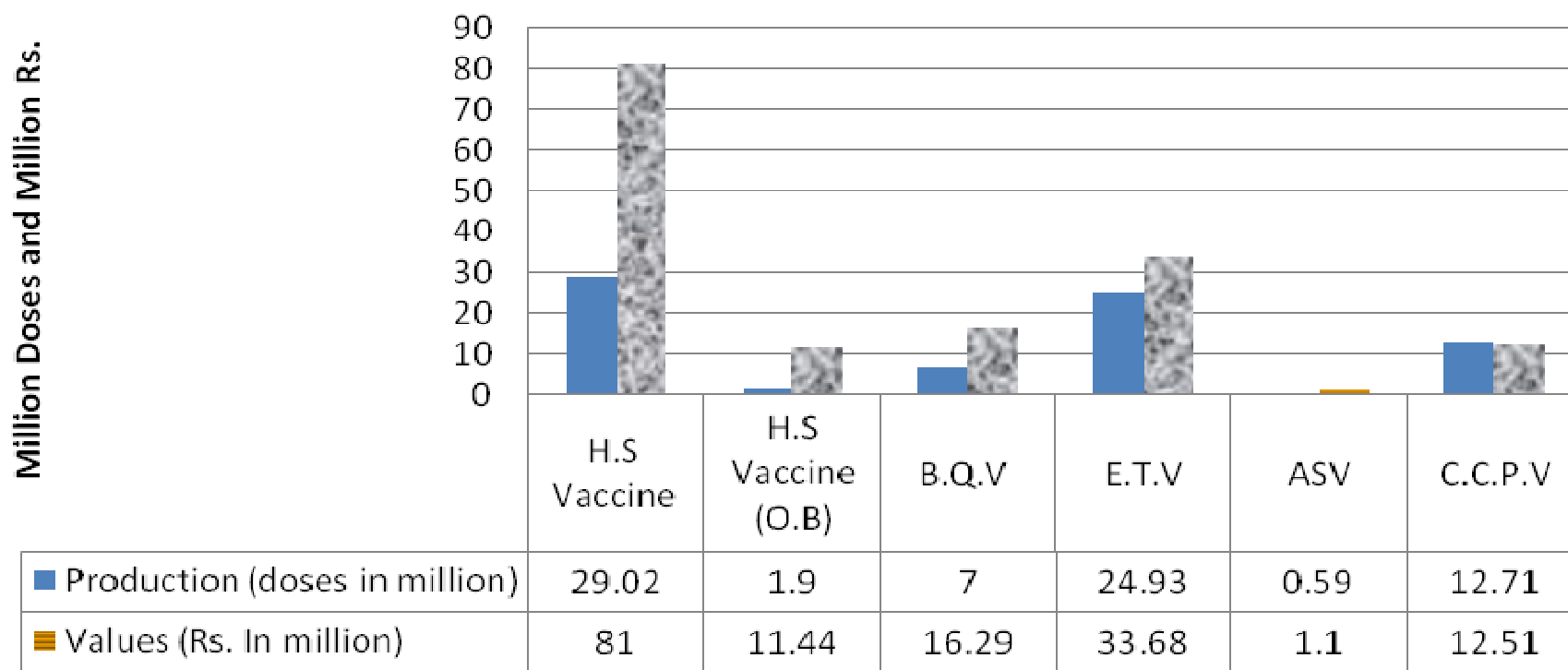
Activities of Quality Control Lab**Biologics and Diluent Tested for Quality Control**

Sr. No.	Name of biologics	No. of batches tested
Bacterial Vaccines		
1.	H.S Vaccine	86
2.	B.Q.V	15
3.	E.T.V	22
4.	Anthrax Vaccine	02
5.	C.C.P.V	09
Viral Vaccines		
6.	Sheep Pox	08
7.	Goat Pox	06
8.	P.P.R.V	44
9.	Avian Influenza +ND	01
10.	N.D.V	60
11.	Hydro	01
Diagnostic Agents		
12.	Mallien	03
13.	Tuberculin	01
14.	Brucella antigens	02
15.	Diluent	44

Activities of Feed Testing Lab

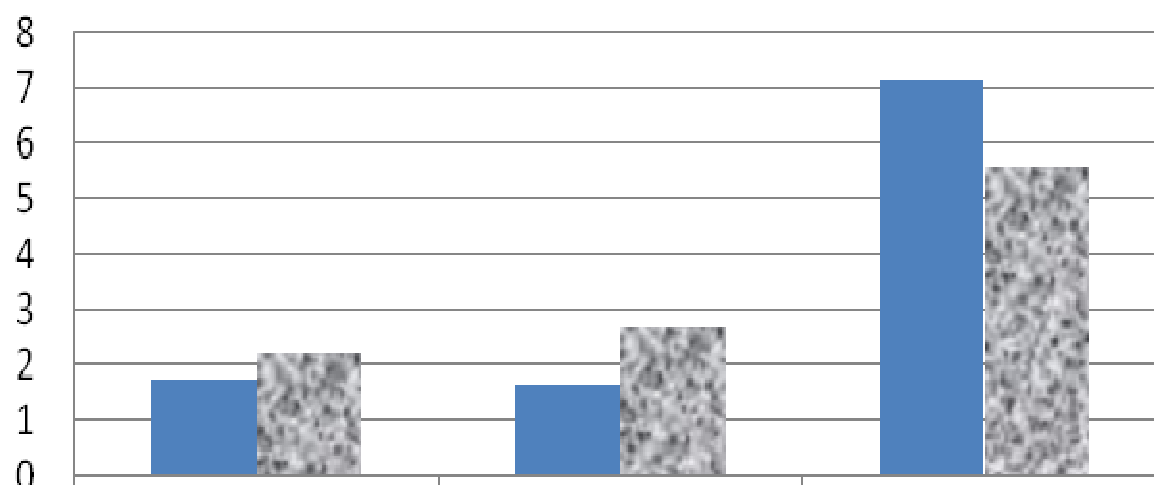
Sr. No.	Tests Performed For	No. of feed/fodder & miscellaneous samples tested
1.	Ether Extract	26
2.	Crude Protein	117
3.	Crude Fiber	10
4.	Purity of Formaldehyde	08
5.	Aflatoxin Level	148
6.	Ash	10
7.	Moisture	08
8.	Ca	84
9.	P	84
10.	Na	80
11.	K	80
12.	Dry Matter	04
13.	True protein	07
14.	Nitrates	04
15.	Milk tests for adulteration	88
16.	Others	15

Bacterial Vaccine Production in 2014-15 and their worth (Rs in Millions)



Viral Vaccines for Livestock Produced in 2014-15 and Values (Rs. In Million)

