Pak. J.L.Sc.Vol .(1) No.1-2009

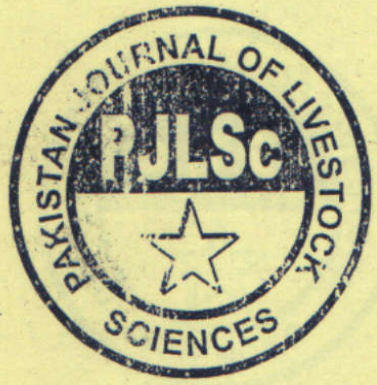
Volume 1 December, 2009 No. 1

ISBN: 978-969-9219-
ISSN 2077-933X

PAKISTAN JOURNAL OF LIVESTOCK SCIENCES

Established 2008

FIRST PUBLISHED
2009



ISLAMABAD

DR. MUHAMMAD HAFEEZ
B.V. Sc, B.Sc AH, M.Sc (Hons) AH, M.Sc. Vety: Sciences U.S.A
CHIEF EDITOR
Author ISBN-978-969-9219

PUBLISHERS
Pak: Livestock Welfare Organization®

Basement Rehman Plaza, Main Murree Road, Bhara Kahu, Islamabad.
Cell: 0345-9727722, 0300-5193723, E-mail: dr.mhafeez1949@gmail.com

Volume 1 December, 2009 No. 1

ISBN: 978-969-9219-
ISSN 2077-933X

PAKISTAN JOURNAL OF LIVESTOCK SCIENCES

Established 2008

FIRST PUBLISHED
2009

ISLAMABAD

DR. MUHAMMAD HAFEEZ
B.V. Sc, B.Sc AH, M.Sc (Hons) AH, M.Sc. Vety: Sciences U.S.A
CHIEF EDITOR
Author ISBN-978-969-9219

PUBLISHERS
Pak: Livestock Welfare Organization®

Basement Rehman Plaza, Main Murree Road, Bhara Kahu, Islamabad.
Cell: 0345-9727722, 0300-5193723, E-mail: dr.mhafeez1949@gmail.com

ISBN ISBN Registered with National Library of Pakistan Islamabad – 978-969-9219-

ISSN Applied

Chief Editor's Registration Registered as AUTHOR with National Library of Pakistan ISBN No. 978-969-9219

Registration with Higher Education Commission Case of Registration in HEC will be filed after the publishing of First Volume, First Issue in 2009

Registration with Pakistan Science Foundation Case of Registration in will be filed with PSF after publishing of First Volume, First Issue in 2009.

SUBSCRIPTION RATES

Pakistan (inland)	Rs. 200/- (Plus Mailing Cost)
This is for one copy per year	
Foreign	US $ 30 (Plus Mailing Cost)
UK Pound Sterling	03 (Plus Mailing Cost)

Commercial and Advertisements
(One Payment for one Volume/issue)

1.	Full out side Cover	Rs.20,000/-
2.	Full inside Cover	Rs.15,000/-
3.	Full page inside	Rs.10,000/-
4.	Half page inside	Rs.8,000/-
	(100 prepared ads be submitted)	

PAK. LIVESTOCK WELFARE ORGANIZATION® (PLWO)

REGD. NO. VSWA/ICT/1274-701

A BRIEF INTRODUCTION

DOCUMENTATION

- PC-Is Preparation
- Feasibilities
- Technical Reports
- Company Profiles
- Training Manuals
- Lectures
- Text Books

ADVISORY SERVICES AND CONSULTANCIES

- Animal Health Coverage
- Animal Production Supervisory Services
- Livestock Management Services/Organizing Trainings
- Establishing Livestock Farms

CONSULTANCY FOR SURVEY STUDIES

- Livestock Markets Dairy/Milk & Meat Animals
- Fodder Production & Marketing
- Milk Marketing
- Animal By-products
- Food Processing

HEAD OFFICE:
BASEMENT REHMAN PLAZA, MAIN MURREE ROAD, BHARA KAHU, ISLAMABAD.
CELL: 0345-9727722, 0300-5193723 , E-mail: drhafeez1949@gmail.com

FARSALAN CONSULTANCY SERVICES

DOCUMENTATION: - PC-Is Preparation - Feasibilities - Technical Reports - Company Profiles - Training Manuals - Lectures - Text Books

CONSULTANCY SERVICES: - Animal Health Coverage - Animal Production Supervisory Services - Livestock Management Services. - Establishing Livestock Farms

REGISTERED PUBLICATION OF FARSALAN CONSULTANCY SERVICES ISLAMABAD

AUTHOR DR. M. HAFEEZ ISBN CODE: 978-969-9219

S.#	Title	Year	Cost (Rs)
1.	**Livestock Industry** – Code 782,	2006-07	AIOU Book Series
2.	**Animal Health and Production Workers (AHPWs) Training Manual** – Urdu - 80 pages ISBN: 978-969092190009 Registered with Copyright IPO Karachi	2006	Rs.100/- Each + Mailing Cost
3.	**Livestock Industry 1st Edition** – English 230 pages ISBN: 978-969-9219-01-06 Registered with Copyright IPO Karachi	2007	Rs.360/- Each + Mailing Cost
4.	**Livestock Economics and Business Management** – English, 225 pages ISBN: 978-969-9219-02-3	2008	Rs.360/- Each + Mailing Cost NBF
5.	**Livestock Sector Development in Pakistan** – English, 215 pages (ISBN and Copyright handed over to HEC Islamabad under agreement)	2008-09	HEC Pakistan Rs.400/- Each + Mailing cost
6.	**Investment in Livestock Sector: A Sustainable Business in Pakistan – English,** 220 pages ISBN: 978-969-9219-03-0	2009	Rs.400/- Each + Mailing Cost
	NEW ARRIVALS/UNDER FINAL STAGES OF PRINTING		
7.	**Livestock Industry 2nd Edition** English, 220 pages ISBN: 978-969-9219-	2010	Rs.360/- + Mailing cost
8.	**Economic Losses due to Major Livestock Disease in Pakistan** English, 150 pages ISBN: 978-969-9219-04-7	2010	Rs.400/- Each + Mailing Cost
9.	**Livestock Micro-Economics** English, 250 pages ISBN: 978-969-9219-	2010	Rs. 450/- + Mailing Cost

TERMS AND CONDITIONS

1. Minimum order 02 books each
2. Delivery of books within FORTNIGHT time after receiving the written order.
3. Payment acceptable by DDO or advance cheque/cash in the name of Dr. Muhammad Hafeez Chief Executive, Farsalan Consultancy Services, Rehman Plaza, Main Murree Road, Bhara Kahu, Islamabad.

Dr. Muhammad Hafeez
Chief Executive FCS
Cell: 0345-9727722

FARSALAN CONSULTANCY SERVICES

KEY NOTE BY THE PATRON IN CHIEF

It gives me an immense pleasure and satisfaction to see the efforts of Dr. Muhammad Hafeez The Chief Editor and his colleagues in bringing out this First issue of Pak. Journal of Livestock Sciences (PJLSC) under the Pak. Livestock Welfare organization (PLWO) Islamabad. The Editorial Board comprises competent Researchers and highly qualified Veterinary Scientists. The Referees are also subject specialist from various Teaching and Research Organizations of the country.

Dr. Muhammad Hafeez and his colleagues started these efforts in 2008 when the Board of Editors & Referees were finalized. First call for the Scientific papers / Articles was made to various Universities of the Country, all DGs of LDDDs in provinces including the VRIs requesting for the scientific papers for publication.

Dr. M. Hafeez is a competent Veterinary Scientist, a mature researcher and a seasoned administrator with a background of DVM of 1972, an MSc (Hons) from NWFP Agricultural University 1983 and a US Graduate of 1988. He remained a Research Office

For 20 years at VRI Peshawar (1972-1992). He remained Assistant Animal Husbandry Commissioner in MINFAL for 10 years (1992-2001).He remained Consultant / Assistant Professor and Program Coordinator for MSc (Hons)/M.Phil Livestock Management Department of Agricultural Sciences of AIOU Islamabad (2002-2005).He has 42 publications and 05 books on his credit and remained thesis supervisor of 13 MSc (Hons)/M.Phil students of various Universities of the Country. He remained practically involved in the publishing of Journal of Animal Health & Production (JAHP) in the capacity of Co-Editor for 7-8 Years, from VRI Peshawar.

He is now running an independent NGO The Pak Livestock Welfare Organization ® in Islamabad and doing tremendous work for Livestock Sector (details available in the Breif introduction of PLWO). The sister Organization of Farsalan Consultancy Services(FCS) is exclusively working in publishing Textbooks for Students of Livestock Management . A list of Books is available in this write-up.

A total of Nine(09) Research papers/Articles were received , sent to Concerned Referees and Advices, Suggestions ,amendments thus recommended were conveyed to the Principal authors Contributors. Final drafts were placed before the Board of Editors and Eight(08) articles /research papers were approved for this issue. Foreign Referees are being consulted for submitting their acceptance, in each discipline of Livestock Sciences.

HEC and PSF authorities were taken into confidence for possible funding but it was subject to Publishing of first Volume on Self Finance basis.