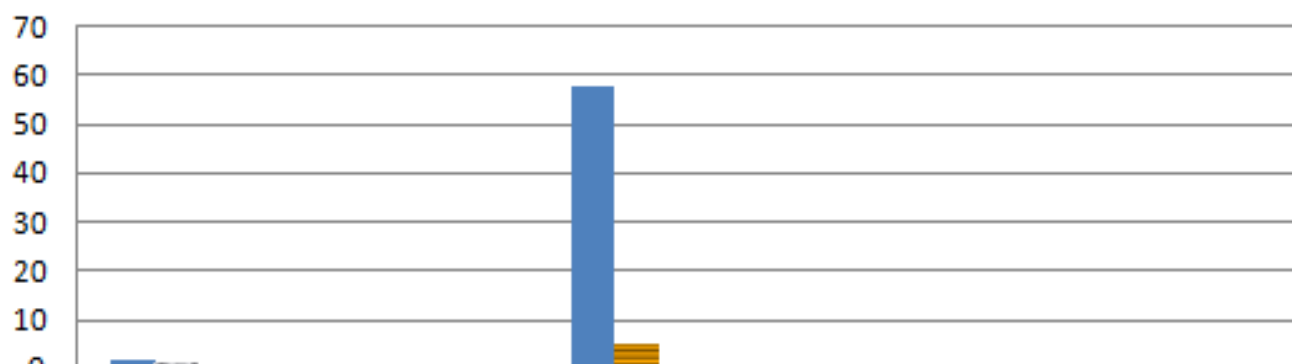
Doses & Values in Million



	Sheep Pox	Goat Pox	P.P.R.V
■ Production (doses in million)	1.75	1.66	7.07
■ Values (Rs. In million)	2.18	2.67	5.56

Viral Vaccines for Livestock Produced in 2014-15 and Values (Rs. In Million)

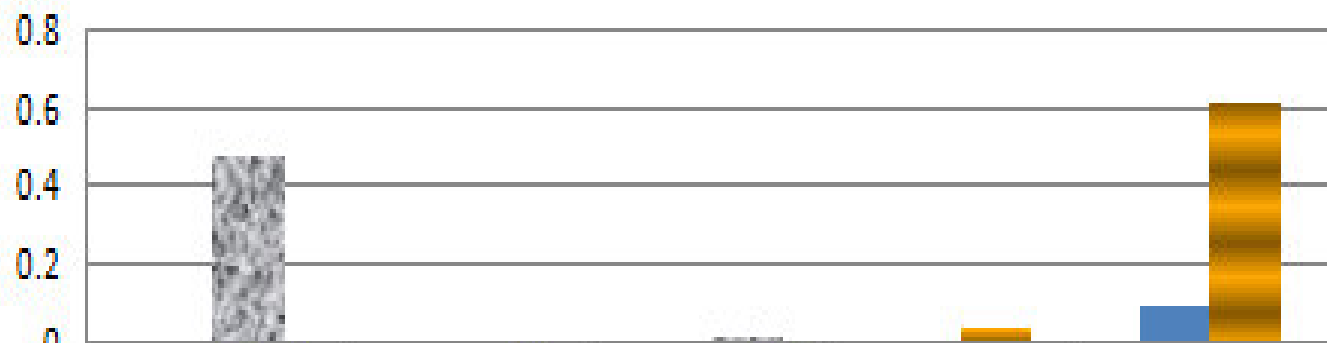
Doses & Values in Million .



	ND+H9	A.I.V (aq)	A.I.V(O.B)	N.D.V	N.D.V(O.B)	Hydro	IBD	ND Flue(O.B)
Production (doses in million)	1.75	0.28	0.01	57.67	0.1	0.02	0.06	0.59
Values (Rs. In million)	0.88	0.16	0.02	4.73	0.18	0.01	0.02	0.18

Diagnostics Produced in 2014-15 and their Worth (Rs. In Million)

Doses & Values in Million.



	Mallien (IDP)	Tubeculin(M)	Tubeculin(A)	Br.ab	Rose Bengal
■ Production (doses in million)	0.01	0.01	0.01	0.007	0.0957
■ Values (Rs. In million)	0.48	0.01	0.02	0.04	0.61

TRAINING

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

Particular	Nos.
No. of trainings	7
No. of participants	24

Executive Summaries of Research Activities Undertaken During Year 2014-15

Isolation and Identification of *Mycoplasma agalactiae* in Sheep / Goats

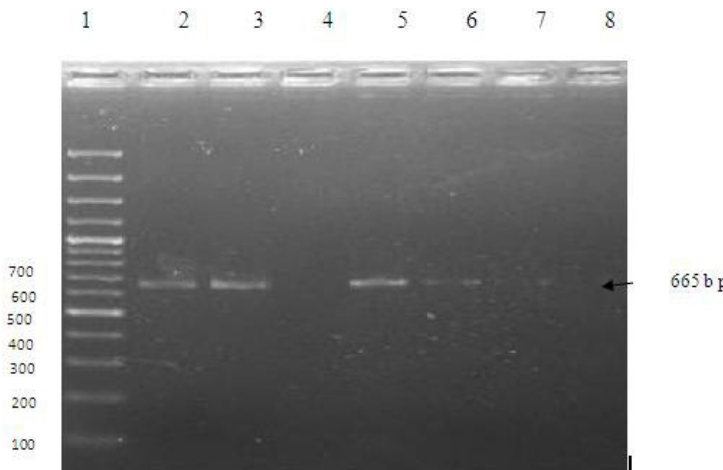
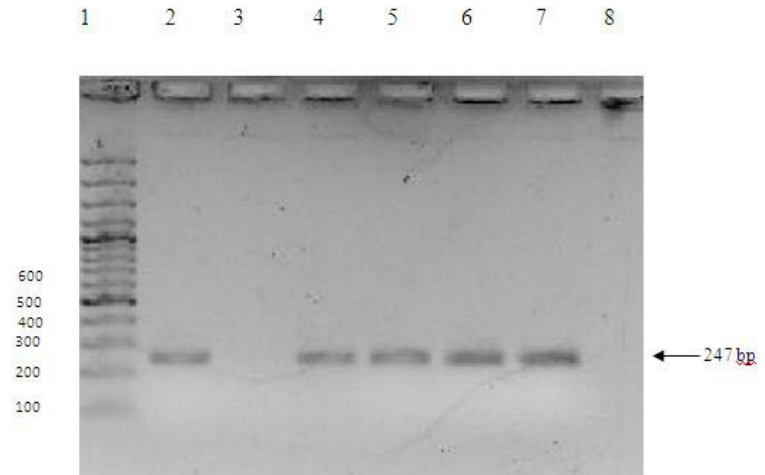
A total of 731 samples collected/received from sheep and goats suspected for mycoplasmosis were processed for isolation of *Mycoplasma agalactiae*. The samples were cultured on PPLO broth and agar medium supplemented with equine serum, yeast extract, benzyle penicillin and thallus acetate and incubated at 37°C in CO₂ atmosphere in moist conduction for 7-12 days. The cultured plates were observed after 7-12 days. All the cultured plates did not show any growth of *Mycoplasma agalactiae*. All the samples were found negative for *Mycoplasma agalactiae*.

Public Significance: Isolation of local strain of *Mycoplasma agalactiae* for development of vaccine.

Clinico-Pathological Findings of *Clostridium perfringens* Type D Enterotoxaemia in Goats and its Hemolytic Activity in Different Erythrocytes

The present investigation was conducted to study the effects of experimental *Clostridium perfringens* type D enterotoxaemia in teddy goats. Clinical signs started to appear after 30 min of experimental infection like anorexia, diarrhea, dehydration, frothing and dyspnea. Gross lesions consisted of severe congestion in tissues of varying intensity with enlarged mesenteric lymph nodes while histological examination revealed edema of lungs, kidney, and lymph nodes and to some extent in brain along with hemorrhages in lungs and intestines. *Clostridium perfringens* type D carrying alpha and epsilon toxin genes were amplified with amplicon size about 247 bp and 665 bp, respectively. Human erythrocytes showed the highest hemolysis, 68%, followed by mice, 57%, against culture supernatants. The percentage of hemolysis was significantly higher at 37°C as compared to 25°C except for rabbit and dog.

Agarose gel electrophoresis of PCR product, *C. perfringens* type D. Lane 1: 100 bp DNA molecular marker, Lane 2, 4, 5, 6, 7: Cpa (alpha toxin encoding gene) corresponding in size approximately 247 bp. Lane 8 (negative control)

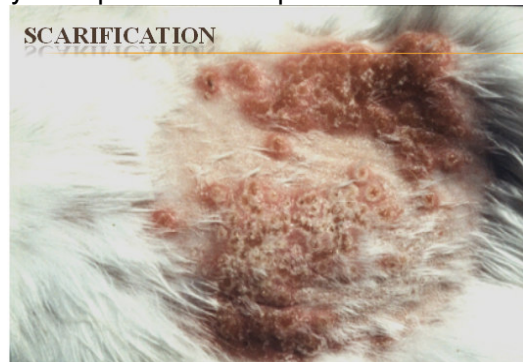
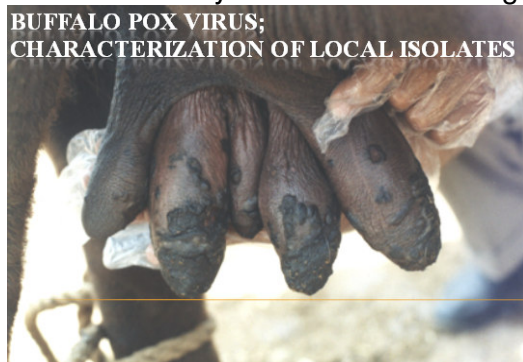


Agarose gel electrophoresis of PCR product, *C. perfringens* type D. Lane 1: 100 bp DNA molecular marker, Lane 2, 3, 5, 6, 7: PCR amplified band corresponding to size of the epsilon (ETX) toxin encoding gene about 665 bp .

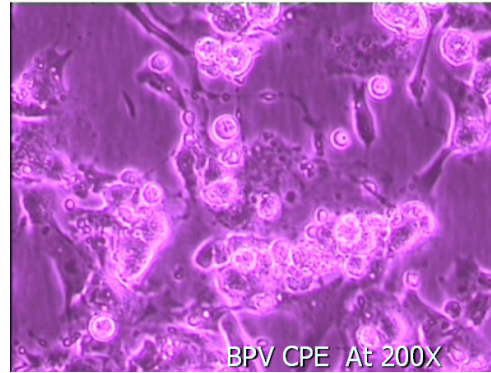
Public Significance: Goat populations show variable symptoms of enterotoxaemia by *Clostridium perfringens* type D, Clinical findings of enterotoxaemia in teddy goats were confirmed and molecular characterization of *Clostridium perfringens* type D were done. The study will help in control of enterotoxaemia by *Clostridium perfringens* type D in teddy goats.

Isolation of Buffalo Pox Virus from Clinical Case and Variations in the Genetics of the B5R Gene Over Fifty Passages

Outbreaks of buffalo pox affect udder and teats, which may ultimately lead to mastitis in dairy buffalo and can significantly compromise the production. In this



study, we have reported 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



at amino acid levels. Phylogenetically, the isolate can be classified closer 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 pox-viruses reported from the neighbouring 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.

Public Significance: Development of Buffalo Pox Vaccine which will help in control of disease.

Production of Diluent for Freeze Dried Vaccines

To provide diluent for reconstitution of freeze dried vaccine of livestock and poultry in the field, mass scale production of diluent is initiated at Biochemistry Section of VRI. Following the protocol recommended by the FAO trials were made to optimize the composition of diluent. The composition of diluent is adjusted to NaCl 8.1gm/L, Na₂HPO₄ 0.2 g/L KCL 0.2gm/L in distilled water with pH 7.2 ± 2. To make the product sterile after filling and sealing of vials the vials are autoclaved at 121°C for 15 minutes. The quality of the final product is checked by culturing on media and animal inoculation tests.

Public Significance: The product will reduce the deterioration of lyophilized vaccine due to non availability of proper diluent and will improve the efficacy of these vaccines.

Development and Comparison of Innocuity and Potentiating Effect of three Oil Adjuvant Vaccines against HS Disease Caused by *Pasteurella Multocida* in Buffalo/Cattle

Haemorrhagic Septicaemia is one of the most common, fatal and acute bacterial disease of livestock which causes mortality above 70% and is caused by

Pasteurella multocida. The only satisfactory and practical method of control and prevention is timely vaccination of all the healthy and in contact animals. Different types of vaccines are being used for the immunity against this disease. In this project three oil based vaccines were produced. Two single emulsion vaccines were prepared by utilizing Montanide ISA-50 and liquid paraffin with lanolin where 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. For immune titre IHA was performed by collecting serum from each and every animal including control animals. The comparison of IHA was done via statistical analysis by using GMT, Single emulsion vaccine prepared from liquid paraffin with lanolin gave maximum immune titre out of all the three vaccines in large animals and in young calves ISA 206 gave a significant titre.



Public Significance: The new vaccines will help to control Hemorrhagic Septicemia in livestock.

Effect of Glucose Saline and Ice Block on the Maintenance/Production of Albino Swiss Mice in Hot Climatic Season

Albino Swiss Mice are reared in Mice Colony Section, VRI, Lahore Cantt for safety testing of different biologics and research studies. They require mild ambient temperature for maintenance of growth and for production.

During summer the hot climatic condition coupled with load-shedding has worsen the situation led mice to reduce feeding, watering and lost their body weights and immunity. Death rate increased due to heat stroke. Ice blocks were placed in rearing rooms and mice were offered glucose saline instead of simple water. Majority of mice regained vigor and death rate decreased.

Public Significance: It is an economical way to maintain the mice population in hot climatic conditions.

Determination of Immune Status of HS Vaccinated Animals in Different Districts of Punjab, Pakistan

A study was conducted to determine the Immune status of HS vaccinated animals in 35 districts of Punjab, Pakistan. A total number 1818 serum samples from cattle/buffaloes were collected to determine the antibody titer through IHA against HS vaccine prepared from *P. multocida* type B:2. The results indicated that plain & desert zones have higher (15.32 & 15.40) GMT values than semi Hilly zone GMT (9.71). Similarly the average GMT value for Veterinary Research Institute, Lahore was higher (14.53) than other sources of vaccines i.e. UVAS & Sindh province (9.81 & 7.52). The results also indicated that there is minute variation in the average GMT of Cattle/buffaloes.

Relationship of Optical Density and Dry Bacterial Weights of *Pasteurella multocida* Type B:2 in Hemorrhagic Septicaemia Montanide ISA-50 Oil Adjuvanted Vaccine

Hemorrhagic Septicaemia (HS) caused by *Pasteurella multocida* type B:2 is an economically important disease of cattle and buffaloes. Which causes heavy economic losses due to sudden death of animals in developing countries like Pakistan every year thus resulting in low productivity from Livestock sector. A study was conducted to standardized the optical density (OD) value according to the dry bacterial weight dose (1.5 mg/ml) of *Pasteurella multocida* type B:2 for Montanide ISA-50 oil Adjuvanted vaccine preparation. Vaccine strain of *Pasteurella multocida* type B: 2 was incubated in Brain Heart Infusion (BHI) broth at 37 °C for 24 hrs and then formalized @ 0.5 %. Hot dried 10 ml glass tubes were weighed before adding 5 ml formalized culture, centrifuged @ 5000 rpm for 30 mints, pellets of different weights prepared after drying at 105 °C to constant weights were used for determination of OD values. At 600 nm wavelength, a linear graph was obtained with OD values of 0.6, 1, 1.4, 1.7, 2.1, 2.2 and 2.4 for concentrations of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75 mg/ml of dry bacterial weights. Similarly a linear graph was also obtained at 540 nm wavelength with OD values of 0.562, 1.044, 1.411 and 1.8 for concentrations of 0.25, 0.5, 0.75 and 1 mg/ml of dry bacterial weights respectively.