I appreciate such an effort and support in letter & spirit that such dedicated Scientists devoted their time for this print out without any remuneration .I wish all success for their future endeavors so that they can keep a liaison between the Scientists , Researches, Teachers Graduate students & farmers, with this positive activity, in private sector

This ,I am sure ,will get every appreciation from within the country and abroad.

December 2009

DR. MASHOOK ALI BHUTTO
Patron in Chief

PAKISTAN AGRICULTURAL RESEARCH COUNCIL

Key Note Address

It is a matter of some satisfaction that Veterinary Personnel have contributed to the "Pak Journal of Livestock Sciences", an innovative effort of its Chief Editor, Dr. Hafeez. The fact that some documentation is being produced in a society which is otherwise a believer of verbal innuendos. It is in this sense that this Journal coming out of PARC-Private Sector collaboration is a welcome change.

The fact that the written word is a permanent document means that for the first time these personnel have put their convictions on paper and have tried to get their colleagues to do the same. This is a beginning and a very positive one and how far it will go towards innovative work is yet to be seen. As a nation, we were subjected to controlled mechanisms created during the colonial times and these colonial mechanisms are again prevailing here. When you write with the heart and head you don't have to seek any reaffirmation from any source. The concept of refereed articles is to control our minds that the West has imposed on us by suggesting that their minds are superior to ours. Our minds are not debilitated. But as the unused land is a waste-land so the unused mind is a wasted mind.

The more you exercise your mind the better it will become. In quantum terms mind of human being is 31.5 gm whether it is of Einstein or of our colleagues. Given this the human beings that apply their mind, they are supposed to exercise this facility in different innovative ways.

In saying the above, I am only mentioning the fact that the beginning has been made. One can keep on praising oneself or we can redouble the efforts to make the quality of the material that it contains. I don't want to take anything away from the people who have done this. It is a great activity but there is always room for improvement. I wish them well! I don't know whether that will continue but I sincerely hope it will.

(Dr. Zafar Altaf)
Chairman

P.O. Box 1031, Plot 20, G-5/1, Islamabad, Pakistan. Tel: +(92-51) 9203966, PABX No: 8442000
Fax: +(92-51) 9202968, E-mail: chair@comsats.net.pk

SCREENING POTATO VARITIES FOR TOTALGLYCOALKALOIDS AND SALT TOLERANCE

Abdullah, Z. ; Ahmad ,R.; Nowshad,K. & *B. Raja .
Department of Agricultural Sciences, Allama Iqbal Open University , Sector-H-8 Islamabad, Pakistan
National Agricultural Research Centre, Islamabad, Pakistan
Email of corresponding author: aiouniversity@hotmail.com

Potato is a good source of carbohydrates in our diet. Unfortunately it contains two main Glycoalkaloids solanine and chaconine which are present more than 20 mg/100mg Fresh Weight (F.wt.). The tubers render them unfit for human consumption (1-4). Such alkaloids containing potatoes if eaten, more than this limit, may cause headache, fever, vomiting, gastrointestinal disorders, diarrhea, drowsiness, cardiovascular and respiratory depression including deterioration of muscles, anemic and hyperglycemic conditions (5-12). Concentration of these Glycoalkaloids generally remains below the maximum permissible limits but occasionally genetic setup, climatic conditions and bad cultural practices increase their concentrations (13-14 ,20, 21,22). Hence there is a great necessity to keep on monitoring glycoalkaloids contents of the potato tubers grown under various climatic regions. Potato tubers were collected from various ecological regions of Pakistan for this investigation. Their TGA concentration was determined based on the methodology of Baker *et al* (15).Table (1) shows that maximum concentration of TGA within the permissible limit, in the potato tubers grown at Punjab and N.W.F.P planes in both the seasons whereas it is slightly on the higher side in the potato tubers collected from Sindh.

Table-1. Concentrations of TGA in potato tubers collected from different ecological regions of Pakistan.

Place	Corps	Range of TGA (mg/100g. F.Wt.)	Mean ± SE
Sind (Mirpurkhas)	Autumn	5.7-33.4	12.18±0.72
	Spring	5.0-27.5	11.56±0.63
Punjab (Faisalabad)	Autumn	5.5-20.1	12.34±0.52
	Spring	4.2-20.8	10.78±0.60
N.W.F.P (Tarnab)	Autumn	5.5-19.4	11.68±0.59
	Spring	6.6-15.3	9.72±0.60
Punjab (Murree Hills)	Summer	9.0-24.4	15.45±1.17
Baluchistan (Sariab)	Summer	8.9-20.1	13.58±0.61
N.W.F.P	Summer	13.2-16.9	14.88±0.89
Parachinar	Summer	18.5-58.7	30.32±8.32
Kagan Velly Swat	Summer	7.7-53.7	23.39±6.23
Azad-Kashmir (Rawalakot)	Summer	5.6-31.2	13.69±1.45
Gilgit	Summer	7.7-14.5	11.75±1.41
Chitral	Summer	7.7-17.6	12.94±1.77

However, this increase is due to the TGA values of few varieties of test trials which are not commonly grown by the farmers in this provinces (e.g. Bintje, 29.00 and Kufri Sindhuri, 33.48 mg/100g. F.wt.). Slight increase of TGA content in var. Multa (Autumn crop 22.00 and Spring crop 20.00mg/100mg F.wt.), which gives a good tonnage in Sind, does not carry any genetic bases of increasing TGA content. This variety has shown much lower TGA values in other part of the country. Neither climatic factors were found to be responsible for this effect. It appears that some faulty cultural practices have caused greening of potato tubers which has resulted in slight increase of TGA levels.

Potato tubers collected from the summer crops grown at higher altitudes show normal TGA values at Sariab, Parachinar, Gilgit and Chitral, but that of Murree Hills, Kagan Valley, Swat and Azad Kashmir are at higher side. The maximum concentrations of TGA found at Murree Hills (24.4 mg/100g. F.wt.), Kagan Valley (58.74 mg/100g. F.wt.), Swat (53.74 mg/100g. F.wt.) and Azad Kashmir (31.24 mg/100g. F.wt.), are very high and alarming. This tendency is well demonstrated by the mean TGA values of these areas. Except var. Veltocenia (TGA-50.75 mg/100g. F.wt.), which was grown for trail at Swat, all the other varieties (e.g. Desiree, Cosima and Multa) have been giving a good tonnage and normal TGA values in the other parts of the country. Hence increase of TGA contains at higher altitudes could not be taken as genetic effect. Variety Ultimus which is vary popular variety at mountains has never shown a higher TGA values. However there have been occasional heavy rainfalls in these areas which often remove soil from the root zone exposing developing tubers. Sunshine following rainfall turns the exposed tubers green thus providing conditions for the synthesis of glycoalkaloids in potato tubers.

Once these glygoalkaloids are syntheses in tubers, they do not disappear even after the exposed greened potatoes are covered with soil. The authors happened to visit these placed one week after the rains and found many fields covered with exposed greened potatoes. A timely earthing of exposed potatoes after each rain would avoid greening of developing potato tubers and synthesis of glycoalkaloids in these areas. Occurrence of high glycoalkaloids containing potatoes in certain areas of Pakistan suggests the need of continuous monitoring for this parameter to avoid the supply of these toxic potatoes for human consumption.

Studies on Salt Tolerance

Potato has been classified as moderately salt tolerant crop. It is capable of withstanding 0.2 to 0.5% salts (EC.4-10 m. M), in dry soil of average texture and that of 1.5 to 3.0 g/l salts in irrigation water (EC.2-5 m. M) under the most permeable soil conditions with adequate drainage (16). Chemical amendments in saline water or soil and suitable cultural practices extend the range of salt tolerance beyond the above mentioned limits. Furthermore, potato being a crop of vegetative phase, does not cross the barrier of reproductive phase which is most susceptible to the salinity.

Growth of the tubers is dependent upon translocation photosynthetic which does not face a direct conflict with saline environment. Our experiments on normal soil have

shown that a critical amount of foliage is essential for producing tubers whereas a luxuriant foliage growth reduces the production of potato tubers.

Salinity is reported to slow down plant growth. If a potato variety of good growth potential is raised under saline environment, even after reducing down the vegetative growth, it would have sufficient foliage to meet critical requirement for producing potato tubers. This fact has made potato different from the other crops and it has been possible to grow them under saline conditions.

Many varieties of potatoes were grown in 15 kg earthen pots salinized with various range of salinities at par with saline soils of Pakistan. Changes undergoing in chemical constituents of potato tubers under saline conditions were also investigated (17,18). TGA contents of the tubers grown at saline soil were found slightly lower than those grown at normal soil. A comparative performance of different varieties with regard to tonnage under normal and saline soils is given below:-

Garding	Performance under None saline conditions	Performance under saline Conditions (about 1% salinity)
1.	Multa	Red Lasoda
2.	Red Lasoda	Multa
3.	Norland	Patrones
4.	Chieftain	Norland
5.	Patrones	Chieftain
6.	Cardinal	Cardinal
7.	Red Bed	Red Bed

Sandy loam provide a good strata for potatoes cultivation using brackish water for irrigation. Saline water with chemical additives percolates down the root zone thus reducing its toxic effect. Coastal belt is generally rich in dewfall during the autumn crop, which provide fresh water for foliar absorption. These factors help in potato growth at coastal sandy belt. Var. Multa and Atom value were grown on coastal sand through irrigation with water having a salt concentration up to 10000 ppm, (EC 4-10 m. Mohs) (19). There was about 50% reduction in yield of tubers at above mentioned highest salt concentration, reduction in yield in irrigation water containing 4000 and 6000 ppm salts being about 25 to 35% may still be of some economical proposition where other wise water is not available and any other crop of commercial importance could not be grown. Sub-soil water of lower salt concentration with chemical additive could be used for irrigation at sandy strata for growing potato crop.