Public Significance: It will help to determine a convenient method to adjust dose of HS oil adjuvanted vaccine.

Preparation and Evaluation of Hemorrhagic Septicaemia Vaccine with a New Oil-Adjuvant for Cattle and Buffaloes in Pakistan

Hemorrhagic Septicaemia (HS) caused by *Pasteurella multocida* type B:2 is an economically important disease of Cattle and Buffaloes, which causes heavy economic losses due to sudden death of animals in developing countries like Pakistan every year thus resulting in low productivity from Livestock sector. At present, alum (adjuvant) precipitated vaccine is being used as mass scale vaccine in Pakistan. Immunity induced by this vaccine lasts for 3-4 months only, which reflect

an un-protective state of the vaccinated animals between two vaccinations. A new HS oil based vaccine has been developed by adding a new adjuvant i.e. Montanide ISA-50. Earlier the dose of oil based vaccine was 5 ml/animal. Now the dose has been adjusted to 1.5 mg/ml with the dose rate of 2ml/animal. This new vaccine has passed sterility, safety & potency tests as per OIE, 2014. This new vaccine will confer solid immunity for whole one year, easy to inject, have low viscosity & single shot will be



enough to protect the animals for whole year instead of two shots. Easy to administer no side effects and no swelling at the injection site will motivate the livestock owners for vaccination of animals against the HS disease which will be helpful for reduction of economic losses due to HS disease and will result in increase in milk & meat production in the country.

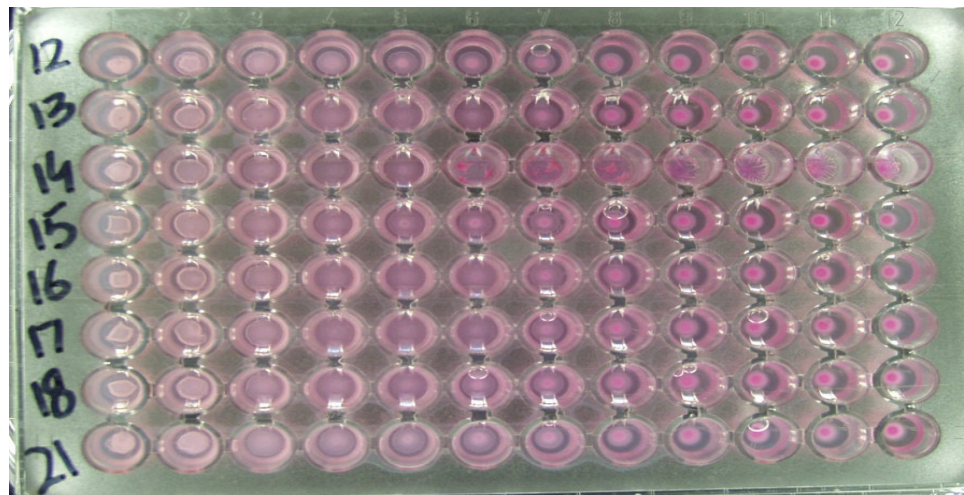
Public Significance: The vaccine will help to reduce the burden of Hemorrhagic Septicemia disease in livestock.

Development of Microagglutination Test (MAT) for Assaying the Antibodies in Sheep and Camels Vaccinated with *Bacillus anthracis* Sterne Live Attenuated Spore Vaccine.

Sheep, goat and cattle in hilly and desert areas of Pakistan are immunized with anthrax vaccine (spore suspension of *Bacillus anthracis* Sterne in 50% glycerin saline) prepared by VRI, Lahore to control the disease. In 2015 in early summer, camel stock in the district Khushab, Punjab, Pakistan and adjacent areas presented

sudden onset of death in animals. Affected stock showed few or no signs of illness before they died. The disease began with the sporadic deaths of single animals in different groups of animals over a few days, led to huge losses in a very short period of time. Any micro-organism suggestive of causative agent could not be isolated from the morbid material submitted to provincial diagnostic laboratory for diagnosis of prevailing problem in camel. Current situation and topography of the area endorsed the immunization of susceptible livestock population including camel stock with anthrax spore vaccine.

In order to evaluate and compare the immune response mounted by anthrax spore vaccine in camel and sheep population microagglutination test (MAT) was developed. Serum samples of sheep and camels were collected 14 days post vaccination. Antigen was prepared from heat-killed, *Bacillus anthracis Sterne* strain whole-cells stained with Rose Bengal dye. MAT was performed as per the standard method. Serum samples of sheep showed the agglutination titers: 79% samples 1/128, 12% samples 1/256, 5% samples 1/512 and 4% samples showed 1/8 titer. Serum samples of camel showed the agglutination titer: 87% serum samples 1/256 and 13% showed the agglutination titer 1/64. The agglutination titer of serum samples of camels were almost comparable to titers observed in serum samples of sheep. At the spot or later no untoward reaction was observed in camels immunized with anthrax spore vaccine. Two to three weeks post vaccination of anthrax spore vaccine mortality was stopped in camel and no case of sudden death was reported later.

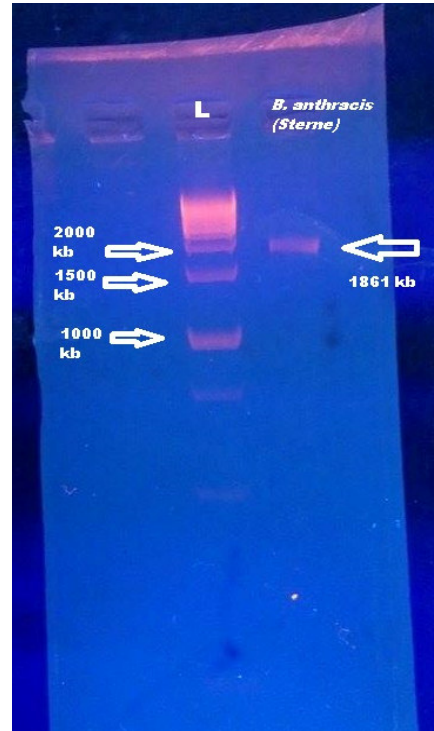


Mat formations indicate antibody titers produced by *Bacillus anthracis Sterne* spore vaccine & button formation indicates absence of antibodies

Public Significance: Anthrax vaccine prepared at VRI Lahore is safe and effective for use in camels.

Molecular Characterization of *Bacillus anthracis* Sterne Vaccinal Strain

Anthrax live attenuated spore vaccine prepared from *Bacillus anthracis* Sterne strain at VRI Lahore is used for immunization of domesticated animals against anthrax in Pakistan. Sterne is predominantly avirulent strain of *Bacillus anthracis* and has been used worldwide to immunize livestock against anthrax since its discovery in the 1930s. In Veterinary Research Institute Lahore, Pakistan *Bacillus anthracis* Sterne strain has been maintained for preparation of vaccine against anthrax for many decades. The seed of *Bacillus anthracis* Sterne strain is maintained on seed lot system and its purity is confirmed time to time following the standard microbiological techniques. Conventional microbiological techniques pose difficulty in differentiating Sterne strain from other bacilli. Lyophilized culture of *Bacillus anthracis* Sterne strain procured from the



seed bank of Bacteriology Section, VRI Lahore was propagated following the SOPs and its purity was confirmed on colony characteristics on blood agar with 5-7% sheep blood, microscopically, biochemical tests and sensitivity to penicillin. *Bacillus anthracis* Sterne isolate was subjected to genomic DNA isolation and amplified using forward & reverse primers conserved to 16SrRNA gene an amplicon of 1861bp was recovered..PCR amplified fragment was analysed by sequencing. Sequencing analysis revealed almost complete homology with 16S rRNA of *Bacillus anthracis* Sterne strain. Sequence was submitted to Gen Bank with accession no. KP942847.