Literature cited

1. Wood, F.A. and Young, D.A. (1974). TGA in potato. Agric. Canada Bull. 1533.
2. Ruddick, J.A., Harwing, J.J. and Scott, P.M. (1974). Non-teratogenicity in rats of blight potatoes and compounds contained in them. Teratology, 9, 1765-168.
3. Wu, M.T. and Salunkhe, D.K. (1976). Changes in glycoalkaloids contents following mechanical injuries of potato tubers. J.Am. Soc. Hort. Sci. 101: 329-331.
4. Mcmillian, M. and Thompson, J.C. (1979). An out break of suspected solanine poisoning in school boys. Quarterly. J. of Medicine. 48: (190): 227-243.
5. Willimett, S.G. (1974). An investigation of solanine poisoning Analyst, 58: 431-439.
6. Wilson. G.S. (1959). A small out break of solanine poisoning Monthly Bull. Minist. Healt Lab.Serv. 18: 207-201.
7. Jadhav, S.J. and Salunkhe, D.K. (1995). Formation and control of chlorophyll and glycoalkaloids in tubers of S. tuberosum. Adv. Fds. Res. 21, 3070354.
8. Nicholson, J.W.G.; Mcqueen, R.E. and Burgess, P.L. (1977). Preservation of feed potato by ensuling with dry forage. Can. J. Anim. Sci. 57, 289-294.
9. Osman, S.F.; Herb, S.F.; Fitzpatrick, T.J. and Schmiedeiche, P. (1978). Glycoalkaloids composition of wild and cultivated tuber bearing solanum species of potential value in potato breeding programme. Agric. And Fd. Chem. 26: 1246-1248.
10. Nishie, K.; Gumbmann, M.R. and Keyl, A.C. (1971). Pharmacology of solanine. Toxicol and App. Pharmacol. 19. 81-92.
11. Satoch, J. (1967). Glycemic effects of solanine in rats. Jap. J. Pharmacol. 17, 625-658.
12. Ahmad, R.; Abdullah, Z.; Azim,A.; Ali,F. and Akhtar, P. (1979). 3rd Annual report on "Glycoalkaloids of Pakistan Potatoes" (FG-Pa-262), Botany Department, Karachi University.
13. Sinden, S.L. and Webb, R.E. (1974). Effect of environment on glycoalkaloid content of six potato varieties at 39 locations. Tech. Bull. 1472, Agric. Res.Ser., U.S.A., 1-28.
14. Cronk, C.T. Khun, G.D. and McArdle, E.J. (1974). The influence of stage of maturity, level of nitrogen, fertilization and storage on the concentration of solanine in tubers of three potato cultivars. Bolletin of Environ. Cont. Toxicol.1, 1: 163-168.

15. Baker, L.c.: Lampitt, L.H and Meredith, O.B. (1995). Solanine glycoside of potato III. An improved method of extraction and determination. J. Sci. Food. Agric. 6,197-202.

16. FAO/UNESCO. (1973). Irrigation Drainage and Salinity. An International Source Book (ed. By N.A. Kovda). Camelot Press Ltd. London, 254-290.

17. Ahmad, R. and Abdullah, Z. (1979). Salinity induced changes in growth and chemical composition of potato. Pak . Jour. Bot. II. 103-112.

18. Ahmad, R. and Abdullah, Z. (1982). Potato cultivation using sea water dilutions for irrigation. J. Agron. & crop. Sci. 151, 75-80.

19. Abdullah, Z and R. Ahmad. (1981). Effect of saline soils amended with calcium nitrate on the growth and chemical constituents of potato plants. Pak. J. Bot.13: 55-67 .

20. Everad J Edwards, Robert E Saint and Andrew H.cobb. (1998).Is there a link between greening and light enhanced Glycoalkaloids accumulation in potato (*Solanum tuberoum* L.) tubers? J.Sci. Food Agric. 76:327-333.

21. Everad J Edwards and Andrew H.cobb. (1997). Effect of temperature on glycoalkaloids and chlorophyll stored at low photon flux density, including preliminary modeling using an artificial neural net work. J.Agric. Food Chem. 45:1032-1038.

22. Nitithamyong A, Vonelbe JH, Wheeler RM, Tibbitts TW. 1999. Glycoalkaloids in potato tubers grown under controlled environments. Am.Potato J. 76(6):337-43.

Article No. 006/1/9

THE CONCEPT OF ESTABLISHING CBO BASED ANIMAL HEALTH AND PRODUCTION CNETRES (AHPCs) IN PAKISTAN

Dr. Muhammad Hafeez
Chief Executive, PLWO Islamabad.

ABSTRACT

Ministry of Social welfare GOP encourage the establishment of Community Based Organizations (CBS) Registered as NGOs under the Registration Voluntary and Social Welfare Associations (VSWA) Act 1961. Trust for Voluntary Organization (TVO), a subsidiary of M/O Social Welfare and a Funding agency provides grant assistance upto one million rupees (Rupees ten lacs) to NGOs for their social welfare activities in at least 170 areas. Based on Project proposals on TVO format.

Pak Livestock Welfare Organization ® (PLWO) assists preparation of farmers both in Agriculture and Livestock in project proposal (on TVO format) in which the establishment of Animal Health and Production Centres (AHPCs) is an activity which is confined to any Community Based Organization (CBO). These centres are functioning on sustainable basis. Provision for funds for veterinary medicines, veterinary vaccines and fodder crops seeds to a tune of Rs. 0.3 millions (Rupees three lacs) in ear-marked. Such centres are operational in Sindh, Punjab, NWFP and North Areas and Flourishing on Self Reliance basis. A Component of income generation in built, in the body of the project proposal in getting appreciation from donor agencies, It is also an approach towards poverty alleviation.

Key words:- NGOs Livestock AHPCs CBOs - Rural Dev. Poverty Alleviation

INTRODUCTION

With the increasing demand of animal proteins (through milk and meat) and supportive marketing indicators as sustained growth in purchasing power of our people despite price hike, livestock production is showing increasing trend of growth.

This can be seen through population and production figers of livestock in recent years as reflected in the Economic Survey Report 2008-09 [1], as compared to previous years reflected in the Economic Survey Report 2007-08 [2]

Ministry of Livestock and Dairy Development (FMLDD) Govt. of Pakistan (GOP) Islamabad and Provincial Livestock and Dairy Development Departments (L&DDDs) of Punjab, Sindh, NWFP and Balochistan including AJK and NAs are continuously providing Animal health and Livestock production services through an establishment of 800 plus Central Veterinary Hospitals (CVHs), 2000 Veterinary Dispensaries and 3000 Veterinary Centres manned with 1800 Veterinary Graduates working as Veterinary Officers Health (VOH) supported with upto 6000, Para-Veterinary Staff together with 350-360 Veterinary Officer Production (VOP).

For the last 20-25 years it is being advocated that with this establishment Animal Health Services can beand are being provided to Not more than 15% of the total livestock population of the country.

Three Veterinary Research Institutes (VRIs) at Peshawar, Lahore and Quetta are producing bacterial, viral and rickettcial vaccines for cattle, buffaloes, sheep/goats and poultry while two Poultry Research Institutes (PRIs) at Rawalpindi and Karachi are not only carrying out Research work but also exclusively producing vaccines for Poultry and Fetching millions of rupees, yearly, based on receipts as Revenue for the state as presented in Annual reports e.g. of

VRI Peshawar have sold only NDV worth 9 million rupees in a single year i.e 2007-08. [3], While Annual reports of VRI Lahore claims to have earned Rs. 20-21 million in the year 2007 for sale of HSV, BQV, ETV and ASV [4], Annual Report of VRI Quetta [5] and Annual Reports of PRI Rawalpindi 2007-08 [6], and Poultry Vaccine Production Centre Karachi 2007-08 [7] Livestock and Poultry Vaccines are directly sold to farmers while most of the farmers are getting their livestock and poultry vaccinated each year.

A recent effort was made by the Ministry of Livestock and Dairy Development GOP to formulate a "Farmer Friendly" policy based on a consultative group of various subject specialists. Deliberations were made and complied as recommendations in the "Stake-holders workshop on Development of Livestock Policies and Action Plans" in 2004 [8] and implementation is being on its way.