Public Significance: An advanced molecular technique is adopted for characterization of *Bacillus anthracis* Sterne strain use for Anthrax vaccine production.

PUBLICATIONS

Avian Influenza and Its Mass Depopulation Strategies in Infected Poultry Birds

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Khushi Muhammad, Department of Microbiology, University of Veterinary & Animal Sciences, Lahore, Pakistan

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Taiwan Vet J **41**, 51 (2015)

Abstract

Avian influenza (AI) is a highly contagious and zoonotic viral disease that affects several animal species. It causes heavy economic losses in the domestic poultry. A quick response is always desired in the event of any disease outbreak. The principal approach to control a contagious disease involves the killing of diseased animals along with the bio containment of infectious agent. Mass depopulation of the infected birds plays an important role in the eradication of the disease. The possible strategies for mass depopulation include maceration, electrocution, cervical dislocation, gassing and foaming. All of these procedures are much intensive and time consuming because it involves a lot of man power, biosecurity risks, applicability for all house types and suitability for large-scale emergency implementation. The basic objectives of these strategies include (1) To reduce pain and suffering to the birds, (2) To minimize disease spread and (3) To ensure of protection to human operators from potential biohazards. A suitable depopulation technique can only be suggested keeping in view the species and type of bird involved, and differences in husbandry practices like management, housing and stocking density. Mass depopulation is an important tool to control the spread of any disease but the selection of procedure depends upon the prevailing circumstances. In this paper, various mass depopulation strategies and their selection in different conditions is reviewed and discussed.

Detection of *Escherichia Coli* and *Salmonella* from Retail Quail Meat through Optimized Multiplex PCR

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²Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Pak. J. Agri. Sci., Vol. 52(3), 809-813; 2015

Abstract

The conventional and multiplex PCR was developed for rapid and simultaneous detection of multiple food-borne pathogens in single reaction mixture. Conventional PCR was optimized with annealing temperature at 50°C, each primer concentration of 10 pmol, DNA template 200 ng and 10 µl Fermentas master mix with 35 PCR cycles. Similarly multiplex PCR was optimized with annealing temperature 56°C, each primer concentration of 20 pmol, DNA template 200 ng using 23 µl Qiagen multiplex master mix and 35 PCR cycles. A total 100 slaughtered quails from various chain stores (n=40), retail market (n=40) and University of Veterinary and Animal Sciences quail farm (n=20) were processed for conventional and multiplex PCR for direct detection of *Escherichia coli* and *Salmonella*. Prevalence of *Escherichia coli* and *Salmonella* through conventional culture method and biochemical testing was detected to be 82.5% and 66.6% respectively. While through conventional and multiplex PCR recovery of *E. coli* was 90% and *Salmonella* was 82%. Statistically no significant difference ($p>0.05$) was found between conventional culture method, conventional PCR and multiplex PCR. Similarly no significant difference ($p>0.05$) was observed in recovery rate of *Escherichia coli* and *Salmonella* in quail meat collected from chain stores, retail market and UVAS quail farm.

Effect of Lentogenic Newcastle Disease Virus (Lasota) on Low Pathogenic Avian Influenza Virus (H9N2) Infection in Fayoumi Chicken

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J Avian Res, 2015, 1(1):1-4

Abstract

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 NDV on the infectivity and excretion of LPAIV in fayoumi chicken. Four week old fayoumi chicks were inoculated intranasally with 10⁶ median embryo infectious of NDV vaccine strain (LaSota) and a H9N2 LPAIV (A/Chicken/Pakistan/UDL/08 H9N2) simultaneously. No clinical signs were observed in chickens infected with the NDV. All chicken showed mild to moderate respiratory distress with LPAIV alone or in combination with NDV. Clinical and necropsy findings revealed non synergistic behaviour of two viruses for the development of clinical signs and lesions. However, the pattern of virus shed was different with co-infected chickens, which excreted lower titres of NDV 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 oropharangeal and cloacal swabs detected positive for LPAIV and lower number for NDV. The knowledge obtained from the study serves the dual purpose of shedding light on the different replication behaviours of LPAIV in early days of experiment which may be due to competition for receptor binding with NDV, as well as the more pathogenic behaviour of LPAIV (H9N2) in fayoumi chickens of Pakistan

Molecular Diagnosis of Bovine Anaplasmosis in District Lahore

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Veterinaria 2015; 1: 7-12.

Abstract

Bovine anaplasmosis is a tick borne rickettsial disease wide spread in tropical and subtropical areas. A comparative study was conducted to check the prevalence of bovine anaplasmosis in different age groups of cattle and buffaloes. A total of 160 samples were collected (80 from each) during May-August. Screening was done by blood smears, stained by Giemsa-wright staining technique and later the blood

samples from the same animals were also processed by PCR. On the basis of microscopic examination, overall 11.25% (18/160) disease prevalence was recorded. On the basis of polymerase chain reaction (PCR) prevalence of *Anaplasma marginale* 25.6% (41/160) was recorded, showing the presence of carrier animals in District Lahore. The polymerase chain reaction showed that the prevalence of bovine anaplasmosis is more in cattle 32.5% (26/80) than in buffalo 18.75% (15/80). The results have demonstrated the high efficacy of polymerase chain reaction assay as compared to microscopic examination.

Comparison between Haemagglutination Test and Polymerase Chain Reaction for Diagnosis of Canine Parvovirus Infection

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Veterinaria 2015; 3(2):5-8.

Abstract

The aim of this study was to compare the efficacy of two most commonly used diagnostic tests for canine parvovirus (CPV) diagnosis: haemagglutination test (HA), and polymerase chain reaction (PCR). A total of 50 fecal samples from dogs showing clinical signs suggestive of parvovirus enteritis were collected aseptically from different pet clinics of Lahore. Fecal samples were processed for cCPV antigen, required for HA and PCR. The HA was able to detect CPV antigen in 35 samples, 32 samples tested highly positive with titers >128, 3 tested weakly positive with titers ranging from 32 to 64 and 15 were negative (titers <16). Using PCR, 39 samples were found positive including 6 HA-negative samples. Chi square analysis showed that there was no significant difference ($P > 0.05$) between the results of HA test and PCR. Thirty percent of dogs presenting bloody diarrhea did not show infection by HA. It is concluded here that specificity and sensitivity of PCR detection is non-significantly higher ($P > 0.05$) than HA. These findings have confirmed that HA test could be employed for the preliminary screening of the agent in field because of its less cost and rapid results but negative results from HA tests of suspected cases should be confirmed through molecular methods.

Microbiological Analysis of Different Snack Foods as Public Health Significance

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Infectious Diseases Journal of Pakistan Vol. 24(1): 2015

Abstract Background

Food borne illnesses are considered as a an important challenge to the public health and significantly contribute to the cost of mhealth. 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.

Methods

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

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.