The Prime Minister of Pakistan, showing his concern in July -2009 directed the MLDD GOP to prepare a new Livestock Policy for the betterment of Livestock Sector.[17] In the previous policy announced in 2007, an important aspect was to encourage private sector to enhance livestock production, NGOs took the initiatives in encouraging CBO oriented Livestock Societies to establish Animal Health and production Centres (AHPCs) where small farmers have identified themselves as Community Based Farmers Organizations.

This concept is also being encouraged under the EC assisted 'Strengthening of Livestock Services Project" (SLSP) being operational in the country w.e.f 2003 which is near completion in 2009 (Several Societies have since been developed and farmers are working in a coordinated atmosphere with full support of Govt. Institutions as detailed in SLSPs Annual Report 2007-08 [9]

Pak. Livestock Welfare Organization ® (PLWO) was established to assist farmers in the preparation of various documentation such as feasibilities, project proposals, establishing AHPCs, organizing Animal Health and Production Workers (AHPWs) Trainings and providing services in Animal Health and Production. Details can be seen in the First and Second Annual Reports of PLWO [10], [11] as well Project Proposals submitted to Trust for Voluntary Organization (TVO), Islamabad, funding agency of Ministry of Social Welfare and Local Govts. Islamabad [11] not only for ICT Islamabad but also for other organizations such as Livestock Development Foundation ® in Tando Allahyar as detailed in their First and Second Annual Reports of LDF [12], [13].

Project Proposals For Establishing An AHPC

Project proposals are prepared for establishing an Animal Health and Production Centre (AHPC) which is run by the Farmers on Self reliance basis on income generation. Such project proposals are prepared by PLWO Islamabad and submitted to funding agency by the Farmers Community as already prepared for ICT Islamabad, Tando Allahyar Sindh, Peshawar, Tangir, NAs and Lahore [14] [15] [16] The Total cost of the project is Rs. One million with no recurring cost, as the project is run by the Farmer Community on income generation basis.

AHPCs Organization

Under their Constitution Membership is based on a token fee of Rs. 50/- per year and farmers meet EVERY WEEK to discuss problems and find solutions through their Resolutions. The office bearers form an Executive Body (with equal member 50% of members from office bearers and 50% nominated. This body meets monthly.

Working of An AHPC

In such a proposed centre (already working in countable places in the country) Farmers of a Union Council numbering 60-80 with 1000-2000 cattle and buffaloes together with sizeable sheep and goat population form a farmers society/NGO/ CBO with their elected office bearers, work within their constitution and get Registered under Social Welfare and Voluntary Organizations Act 1960 of GOP and Provincial Govts. This comprises the following itemized actionable components:-

(i) AHPWs Training

There is an allocation of Rs. 80000/- for this purpose, fifty – seventy interested farmers are provided ten (10) days training. This training is based on a Manual written in Urdu with some important English terminology with three lectures per day and two practical /on hand training in the nearest animal clinic and a Running Dairy Farm.

(ii) AHPWs Training Manual

This is a Registered Manual of 70 pages with cost of Rs. 100/- written by Dr. M. Hafeez, in 2006 with ISBN-978-969-9219-009 and copyright No. 17213- copr. Th cost of 50 AHPWs Training Manual is included in the project proposal.

(iii) Revolving Fund (Rev-I) for Veterinary Medicines

There is a provision of Rs. 2,00,000 (rupees two lacs only) for the purchase of veterinary medicines. There will be sold to the farmers, based on the prescription of the Veterinary Doctor. The medicines are purchased on the whole sale rates and marginal saving goes to the AHPC.

(iv) Revolving Fund (REV-II) for Veterinary Vaccines

A minimum of Rs.One lac only (Rs. 1,00,000) has been allocated for the purchase of Four-Five Veterinary Vacines used against endemic diseases of cattle/buffaloes namely HSV, BQV ASV ETV and FMD while in sheep and goats ETV and CCPPV. The vaccinations will be done by the SA/Vas and the farmers as a combined team of vaccinators. The amount of income generated i.e. Rs.50/- per cattle and buffaloe and Rs.30/- per sheep and goat will again become revolving fund for next year.

(v) Revolving Fund (Rev-III-F) for Fodder Crops

A minimum of Rs. 50,000/- (Rupees fifty thousand only) are always earmarked to provide Rs. 5000/- to Rs. 6000/- to famrers with 100-200 Kanals of Agricultural land for growing fodder crop. After paying the labor cost and costs of pesticides and fertilizer (Natural fertilizer from the farmer community is preferred) Fifty (50) percent fodder grow and harvested will go to the AHPC. In come places 40% of the fodder goes to AHPC.

(vi) Budget Break-Up

Salaries/Allowances to AHPC

a. There is a provision of an allowance to Veterinary Doctor on weekly or twice a week visit to the AHPC for Routine Veterinary services and provision of allowances to two Veterinary Assistants on weekly visit basis including one AHPC Assistant/Computer Operator is also earmarked in the budget break-up.

b. Fifty percent (50%) of the building rent not more than Rs.36,000/- Rupees thirty six thousand only is also included in the first year budget.

c. One potential farmer who is trained as AHPW and has sufficient place for opening of AHPC with water electricity, security of medicines, Animal Health and Production

Workers Kit (AHPW Kit) and a meeting space for farmers is selected by the Community and made him responsible under an Agreement (available in the AHPW Training Manual). Between farmers community and the sponsors.

d. Allocation for Animal Health and Production Workers Kit (AHPW Kit) is a provision of 42-43 items AHPWs Kit used for Animal Health and Production under the Direct Supervision of the Veterinary Doctor and Veterinary Assistant. The cost of these times was Rs.30,000/- in the year 2008 and Rs.4000/- in (May-2009) which is subject to revision.

e. Equipment such as one Office Computer, Telephone (PTCL) or mobile set is also included along with Record keeping Registers, Receipts and prescription chits etc

f. Provision of one Fridge for keeping medicines and vaccines is also included some utility bills such as are also included electricity, phones, CNG and Travelling allowance for filled work is included.

g. The Centre (AHPC) is also supposed to keep record for various vaccinations as laid down in the project proposal (vaccination proformas ABCD-E and for fodder crops F, a cash book for financial record and all finances must be transacted through a Bank where farmer community keeps its accounts.

INCOME GENERATION

The proposed AHPCs where functioning are running on income generation basis. The income generation and sustainability plan is in-built in the project proposal. This includes the following heads/sources of income generation:-

(a) Registration of 1000-2000 buffaloe/cow @ Rs.50/- each
(Farmer will get 4 (Four Vaccinations free of Cost) in First Registration.)

(ii) Second Vaccination will be @ Rs. 50/- Each / year

(iii) Registration of Sheep and goat @ Rs. 30/- each / year
(Farmer will get three vaccination free of cost)

(iv) Sheep and goats will be vaccinated @ Rs. 30/- each/year

(v) Deworming of adult large animals @ Rs. 50/- each/yearly

(vi) Deworming of sheep and goats @ Rs. 30/- each/yearly.

(vii) Spray of insecticides/Parasiticides for large cattle and buffaloes @ Rs.50/- each, once

(viii) Spray of insecticides/Parasiticides for sheep and goats @ Rs. 40/- each

(ix) Horses spray @ Rs. 70/- each once

(x) Camel spray @ Rs. 100/- each once

(xi) Dogs cats spray @ Rs. 40/- each once

(xii) Animals Clinical exam Cattle/Buffaloes @ Rs. 50/- each (weekly)

(xiii) Animal Clinical exam sheep and goats @ Rs. 40/- each (weekly)

(xiv) Surgical operations – according to nature of the case

(xv) Fodder production 60% will go to the farmer while 40% of the cost of fodder will go to the AHPC

SUSTAINABILITY

With the Registration of 1000-2000 cows/buffaloes and equal number of sheep and goat with 50 farmers growing 50,000 monds seasonal crops twice a year can easily run such centres and will play important role in increased healthily milk and meat production in the country. It has also been observed that such centre are not only progressing well but famers are getting inclined

toward CBO based Community Centres/AHPCs and becoming models for the neighboring villages/farmers.

RECOMMENDATIONS

1. Small farmers can identify themselves get registered as CBOs/NGOs/Famers societies and establish such a centre.
2. Such a society where 1000-2000 milch cows/buffaloes together with equal or double the number of sheep/goats are available can run such a centre on sustainable basis.
3. Agricultural land of 50 farmers with not less than 100-200 kanals each can establish an AHPC and prove agro-livestock coordination model.
4. Each year 50-100 farmers can be trained without recurring cost from the sponsors.
5. Services can be hired from one Veterinary Officer and two Veterinary Assistants to run one centres on Regular basis.
6. Such model centres will be followed by other interested farmers which will lead not only to self reliance but to poverty alleviation and increased healthy livestock production.
7. Animal Health Coverage will also reach to far flung areas.

REFERENCES

1. Anonymous (2008-09) Economic Survey of Pakistan Economic Advisory Cell Ministry of Finance, Economic Affairs GOP Islamabad.
2. Anonymous (2007-08) Economic Survey of Pakistan Economic Advisory Cell. Ministry of Finance and Economic Affairs GOP Islamabad.
3. Anonymous (2008-09) Annual Report of Veterinary Research Institute – Bacha Khan Chowk Peshawar.
4. Anonymous (2007-08) Annual Report of Veterinary Research Institute – Ghazi Road Lahore.
5. Anonymous (2007-08) Annual Report of Veterinary Research Institute – Brewary Road – Quetta.
6. Anonymous (2007-08) and (2008-09) Annual Reports of Poultry Research Institute, Shamsabad Rawalpindi.
7. Anonymous (2007-08) Annual Report of Poultry vaccine Production Centre, Karachi.

8. FAO (2004) proceedings of the Stake holders workshop on development of livestock policies and action plans – Islamabad.

9. Anonymous (2007-08) Annual Report of Strengthening of Livestock Services (SLSP) of Pakistan Project MINFAL– Islamabad.

10. Hafeez M. (2007) Establishment of Animal Health and Production Centre Bhara Kahu ICT Islamabad.

11. Hafeez M. (2008) (2009) First and Second Annual Reports of Pak: Livestock Welfare Organization ® Islamabad.

12. Hafeez M. (2007-08) Improved Livestock Production through establishing AHPC – Tando Allahyar Sindh.

13. Hafeez M. and Mashook Ali Bhuttu (2007-08) Second Annual Report of Livestock Development Foundation (LDF)

14. Hafeez M. (2008-09). Poverty alleviation through improved livestock production in the Rural areas of ICT- Islamabad. An activity based project proposal submitted to TVO for funding

15. Hafeez M. (2008-09) Strengthening Animal Health and Production Activities of PLWO in various Union Councils of ICT Islamabad – Project proposal submitted to TVO for funding.

Hafeez M. (2009-10) project proposal for establishing Animal Health and Production Centre for Gabber Social Welfare Society Tangir Distt: Diamer.

FACTORS AFFECTING MORTALITY IN BUFFALOES AND CALVES

H.A Hashmi, M. T. Tunio, S.H.Abro and R. Wagan

1.and 2. Department of Agricultural Sciences, Faculty of Science, Allama Iqbal Open University, Islamabad. .3 Department of Biomedical Sciences and Veterinary Public Health (SLU) Uppsala, Sweden. .4 Faculty s of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam Pakistan.

ABSTRACT

The present project was designed to find the effect of dam on calf mortality, the number of still births and abortions , the effect of environment difference on the calf mortality, the relation of age and parity number to calf mortality. Certain managemental and aetio-pathological factors contributing mortality were also studied from data of two dairy farms one located in Okara (Punjab Province) and other in Malir (Sind Province) from March 1997 to February 2002. The unadjusted means and standard deviation (SD) of still birth was 7.59+- 13.49 percent and calf abortion was 2.64+-3.59 percent. Mean value of abortion reveals that herd (farm) and sex were the significant ($P < 0.01$) sources of variation for still birth in buffalo calves. However, herd (farms) year of birth did not affect abortion rate. The variation noted was attributed to the managemental practices. The feeding regime and the age of the dam seem to have contribution in this regard.

Key words: Mortality, causes, calves, buffaloes

INTRODUCTION

Calves are the future herd and keeping them in fit and healthy condition not only makes an effect on a livestock farms' efficiency but also contributes to the economy and production out puts in the years to come. Mortality in the calves renders not only economical losses but also contributes to the low production of the herd when taking futuristic terms for the farming scenario (Ablaha *et al* 1995). This situation contributes to the low per capita consumption of dairy produce and the meat on the national level: which has been estimated to be 150 liters of milk and 19 Kg of meat per head per annum in Pakistan and is far below the recommended dietary allowances level of an individual in most of the advanced countries (Husnain, 2006). Hence, diet of an average common man in Pakistan is critically deficient in animal's protein. This serious shortage in protein supply can be met by enhancing the meat and milk production in the country. Apropos the present study was planned to explore the factors, which contribute to mortality in calves and adult buffaloes.

MATERIAL AND METHODS

Source of data

Records of buffaloes and calves maintained at two Government Dairy Farms situated in Okara (Punjab Province) and Malir (Sind Province) during the period from March 1997 to Feb 2002 were utilized for the present study. Malir is Arid irrigated and weather is hot humid throughout the year with relative humidity from 55±5 to 5±5. While the Okara is canal irrigated and climate is relatively dry. Rains usually occur during the months of July to September with relative humidity ranging from 50±1.5 to a maximum of 82±1.6.1.2 %.

Collection of data

Postmortem examination reports/death reports were consulted thoroughly and related observations were recorded like name and number of the calf, cause of death, sex of the calf and age of the calf at death .Additional information like, date of birth of calf, type of birth, season of birth weight of calf at birth, dam's number and number of abnormal calving (still births and abortions) were also recorded. Other related records were analyzed and observations thereto recorded were, age of dam at the time of parturition, parity number of the dam, herd size, feeding regimes and husbandry practices. Similarly, three age groups of calves made to determine the most critical stage where the mortality was the higher included up to 1 month, 1 - 3 months and above 3 months. Miscellaneous group included bloat, heatstroke, heart attack, diarrhea, dystokia, white scour, snake bite and retention of urine etc.

Statistical analysis

An effort was made to estimate the various factors causing abortion and stillbirth in buffalo calves and mortality in buffaloes. Effect of environmental factors viz, herd, year of birth, type of sex and parity number was calculated by Least Square Analysis of Variance using Mixed Model Least Square and Maximum Likelihood Computer Program (Harvey,1987).

RESULTS

Calf mortality

The data of calves born from March 1997 to February 2002 showed that the mortality rate of 48.26 and 19.18% among 153 and 840 calves at Malir and Okara farm respectively. Stillbirth rate at Malir farm was 30.64 and 34.88 where as it was 22.31% and 23.66% at Okara farm in male and female calves respectively. The unadjusted means and standard deviation of stillbirth was 7.59± 13.49%.The percentage of abortion cases was 67.08, 66.19, 15.68, and 14.06 in male and female calves at Malir and Okara farms respectively. The least square analysis variance for abortion indicated that lactation number (parity number) significantly ($P < 0.05$) affected abortion rate in calves. The calving during the off-breeding season (November – June) resulted mortality in 18.91, 14.13 in males whereas it was 27.00 and 12.31% in females at Malir and Okara farm calves respectively. The greatest losses (13.21 and 2.24%) were caused due to pneumonia followed by enteritis (9.73 and 4.18%) in female and male calves at Malir and at Okara farm.

Dam mortality

There was a marked and significant rise in no of death among third and sixth lactation at both the farms. The highest cause of death remained dystokia, septicemia and pneumonia beside miscellaneous causes.

Table 1. Still birth rate of buffalo calves at two military dairy farms

Farms	Male	Female
	Mortality (%)	Mortality (%)
Malir	30.64	34.88
Okara	22.32	23.66

Table 2. Abortion rate of buffalo calves at two dairy farms

Farms	Male	Female
	Mortality (%)	Mortality (%)
Malir	67.09	66.19
Okara	15.68	14.06

Table 3. Effect of parity number on mortality % in adult buffaloes

Farm	Parity Number					
	1st	2nd	3rd	4th	5th	6th
Malir	5 (5.61)	9 (10.11)	21(23.59)	16 (17.97)	12 (13.48)	27 (30.33)
Okara	20(7.78)	21(8.17)	54 (21.01)	49 (19.06)	51 (19.84)	62 (24.12)

Table 4a. Causes of mortality in the dams of three age groups at Malir farm

Cause of death	Gp-I		Gp-II		Gp-III	
	Mortality	%age	Mortality	%	Mortality	%
Gastroenteritis	4	4.49	5	5.61	5	5.61
Dystokia	2	2.24	3	3.37	2	2.24
Septicemia	6	6.74	8	8.98	10	11.23
Pneumonia	3	3.37	4	4.49	4	4.49
Miscellaneous causes	5	5.61	8	8.98	18	20.22

Gp-I = 3 years Gp-II = 3.1 – 8 years Gp-III = above 8 years

Table 4b. Causes of mortality in the dams of three age groups at Okara farm

Cause of death	Gp-I		Gp-II		Gp-III	
	Deaths	%age	Deaths	%age	Deaths	%age
Gastroenteritis	4	1.55	10	3.89	20	7.82
Dystokia	20	7.82	30	11.67	37	14.39
Septicemia	10	3.89	12	4.66	33	12.84
Pneumonia	4	1.55	10	3.89	8	3.11
Miscellaneous causes	12	4.66	28	10.89	39	15.17

Gp-I = 3 years Gp-II = 3.1 - 8 years Gp-III = Above 8 years

DISCUSSION

Overall Calf Mortality

The data showed that the mortality rate was 48.26 and 19.18% among 153 and 840 calves. Both the farms were in two different climatic zones. In our study the farm at Okara showed overall low mortality i.e. 19.18% as compared to at Malir i.e.48%.

Still birth

The pattern of still birth was same as the overall pattern of mortality among the two farms however higher mortality was observed in female calves at Malir. The unadjusted means and standard deviation of stillbirth was 7.59± 13.49. The Least square analysis of variance for stillbirth shows the significant difference ($P<0.01$) among the two farms. In our study no significant difference among the sex was observed which was in accordance with Parekh and Singh (1981). However, the still births reported by these scientists ranged between 0.67 to 9.2%. Whereas in our study much higher still birth rate (up to 34%) was observed. The variation among the records of the present study may be due to the age and body weight of the dam or the birth weight of the calf as described by Hearnshaw *et al.* (1984).