Conclusion

Snack foods showed detectable levels of microorganisms of public health significance. These foods are contaminated due to poor hygiene practices. Necessary hygienic measures should be recommended to reduce the ontamination level.

Mild form of Peste Des Petits Ruminants Virus (PPRV) In Pakistan

Ullah RW, A B Zahur, A Latif , JI Dasti , R Zahra and S H Khan. Pakistan J. Zool., vol. 47(1), pp. 0-0, 2015 (PJZ-1730-14)

ABSTRACT

An outbreak of Peste Des Petits 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. Nasal and ocular swabs were analysed by RT-PCR for the presence of PPRV specific genome and their sera were analysed 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.

Molecular Detection of Chicken Infectious Anemia in Day Old Broiler Chicks from Faisalabad Pakistan

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Proceedings of the "International Seminar on Poultry Diseases" 14-15 Dec, 2015, Department of Pathology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Chicken infectious anemia (CIA) is an emerging immunosuppressive infectious disease of poultry in Pakistan. It results in high mortality, poor growth, immunosuppression and poor response to vaccine in young birds. In Pakistan, information regarding disease status of CIA in different types of birds including commercial layers, broilers and parent stock is not yet available. Therefore, present field study was designed to investigate the molecular epidemiology of chicken infectious anemia in day old broiler chicks, because the detection of CIA in young chicks may also represent the status of disease in parent flocks. For this purpose, a total 254 blood samples were collected from different farms and hatcheries located in Faisalabad, Punjab, Pakistan. These samples were analyzed for chicken anemia virus (CAV) through PCR, using specific primers (CAV1 & CAV2) of highly conserved VP-2 coding gene. Total 38 (14.96 %) samples from 17 farms were found positive for CAV through PCR assay. The hematological parameters like RBC, Hb and PCV of all samples were also determined and the hematological values of CAV

positive birds showed (RBC($\times 10^6/\mu\text{l}$) 1.99 ± 0.37 , Hb (g/dl) 5.88 ± 0.77 , PCV (%) 18.74 ± 2.97) severe anemia. The results of present study suggested that CIA is prevalent in Pakistan. Further epidemiological and molecular investigations are required to design and implement control strategies for this important immunosuppressive disease

Growth Promoting and Possible Toxicopathological Effects of Sanguinarine (Sangrovit®) In Growing Broilers

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Department of Pathology, University of Agriculture, Faisalabad; ¹Department of Livestock and Dairy Development Punjab, Pakistan; ²Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad; ³Department of Parasitology, University of Agriculture, Faisalabad; ⁴Veterinary Research Institute (VRI), Zarar Shaheed Road, Lahore, Pakistan

Proceedings of the "International Seminar on Poultry Diseases" 14-15 Dec, 2015, Department of Pathology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Sanguinarine is a plant extract obtained from *Macleayacordata* plant belonging to family Papaveraceae. It is used as antibacterial growth promoter for poultry, livestock and pig industry. The present experimental study was planned to investigate the growth promoting and possible pathological effects (if any) of Sanguinarine available as commercial product Sangrovit®. One hundred day old broiler chicks were procured from commercial hatchery and divided into 5 equal groups, i.e., A-E. The commercial feed and water was offered to the chicks *ad libitum*. The group E was kept as control group, while group A Sangrovit @ 1 gm/10 lit drinking water (DW) 24 hours daily, group B Sangrovit @ 1 gm/10 lit for 12 hours daily, group C 50 mg/kg feed, group D 1 gm/5 lit DW 24 hours daily. The duration of experiment was 42 days. Physical and some hemato-biochemical and pathological parameters were studied. The data thus obtained was subjected to analysis of variances (ANOVA) test and group means were compared by Duncan's multiple range test (DMR).

Birds administered with Sangrovit 1gm/5 lit drinking water 24 hours were depressed, less attractive towards feed, water and loose drooping were observed and this situation remained for two weeks. Mortality in group A and D was 25 and 35%, respectively. Feed intake of group D was significantly lower than the control group E. The body weight of group B and C were significantly higher than the control group while group D showed lower body weight as compared to control group. In serum biochemical parameters total protein and globulin were significantly higher in groups

C and D as compared to control group. Urea of groups B, C and D were significantly higher than control group. ALT was lower in C group while AST was lower in groups A, C and D. cholesterol of groups A and C were significantly higher and group D was significantly lower than the control group.....

Emerging Threat of Necrotic Enteritis in Poultry Birds: A Review

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Proceedings of the "International Seminar on Poultry Diseases" 14-15 Dec, 2015, Department of Pathology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Necrotic enteritis (NE) is an emerging economically significant problem of broiler industry caused by a bacterium *Clostridium Perfringens*. NE is one of the top ranked intestine damaging bacterial disease of poultry birds. Under normal conditions, the bacteria live harmlessly in the gut but whenever there are drastic changes in the environment of gut, it quickly leads to proliferation of bacteria. *C. perfringens* possess novel toxins such as alpha and β toxins which are considered key virulence factors for the pathogenesis of NE. Moreover. It is clearly known that Necrotic enteritis is produced under specific conditions only by specific strains of *C. perfringens*. Favorable environment for the growth of *C. perfringens* is produced by mucosal damage inducing factors such as parasitism (coccidiosis) high fiber diets, poor hygienic and housing conditions in addition to toxins. Moreover, excessive use of antibiotic growth promoters (AGP) enhance the capability of *C. perfringens* to induce disease. *C. perfringens* possess *plc* gene that encode for the Alpha toxin. A toxoid vaccine using alpha toxin produced antibody response which was transferred to the progeny as well and resulted into partial protection from NE. These toxoid vaccines are still in debate and needs a deep insight of mechanisms involving the role of alpha toxin in development of immunity and pathogenesis. This review has three purposes. First, it is designed to summarize the currently available information about necrotic enteritis in chicken. Second, it is aimed to elaborate the pathogenesis of necrotic enteritis at molecular level. Finally, future prospects of vaccination against

necrotic enteritis and other possible novel methods for the control of necrotic enteritis are suggested.

Pathological Effects of *in ovo* Vaccination against Newcastle Disease in Chicken and Comparison of Its Immune Response with Post-Hatch Vaccination

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Proceedings of the "International Seminar on Poultry Diseases" 14-15 Dec, 2015, Department of Pathology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

New castle disease (ND) is an endemic and prevalent disease in Pakistan. Prevention of disease is mainly practiced by vaccination. Killed and live attenuated vaccines are being used through drinking water or oculo-nasal route. *In ovo* vaccination for ND is not being carried out in Pakistan and this study was designed to investigate the pathological effects of *in ovo* vaccination of different strains of ND and comparison of its immunogenic potential with post hatch vaccination against NDV. Total 150 embryonated eggs were divided in five groups A, B, C, D, and E. Group A was kept as negative control (unvaccinated against ND). Group B (shamed group) injected with 0.1ml normal saline. The embryonated eggs of groups C, D and E were *in ovo* vaccinated with 0.1 ml of Lasota, Mukteswar and Hitchner B1 strains at day 18 of incubation, respectively. After hatching group B was given vaccination at day 7 and 21 while group C, D and E received booster vaccination of ND with Lasota strain through oculo nasal route only at day 21. Group A remained unvaccinated. Development of immunity following pre-hatch or post-hatch vaccination was examined through HI test by collecting the serum samples from all the birds in all the groups on day 1st, 7th, 14th, 21st, and 28th of age. Six birds from each group were slaughtered at 1st day of age and day 35 of the experiment. Organs were observed and collected for any pathological lesions. Results of the experiment revealed hatchability in all groups above 90%. All the birds showed normal behavioral and clinical signs. Lymphoid organs of all the birds including spleen, thymus and bursa of Fabricius were normal in gross appearance. Absolute and relative weights of bursa, spleen and thymus were significantly high in Group D as compared to control and other vaccinated groups. At day 1 and 7 highest antibody titers against ND were

observed in group D followed by C and E. at day 14, 21, 28 and 35 titers were highest in group D followed by C, B, E and A. it can be concluded that *in ovo* vaccination gives better immunological responses as compared to post hatch vaccination and there are no detrimental effect of this procedure on chicks