Abortion Rate

Higher trend of abortion among the male calves was observed at both farms. The data showed that sex of calves and lactation number (parity number) significantly ($P < 0.05$) affected abortion rate in calves. However, herd (farms) year of birth did not affect abortion rate ($P > 0.05$). In our study the higher abortion rate at Malir is clearly in accordance with Drew (1988).

Season and calf mortality

Two seasons i.e. calving season (July – October) and off-calving season (November – June) were selected and observations were made on 317 (male) and 4379 (female) calves born at Malir and Okara farm respectively. In both the seasons the mortality in female calves was higher at Malir i.e. 24.25% and 27% whereas it was higher in males in Okara in both the seasons i.e. 25.24% and 14.13%. The season has also been considered as an important risk factor Bebe *et al.* (2001)

Causes of calf mortality

Female calves showed higher death percentages at both the farms. The greatest losses (13.21 and 2.24%) were caused due to pneumonia followed by enteritis (9.73 and 4.18%) in female and male calves at Malir and at Okara farms. results in our study are in line with Verma and Kalra (1974) and Otesile *et al.* (1983)

Effect of parity number on mortality in adult buffaloes

Consistent results at both farms, where high trend of mortality among the sixth lactation was observed. At the sixth lactation the age might have contributed to this high mortality

CONCLUSION

Controlling calf mortality is one of the most important factors for increasing profits from dairy farming Many research workers conducted their research studies on calf mortality in different regions of the world. The unadjusted means and standard deviation of stillbirth was 7.59± 13.49. The unadjusted mean of calf abortions was 2.64± 3.59. Least square means SE of abortion of reveals that herd (Farm) and sex were the significant ($P < 0.01$) sources of variation for stillbirth in calves. However, year of birth of calves did not have any significant effect on stillbirth. The data showed that sex of calves was highly significant. Lactation number (parity number) significantly ($P < 0.05$) affected abortion rate. The variations noted species may be attributed to the husbandry practices including the feeding regime.Age of the dam seem to have contributed in this regard. Other factors observed showed no statistical variation/value viz a viz number of variations and observations noted in the study.

REFERENCES

1. Ablaha, B. S., H. Y. Hussin and A. K. Nassir, 1995. Factors affecting calf mortality. *Iraqi J of Vet Sci*, 1995, 8(1) : 41-46.
2. Bebe, B. O., S. A. Abdulrazak, P. O. Ogore, J. O. Ondiek and T. Fujihara, 2001. A note on risk factors of calf mortality in large scale dairy farms in the tropics: A case study on Rift Valley area of Kenya. *Asian Austr J of Anim Sci;* 14(6): 855-857.
3. Balakrishnan, M., 1996. Factors affecting mortality of buffalo calves in an organized herd. *Indian J of Dairy and Biosci*, 1996, 7: 61-65
4. Bali, M. K., I. J. Juneja, R. M. S. Khanna and R. P. Singh, 1979. A clinical note on buffalo calf mortality. *Indian J of Dairy Sci.*, 32 (4): 370-372
5. Drew, B., 1988. The influence of management factors during roaring on the subsequent performance of Friesian heifers. British cattle breed Club Digest. (*Anim. Breed Abst.*, 57:164, 1988).
6. Ghose, S. K., S. K. Roy and A. R. Samanta, 1994. Effects of metrological factors on calf mortality in subtropical zone of West Bengal. *Indian J of Anim Prod and management,10(4):156-158*
7. Ghosh, S. K., S. K. Roy, A. K. Pyne and R. Dattagupta, 1996. Factors affecting calf mortality. *Indian Vet J,* 73(12):1277-1279.

8. Harvey, W. R., 1987. Mixed Model Least square and maximum Likelihood Computer Program (LSMLMW) PC-1 version
9. Hearnshaw, H. R., Barlow and R. Darnell, 1984. Factors affecting calving difficulty and calf mortality of Hereford and Hereford cross cattle. Proceed. Of the 4th Conf., *Aust. Asso Of Anim Breed and Genetics*, Univ. Adelaide, South Australia, 4-6, 106-107.
10. Herbers, F., 1981. Parturition and postnatal morbidity and mortality of calves in the Thai Swamp buffaloes (Bubalis bubalis). *Thesis Justus* – Liebig Universittat, Giessen German Federal Republic
11. Husnain, H. U., and R. H. Usmani, 2006. Livestock of Pakistan. Livestock Foundation, Islamabad.
12. Islam, S. S.Ahmed AR,Ayesha.Ashraf.,Nargis.Khanam and M.B Ahmed. 2005. Causes and consequences of calf mortality in a dairy farm of Bangladesh. *J of Anim Vet Advances*, 4(2): 260-264.
13. Janakiraman, K., 1986. A system of buffalo calf rearing to reduce mortality rate and achieve early mortality. *Buffalo-J.* 2(1): 75-79.
14. Khera, S. S., 1980. Foetal and young calf mortality among bovine livestock in India. Geographic and temporal pattern of mortality. *Indian J Anim. Sci.*, 51 (3): 292-302.
15. Katoch, S., N. K. Manuja, Y. P. Thakur and S. Singh, 1994. Factors affecting calf mortailty in organized dairy herd. *Indian Journal of Dairy Science*, 46(12):592-594.
16. Parekh, H. K. B. and A. Singh, 1981. Mortality pattern in crossbred of Gir with Friesian and Jersey sires. *Indian J Dairy Sci.*, 51 (4): 419-424.
17. Patil, S. B. and K. R. Gupta, 1980. Studies on mortality rate in Red Sindhi calves under Dhule conditions. *Livestock Advisor*, 5(II):19-22.
18. Otesile, E. B., O. B. Kasalia, A. Dyejjde and M. T. Trimnell 1983. Calf losses on the University of Ibadan farm, N, Nigeria, 1976-77. *Tropical Veterinarian,* 1(3): 164-171.
19. Rao, A. V. N., 1982. Incidence of pre and neonatal calf mortality in livestock farms in Andhra Pradesh. *Indian J Anim. Reprod.*, 2(1): 29-31.
20. Sharma, M. C., N. N. Pathan, N. N. Hung, N. H. Lien and N. V. Vuc, 1984. Mortality in growing Murrahett Buffalo calves of Vietnam. *Indian J Anim Sci.,* 55 (4):4-22.
21. Verma, P. C., and D. S. Kalra, 1974. Mortality in buffalo calves (Bos bubalis). *Indian Jl Anim Sci.*, 44 (3): 163-168.

In Ovo- Vaccination of Chicken Embryos with Infectious Bronchitis Virus Vaccine

M. T. Tunio[1], S.H.Abro[2], R. Rind[3], R. Wagan[3] and H. A. Hashmi[1]

1. Department of Agricultural Sciences, Faculty of Science, Allama Iqbal Open University, Islamabad. 2. Department of Biomedical Sciences and Veterinary Public Health (SLU) Uppsala, Sweden. 3. Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam Pakistan.

ABSTRACT

This Study was carried out to evaluate *in-ovo* vaccination against Infectious Bronchitis (IB) virus. Two hundred forty specific pathogen-free (SPF) fertile chicken eggs of Babcock layer were selected and divided into groups A, B, C, and D (i.e. 60 eggs were kept in each group). The embryonated eggs of groups A, B and C were vaccinated with IBV M-41 strain using the manual micro-injection system at 18 days of incubation period while group D were kept as control. After hatching, hatchability percentage, antibody titre in sera of vaccinated chicks was recorded. *In-ovo* vaccination with different dilutions affected the hatchability in embryos as compared to the chicks did not receive vaccine. The chicks of group A received vaccine at the dose of 0.1ml with virus serial dilution 10^{-6} showed 83.33% hatchability while group B received vaccine the dose of 0.1ml with virus serial dilution 10^{-5} yielded 68.33% hatchability. The chicks of group C were vaccinated with the dose of 0.1ml dilution 10^{-4} had 63.33% hatchability, Chicks of group D receiving no vaccine showed 96.66% hatchability. The blood of all the vaccinated birds was examined for haemagglutination inhibition test at weekly interval and clear variation in the antibodies titre was evident. The chicks received 0.1ml dilution 10^{-6} dose of vaccine had the maximum 5.75 antibody titre followed by 10^{-5} (5.5titre) and 10^{-4} (4.75 titre) at the end of 5th week. Antibody titre of groups A, B and C gradually decreased to 5.00, 4.62 and 3.15, respectively up to the end of 7th week. No antibody titre was observed in chicks of group D.

Key words: Infectious Bronchitis Virus, hatchability, *in-ovo* vaccination, chicks, antibody titre chicken

INTRODUCTION

In Pakistan, intensive poultry farming is increasing day by day and growing with an average rate of about 10-15% every year. During last five years, it gave more profit to the farmers however; the poultry industry is facing a variety of problems, particularly the diseases of viral origin. According to an estimate published in various papers in Pakistan, the poultry industry bears yearly loss of 30% due to various infectious diseases (Anonymous, 1994). The annual turnover in the poultry sector estimated is 7.1 billion rupees or 426 million U.S dollars while the total investment is 65 billion rupees. There were about 300 hatcheries, 140 feed mills and 15,000 commercial poultry farms in the country (Qureshi, 2005). Infectious bronchitis is highly contagious, acute upper respiratory tract disease in chickens. The infectious bronchitis virus (IBV) contains RNA genome as reported by Cavanaugh *et al.,(* 2002).

The clinical signs of the disease are coughing, sneezing, tracheal rales, gasping and nasal discharge. Mortality may occur in very young birds, whereas decrease in weight gain and feed efficiency are usually observed in older birds. A drop in egg production and egg quality observed when birds in layer flocks got infected (King *et al.* 1991). Avian infectious bronchitis virus (IBV) causes tremendous economic losses to the

poultry industry worldwide. Different serotypes of this virus showed little cross-protection Lin *et al.* (2005). Infectious bronchitis virus replicates in tissue of the respiratory and intestinal tracts, kidney, and oviduct. The kidney and other non-respiratory organs are site of persistence of IB infectious virus being periodically shed in nasal secretion and feces (Dhinaker *et al.* 1997).

Chicken responds to infectious bronchitis (IB) field infection or vaccination by producing specific antibodies and cell mediated immunity. Antibodies induced by IBV could be of IgM, IgG, and IgA; although the main antibody found in serum (humoral antibody), is of the IgG class (Cook, 1995). Both live and dead virus vaccines are used in IBV immunization. Live vaccines are initially used to vaccinate broilers, breeder and layer flocks. Massachusetts serotype vaccines are very commonly used. (Cavanagh and Naqi, 2003).

MATERIALS AND METHODS

Two hundred forty Specific Pathogen-Free (SPF) fertile eggs of Babcock Layer were obtained from K&N's, Poultry Diagnostic and Research Institute, Karachi. The eggs were cleaned and disinfected with DS 200 (Divine Pharama Karachi) , a quaternary ammonium formulation and were placed in incubator. Before the incubation, incubator was fumigated and sterilized with 10% formalin solution then incubator was kept closed for 24hours After the sterilization, the eggs were kept in incubator at 37^0C temperature with 80% humidity. The incubated eggs were candled on 7 days of incubation, the dead embryos and infertile eggs were discarded.

Vaccine and vaccination

Infectious bronchitis virus M-41 strain vaccine from Salisbury, USA was selected for *in-ovo* vaccination. The dose of 0.1ml was inoculated with 10^{-6}, 10^{-5} and 10^{-4} dilutions. The shell surface of eggs was cleaned and disinfected by iodine solution. After cleaning and disinfecting of the eggs, two holes of 1mm diameter were made in the shell of egg by dental drill machine, then inoculum was inoculated with 0.1ml of IBV (M41) vaccine by using 26 Gauge×1/2 needle (Socorex Swiss self refilling syringe Cat No.187. 20501). However, two hundred forty-embryonated eggs were divided into 4 groups according to doses inoculated i.e. A, B, C, and D (non-vaccinated considered being control group). The vaccinated eggs were sealed by paraffin wax and then the eggs were transferred to the hatcher. The hatchability percentage of the vaccinated and non-vaccinated embryos was recorded. After the hatching, the newly hatched chicks were counted and percentage was determined.

Haemagglutination test

Two types of haemagglutination tests (i.e. haemagglutination test and spotted haemagglutination test) were conducted for qualitative promotions of the haemagglutination potential of the virus. While, micro-haemagglutination inhibition test was performed as quantitative haemagglutination test.

Spot haemagglutintion test

A drop of 5% red blood cells suspension and a drop of fluid containing virus were mixed on haemagglutination plate. The agglutination of red blood cells with virus within 45 seconds was considered as a positive. (Ammar, 2001)

Micro haemagglutination test Procedure

A 50µl of the saline were added to 96 wells of micro titration round bottom plate. In the first well of each series 50µl virus suspension were added to the wells containing saline and mixed thoroughly with micropipette. A 50µl of diluted virus suspension was transferred from the first to the second well and mixed properly with micropipette. This process was repeated till the 10th well from which 50µl of diluted virus suspension was discarded. No virus was added to the 11th and 12th well and kept as controls. Then 50µl of 1% chicken erythrocyte suspension (prepared from 5%solution of chicken RBCs) were added to wells and incubated for 30 minutes at 4^{O}C. Haemagglutination was recorded the highest dilution and considered to be complete agglutinations.

Haemagglutination inhibition test

Two methods of testing of the antibody titre were tested i.e. constant–serum diluted virus alpha procedure and other constant-virus diluted serum beta procedure. Later beta neutralization test was performed during this study.

Beta neutralization

Procedure A 50µl of phosphate buffered saline (PBS pH 7.2) was added to 96 wells of micro-titration plate with rounded bottom. In the first well of each series, 50µl of serum were added and mixed thoroughly with the micropipette. A 50µl of diluted serum was transferred from the first well to the second and mixed properly. This process was continued till the 10^{th} well from which 50µl of diluted serum was discarded. No serum was added to the 11^{th} and 12^{th} and kept as control wells. A 50µl of 4HA units of the virus was added and mixed from 1^{st} to the 11^{th} well. The 11^{th} well contained antigen was considered as virus control. No serum and antigen were added to well 12 and only 50µl of red blood cells were added and kept as RBC control. The plate was then incubated at 4^{0}C for 30 minutes to record the inter reaction between antigen and antibody. A 50µl of 1% erythrocyte suspension (prepared from 5% RBCs) were added to each well of the plate and incubated for 30 minutes at 37^{0}C. After 30 minutes, a complete haemagglutination in well No.11 (antigen control) and no haemagglutination in well No 12 (diluent control) were recorded. (M.Rabbaani *at el* 2001-2002)

Preparation of vaccine inoculum from M-41 strain of IBV

IBV M-41 vaccine was modified by serial passage in eggs for 27 times to reduce its pathogencity.

Serum samples for antibody titre

After hatching of the chicks, the blood samples were collected with a week interval from 1-5 weeks. The blood was collected from jugular vein and also from wing vein. Blood was collected in disposable syringes (3ml), which were then placed at room temperature (30^0C) in slanting position for two hours and sera were collected in sterile appendrop tubes, which were properly marked with number of the chicks of the group and stored at -20^0C till used. The following three doses were prepared in sterile phosphate buffered saline (PBS) and inoculated.

Phosphate buffered saline (PBS pH 7.2)

The ingredients of buffered solution	Weight in grams
Sodium chloride	8.00g
Potassium chloride	0.20g
Disodium hydrogen Orthophosphate	2.31g
Potassium dihydrogen Orthophosphate	0.20g
Distilled water	1000 ml

Table showing various dilutions of IBV virus vaccine

Group A	60 embryonated eggs were vaccinated with dose of 10^{-6} EID_{50}/ 0.1ml
Group B	60 embryonated eggs were vaccinated with dose of 10^{-5} EID_{50}/ 0.1ml.
Group C	60 embryonated eggs were vaccinated with dose of 10^{-4} EID_{50}/ 0.1ml.
Group D	60 embryonated eggs were vaccinated with dose of 10^{-4} EID_{50}/ 0.1ml.

RESULTS

The present study was conducted to evaluate *in-ovo* vaccination against IBV that would be practical alternative and effective substitute to post-hatch vaccination against IBV infection under field conditions. The result obtained after this study have been narrated as under.

The effect of different dilutions of IBV M-41 strain vaccine on hatchability of embryos

During present investigation the effects of various dilutions of IBV M-41 strain vaccine on the hatchability of the chicks vaccinated at day 18 during embryonating period was recorded and are presented in Table 1.

The results showed that the hatchability of the chicks was affected by different dilutions of vaccine. The dilutions 10^{-6}, 10^{-5} and 10^{-4} were inoculated into the

embryos at day 18 of the incubation, gave 83.33%, 68.33% and 63.33% hatchability, respectively. However, a dilution 10^{-4} that yielded 63.33% hatchability, which was lower as compared to other doses significantly, affected the hatchability of the chicks. The reason could be the high antigen concentration that sometimes may cause antigenic burst in immune cells and produces serious damage in immune cells and also other cells that cause embryo death and ultimately it goes for reduced hatchability in chicks. Therefore, the dilution 10^{-6} is considered and recommended, which can induce better immunity and affects least hatchability in chicks.

Table-1. The effect of different dilutions of IBV-M-41 strain vaccine on the Hatchability of chicks.

Group of chicks	Dose in log serial virus dilution	Dose of vaccine inoculated (CAS)	No. of eggs set	No. of hatched chicks	Hatchability %
A	10^{-6} EID_{50}	0.1ml	60	50	83.33%
B	10^{-5} EID_{50}	0.1ml	60	41	68.33%
C	10^{-4} EID_{50}	0.1ml	60	38	63.33%
D (Control)	No vaccine	Control	60	58	96.66%

The antibody titre in the sera of chicks vaccinated by *in-ovo* technique with IBV M-41 strain during embryonic age at day 18 of their incubation.