Molecular Epidemiology and Pathology of Chicken Infectious Anemia in Layer Chicks in Faisalabad Pakistan

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Proceedings of the "International Seminar on Poultry Diseases" 14-15 Dec, 2015, Department of Pathology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Chicken anemia virus (CAV) is the causative agent for chicken infectious anemia (CIA) disease in poultry which is an economically important disease. CIA is responsible for anemia, immunosuppression, decrease weight gain and low production. CAV is emerging worldwide and has got considerable attention. In Pakistan only one outbreak report is available on CAV implicating study of disease at wider scale. This study was designed to check the prevalence of chicken anemia virus through PCR in layer chicks of less than 7 day of age in district Faisalabad. And it is the first epidemiological study on CAV in layer chicks in Pakistan. For this purpose, 245 samples of different organ (liver, spleen and thymus) and blood from live birds were collected from five tehsils of Faisalabad. The average values of hemoglobin and pack cell volume were 4.72g/d and 18.35% respectively. Out of 245 pooled samples 65 were found positive for PCR assay. An overall prevalence of 26% was found in district Faisalabad. Histopathologically, CAV positive birds showed moderate to severe congestion of blood vessel in liver. Thymus and spleen of CIAV positive birds showed marked lymphocytic depletion. It was concluded that there was high prevalence of disease. The prevalence of CIAV was significantly different among different areas of Faisalabad, in different age group and with respect to different environmental conditions, while there was no significant difference of prevalence in cockerels and females. This study shows that there is need to study this disease at much wider scale in order to access the prevalence of disease in country and to reduce the economic losses occurring due to chicken infectious anemia.

Molecular Characterization of *Mycoplasma Gallisepticum* Isolates from Pakistan

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20th Congress of the International Organization for Mycoplasmaology – IOM

Abstracts

This study was aimed to compare serological and culturing based analysis for detection of *Mycoplasma gallisepticum* (MG) and their molecular characterization in flocks from broiler, breeder and layer farms with history of high mortality. Randomly 200 serum and tracheal swab samples were collected from broiler breeder and layer farms. The samples were analyzed for presence of MG antibodies through ELISA in serum samples while tracheal swabs were subjected for culturing of MG on Frey's medium. Serum analysis revealed that out of 200 serum samples collected from broilers, 50% (n=100) of the samples showed cut off value equal or above positive control, out of 200 breeders serum samples 75% (n=150) showed positivity while out of 200 serum samples collected from layer birds, 70% (n=140) showed positive response towards MG antibodies. In further studies, tracheal swabs were collected from the birds selected for serology and out of 200 tracheal swabs collected from broilers, MG was recovered from 18 swabs (9%), out of 200 tracheal swabs collected from breeders, MG was recovered from 53 swabs (26.5%) and from 200 tracheal swabs of layers, MG was recovered from 42 (21%) tracheal swabs. The tracheal swabs showed positive response on MG culturing was further confirmed for the presence of Mgc2 gene through PCR. Out of 18 positive swabs of broiler birds, 9 samples were found positive through PCR for presence of Mgc2 genes, out of 53 positive swabs of breeders, 20 (37.7%) were positive through PCR while out of 42 positive tracheal swabs collected from layers, 13 (30.9) samples were positive. A total of six MG isolates were phylogenetically analyzed through partial Mgc2 gene to compare with previously characterized Pakistani isolates and worldwide. All MG isolates are clustered with previously characterized isolates of the region and revealed no major genetic variation in the target gene sequence. Furthermore, pairwise analysis was carried out to compare nucleotide sequences of currently characterized Pakistani MG isolates, exhibited 98% to 100% similarities globally and 98% to 99% identity with previously characterized Pakistani isolates

Molecular Characterization of *Mycoplasma Synoviae* Isolates from Pakistan

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20th Congress of the International Organization for Mycoplasmaology - IOM

Abstract

This study was carried out to detect the prevalence of *Mycoplasma synoviae* in breeder farms in clinically reported areas of Pakistan, by culture and molecular based detection. Clinical field samples (Trachea/ tracheal swab n=150, joints n=100) were processed for culturing of MS on Frey's medium. Out of 150 Trachea/ tracheal swabs collected from various breeder farms, MS was recovered from 24 samples (16%) and out of 100 joints collected from breeders, MS was recovered from 36 joints (36%) The samples showed positive response on MS culturing was further confirmed through PCR using 16s rRNA species specific primer. Out of 60 positive samples 40 samples were found positive through PCR. A total of 20 MS isolates were phylogenetically analyzed through partial 16S rRNA gene to compare with previously characterized Pakistani isolates and available sequence data of MS worldwide from GenBank. All MS isolates are clustered with previously characterized isolates of the region and revealed no major genetic variation in the target gene sequence. Furthermore, pairwise analysis was carried out to compare nucleotide sequences of currently characterized Pakistani MS isolates, exhibited 99% to 100% similarities globally and 94% to 95% identity with previously characterized Pakistani isolates.

Awareness about Infectious Hepatitis among Barbers in Lahore, Pakistan

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Infectious Disease Journal of Pakistan Vol. 23(3): 2014

Abstract

Background

Infectious hepatitis is a major public health problem especially in developing countries. The profession of barbers has been implicated in the spread of infectious hepatitis. The present study was conducted to assess the knowledge of barbers regarding how their professional practices may spread viral hepatitis.

Materials and Methods

The study was conducted from July 2012 to June 2013 in different areas of Lahore, Pakistan including Aziz Bhati town, Shalimar town, Data Ganj Bukhsh town and Nishter town. One hundred Barbers were interviewed randomly and data was collected by a questionnaire and checklist. Data entry and analysis was done by using SPSS 16. Quantitative variables (Age and Experience of Barber) were presented by using mean \pm SD. Qualitative variables (Education, Monthly income, Experience of barber and Knowledge about Hepatitis B and C) were presented by using frequency table and percentage. Chi-square test was used to see the association between age and experience of barbers with knowledge of Hepatitis B and C. p-value <0.05 was taken as significant.

Results

Age, experience of barbers, educational status and their monthly income were correlated with their awareness level about hepatitis B and C. A significant association was observed in awareness level with educational status while there was no significant difference between age groups and experience. Barbers who attained education up to matric or higher had better knowledge about the health hazards of their profession including skin diseases, hepatitis B and C than those that were less educated (P < 0.05). It was also noted that barbers with more than five years of experience had better knowledge about infectious hepatitis as compared to those with less experience.

Conclusion

Awareness level of barbers about hepatitis B and C is less. This limited knowledge is affected by their educational status, age and their work experience.

Peste des petits ruminants (PPR) in Small Ruminants – A Clinical, Haemato-Serological and Pathological Aspects

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Inter J Vet Sci, 3(4):206-209. 2014

Abstract

Peste des petits ruminants (PPR) frequently known as “goat plague” or “Small ruminant plague” is a very noteworthy disease for the economy of any nation. The objective of the study is based on clinical signs, physical evaluation, clinical parameters, complete blood count (CBC) and serum biochemistry. Three animals (two bucks and one ram) were evaluated completely as suffering from PPR after critical investigation. All animals were presenting lymphopenia. In the absence of appropriate treatment all three animals were recommended enrofloxacin dosed at 2.2 mg/kg b.w and ketoprofen dosed at 2 mg/kg for 7 days along with fluid therapy and multivitamins powder for regular use. One of the buck died after 3 days but remaining two animals starts presenting recovery signs after 6 days and ultimately recovered up to 15 days but with weak body condition. Post-mortem of dead animal revealed hemorrhagic trachea, necropsied kidneys and mesenteric swollen lymph nodes. After fifteen days recovered animals were again passed through critical examination to investigate recovery progression. Increase in lymphocytic count to standard ranges was a durable mark of recovery.

Chemo-prophylactic and Hemato-serological effect of anti-diarrheal drugs against Neonatal Calf Diarrhea

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Inter Journal of Innovation and Applied Studies Vol. 6 No. 4: 2014

Abstract

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 trial was conducted to check chemo-prophylactic effects of different anti-diarrheal drugs. Thirty neonates were selected and divided into six equal groups treated respectively with Colimune Ora, Cosumix Plus, Streptomagma, 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 Colimune Ora, NMK and Cosumix Plus whereas decrease in case of Streptomagma 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.

Persistence of *Peste des Petits Ruminants Virus* (PPRV) in goats after an outbreak in Punjab Province of Pakistan; a longitudinal study.

Ullah RW, AB Zahur, A Latif , J I Dasti, H Irshad. SL-CARP International Research Symposium Colombo Sri Lanka 11-12 August 2014.

ABSTRACT

Peste des Petits 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 analysing appropriate samples (nasal/ocular swabs, faecal 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 faecal samples of recovered goats (n=96) collected at 4, 8, 12 and 16 weeks after the outbreak. Samples were analysed using real time-PCR. Of 96 goats faecal 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.

Epidemiological Analysis of Peste des Petits Ruminants (PPR) Outbreaks in Pakistan

Zahur, A.B., Ullah, A., Irshad, H., Latif, A., Ullah, R.W., Jahangir, M., Afzal, M., Khan, S.A. and Salaria, S.M. (2014) Journal of Biosciences and Medicines, 2, 18-26. <http://dx.doi.org/10.4236/jbm.2014.26004>

ABSTRACT

The current study reports the outbreaks of Peste des Petits 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.

Clinical investigation of peste des petits ruminants outbreak in sheep and goats at Islamabad, Pakistan

Ullah RW, Latif A, Irshad H, Zahur AB, Samo MH, Khan SA (2014). Res. J. Vet. Pract. 2 (IS): 8-10. <http://dx.doi.org/10.14737/journal.rjvp/2014/2.1s.8.10>

ABSTRACT

Clinical and laboratory investigations were carried out during an outbreak of Peste des Petits 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

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Evaluation of haemagglutination assay (HA) for the detection of peste des petits ruminants virus (PPRV) in faecal samples of recovered Goats

Latif A, Akhtar Z, Ullah RW, Zahur AB, Ullah A, Irshad H, Malik AR, Farooq U (2014). Res. J. Vet. Pract. 2 (IS): 11-13. <http://dx.doi.org/10.14737/journal.rjvp/2014/2.1s.11.13>.

ABSTRACT

This paper reports the findings of evaluation of Haemagglutination Assay (HA) for detection of Peste des Petits Ruminants (PPR) in faecal samples of sheep and goats persistently infected with PPR. Faecal 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 faecal samples of small ruminants, infected with PPRV. Therefore, other sensitive and specific laboratory test should be used for detection of PPRV in faecal samples of persistently infected animals

Snake bite in jersey cattle; a case report

Farooq U, Irshad H, Ullah RW, Ullah A, Afzal M, Latif A, Zahur AB (2014) Res. J. Vet. Pract. 2 (5): 82-83.

ABSTRACT

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.

Isolation and characterization of lineage-IV *Peste des Petits Ruminants* (PPR) virus strains from Pakistan

Zahur AB, A Ullah, H Irshad, A Latif, RW Ullah, M Afzal, SA Khan, MH Samo, K Mahboob. International Journal of Innovation and Applied Studies. Vol. 8 No. 1 Sep. 2014, pp. 185-194. <http://www.ijias.issr-journals.org/abs.php?article=IJIAS-14-201-06>

ABSTRACT

A total of 62 Peste des Petits 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, I_c-ELISA, virus isolation and RT-PCR. Of 397 tissue samples, 65% tested positive by I_c-ELISA. Six PPR virus isolates were obtained through cell culture on VERO or GKC cell from 61 I_c-ELISA positive samples identified by characteristic CPEs and confirmed by testing the cell culture supernatant by I_c-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).

Epidemiology of Bovine Brucellosis- a review of literature

Ullah RW, Waqas MY, Shah MA. Veterinaria 2014; 2: 16-19.

ABSTRACT

Brucellosis is mainly caused by *Brucella abortus* in bovines which results in great effect on economy, reduced milk production, abortions in last trimester, long calving interval. In Pakistan incidence is increases 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 to neighboring countries.

Gross pathological findings of rabbit hemorrhagic disease (RHD) in two (02) cases

Farooq U, Ullah RW, Latif A, Zahur AB, Dasti JI and Irshad H (2013).. Res. j. vet. pract. 1 (4): 39 – 40.

ABSTRACT

Laboratory animals like mice, rabbits, guinea pigs are the key experimental animals in research laboratories. This report is about the domestic angora rabbits which were kept at Animal Health Research Laboratories (AHRL), National Agricultural Research Centre(NARC) Islamabad for research purpose. Suddenly death occurred in two of rabbits. These rabbits were about ten (10) weeks of age. For proper diagnosis, necropsy was performed in two rabbits and this was diagnosed that these were died of Rabbit Hemorrhagic Disease (RHD) which is caused by a Rabbit hemorrhagic disease virus (RHDV); a member of genus Lagovirus from Calciviridae family. The disease is first time reported in Pakistan in angora rabbits kept for research purpose

PROJECT COMPLETED

PARB Project ID 203

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

ONGOING PROJECT

Missing Facilities at Veterinary Research Institute (VRI) and Foot and Mouth Disease Research Centre (FMDRC) Lahore

CONSTRAINTS

- i) Shortage of manpower as compared to workload.
- ii) Non availability of Reverse Osmosis (RO) system.
- iii) Non availability of automatic filling and bottling machine.
- iv) Power supply fluctuations.
- v) Shortage of lyophilizer and cap sealing facility.
- vi) Lack of training of para-technical staff.
- vii) Lack of training of technical staff in advanced techniques.
- viii) Administrative control of supply of vaccines under two directorates.

- ix) Shortage of space for vaccine storage.
- x) Manual data entry in supply section.

FUTURE PLANS

- i) To enhance the quantity and quality of biologics.
- ii) To introduce new vaccine (Lasota) against Newcastle disease in poultry.
- iii) Shifting of vaccine production from conventional method to fermenter technology.
- iv) To make possible the availability of lyophilizer, automatic filling machines, other equipments and machinery required for vaccine production.



Worthy Secretary Livestock inaugurated three newly produced Hemorrhagic Septicemia vaccines HS- LA, HS-50, HS-plus



Worthy Secretary Livestock examining the diluent
(A new product of VRI)