The present results show the humoral immune response of the chicken vaccinated with IBV M-41 vaccine through *in-ovo* vaccination during embryonic age at day 18 of their age. Different doses (0.1 ml of 3 different virus dilutions) of vaccine were inoculated into the embryos and their antibody levels were recorded from the week 1 to week 5 after hatching (Table 2). An increased variation in the mean antibody titre was observed at different weeks in the sera of the chicks in response to different doses of vaccine inoculated at 18 days of their embryonic age. A slight difference in the mean antibody titre was also observed among the groups of chicks in relation to the dose of vaccine administered. However, after 5 week, i.e., 6^{th} and 7^{th} week post-vaccination, a significant difference in the mean titer was observed among the weeks and groups of the chicks in their sera. After that, the mean titre of antibody started declining and reached at such level where it was expected to be not capable to protect the birds from the disease therefore revaccination at this stage should be carried-out to continue protection against the disease.

Table-2. The mean antibody titre of IBV-M41 vaccine in the sera of chickens using a micro-injection determined by haemagglutination inhibition test up to seven weeks post-ovo vaccination.

Groups of chicks	Doses in log	Dose of vaccine inoculated	1st week mean antibody titre	2nd week mean antibody titre	3rd week mean antibody titre	4th week	5th week mean antibody titre	6th week mean antibody titre	7th week mean antibody titre
A	10^{-6} EID_{50}	0.1ml	1.37	2.50	2.87	5.00	5.75	5.25	5.00
B	10^{-5} EID_{50}	0.1ml	1.25	2.25	2.87	4.00	5.50	5.00	4.62
C	10^{-4} EID_{50}	0.1ml	1.50	2.00	2.50	3.75	4.75	4.00	3.15
D	No vaccine was offered	-	0	0	0	0	0	-	-

It was further observed from the study that higher dilution of vaccine that produced lesser antibody titre whereas a lower dilution (considered normal dose of vaccine) produced somewhat higher antibody titre but started declining at weeks six and seven suggesting revaccination. From this study, it is also concluded that with the increase in age of chicks, the HI titre in the sera also increased up to 5 weeks. From the investigation, it was further observed that reasonable protective immunity could be obtained by in-ovo vaccination with IBV M-41 vaccine at 18 days of embryonic age.

DISCUSSION

The results regarding the effects of different doses of IBV-M41 strain vaccine on the hatchability percentage of chicks is presented in Table-1. It is concluded from the present study that the higher dose of vaccine has an adverse effect on the hatchability of the embryos indicating that the dose of vaccine can cause embryonic mortality and needs to be investigated thoroughly.

Wakenell *et al.* (1995) conducted similar kind of study to investigate the effect of *in-ovo* vaccination on the hatchability of chicks. Gagic *et al.* (1999) also conducted similar kind of investigation; both recorded clear effect of *in-ovo* vaccination on the hatchability of the chicks and noted that a lower dose has lesser effect on the hatchability. Whereas, Stone *et al.* (1997) in their study indicated that the needle gauge for inoculation and the inoculum volume also affected the hatchability and oil emulsion vaccine exhibited a lower hatchability as compared to other oil vaccine. Chew *et al.* (1997) investigated that infectious bronchitis virus (IBV), the hatchability of the eggs inoculated

with V-IBV at day 18 was significantly reduced (27%) than the eggs, which were not inoculated with IBV or were inoculated with P-IBV (45-58%, $P < 0.01$). The chicks of all treated groups were survived at day 5 of post-hatch.

Therefore, the results of the present study are in agreement to the results of the above authors who observed the effects of *in-ovo* vaccination on the hatchability of chicks, particularly when a higher dose was given to embryos at day 18 of their incubation. The phenomenon is not clear why such mortality of chicks takes place, however, the reason might be due to some problem in proper sealing of hole and also penetrating of the needle deeply which may cause injury to the growing embryo or might be bacterial contamination takes place during *in-ovo* vaccination process, therefore all these required proper examination to come that point to handle the embryos accordingly to reduce the losses. However, Lee *et al.* (2000) inoculated chicken embryos with eight different strains of infectious bronchitis virus (IBV) representing seven different serotypes at 17 days of embryonation. They investigated that strict epithelio-tropic nature and wide tissue tropism of strains of IBV in the chicken embryos and the universality of ribo-probe. In situ hybridization with this probe will be useful for understanding the tissue tropism and the pathogenesis of IBV *in-vivo*.

The data regarding the antibody titre in the sera of chicks vaccinated by *in-ovo* technique with IBV M-41 during embryonic stage at day 18 of the incubation is presented in Table-2. Gagic. *et al.* (1999) who conducted and observed in their experimental work that *in-ovo* technology could be used to protect chicken against multiple diseases. They recorded both responses through in-ovo that developed humoral and cellular immune responses. While Borzemska and Szeleszuzuk (1999) and Sharma (1999) who were the pioneer of postulating the basis of development of technique of *in-ovo* for immunization in birds at the final period of embryogenesis. They agreed that immunization by *in-ovo* vaccination of embryos that enabled the chicks from any disease and also vaccines to be administered before the transfer of maternal antibodies from the yolk sac to the chicken blood stream. Furthermore that Wakenell *et al.* (2002) who adopted and received the patent for the new *in-ovo* vaccination technique in USA against Mareks disease, which is now common but vaccination against NDV, ILT and Gumboro disease are in progress and better results are achieved yet. However, the present study is just conformation of the above studies of *in-ovo* vaccination in embryonic stage of various strains of virus conducted by different workers. In this survey, better results regarding the presence of antibody in the sera and its efficacy of protection against the disease are achievements of the *in-ovo* vaccination technique in early age of the chicks. Moreover, Wakenel *et al.* (1996) were of the same opinion that embryonic vaccination may be an alternative method of vaccination to protect chicken against infectious bronchitis and other diseases which should be brought in practice to protect the chicks immediate after hatch which are at risk.

From the present study, it is concluded that *in-ovo* vaccination at 18 days of embryonated eggs with IB M-41 vaccine is applicable and has commercial potential. However, hatchability was adversely affected by *in-ovo* vaccination, which could be improved with the use automatic injectovac system capable of handling 60,000 eggs at a time.

REFERENCES

1. Ammar , 2001. In-ovo vaccination of chicken embryos with local Pakistani strain of Newcastle disease. M.Sc. Thesis, Dept. Vet. Microbiology. Sindh. Agri. Univ. Tandojam.

2. Anonymous, 1994. Economic survey of Pakistan, Finance Division, Economic wing, Government of Pakistan, Islamabad.

3. Borzmska,W.B. and P. Szeleszcuzuk, 1999. The in ovo vaccination a new technique for poultry immunization. Ha rj vol. 55 (1) Pp. 15-18.

4. Cavanaugh, D., and S. A. Naqi. 2003. Infectious bronchitis. In: Diseases of Poultry (11th ed.). Iowa State University Press, Ames, IA. Pp. 101-119.

5. Cavanaugh, D., K. Madwort, D. Welchman, P. Britton and R.E. Gough, 2002. Coronaviruses From Pheasants are Genetically Closely Related to Corona viruses of Domestic Fowl (IBV) and Turkeys, Avian Pathology. 31(1) 18-93.

6. Chew, P.H., P.S Wakenell , T.B Farver, 1997. Pathogenecity of attenuated infectious bronchitis viruses for oviduct of chickens exposed in ovo. Avian Dis 41(3) : 598-603.

7. Cook J.K.A. 1995. Serological assay on avian infectious bronchitis virus. Infectious Bronchitis Workshop, Western Poultry Disease Conference, Univ. of California. Pp 1-18.

8. Dhinaker Raj, G. and R.C. Jones. 1997. Infectious bronchitis virus immunopathogenesis of infection in the chicken. Avian pathol 26: 677-706.

9. Gagic. M., C.A StHill and J.M Sharma, 1999. In ovo vaccination of specific pathogen free chickens with vaccines containing multiple agents. Avian Dis 43 (2): 293-301.

10. King, D.J., D. Cavanagh, B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid, and H.W. Yoder, 1991. Infectious Bronchitis. In: Diseases of Poultry (9th ed), Iowa State University Press, Pp. 471-484.

11. Lee, C.W., C.Brown and M.W Jackwood, 2000. Tissue distribution of avian infectious bronchitis virus following in ovo inoculation of chicken embryos examined by in situ hyberdization with antisense digoxienin-labeled universal riboprob. J Vet Diagn Invest 14 (5): 377-81.

12. Lin, K.Y., H.C Wang and C.H Wang. 2005. Protective effect of vaccination in chicks with local infectious bronchitis viruses against field viruses challenges. J Micribial Immunol Infect 38 (1): 25-30.

13. M.Rabbani.,M.A Muneer and K.naeem,2001-2002. Techniques in Virology. Ferozan Publishing company ,Urdu Bazar, Lahore, Pakistan. Pp.26-30.

14. Qureshi, A.A. 2005. A Changing scenario of poultry diseases in Pakistan. Proceeding of Asian veterinarian Association Conference held at Lahore. Pp. 75-78.

15. Sharma, J.M. 1999. Introduction to poultry vaccine and immunity adv Vet. Med. 41: 481-494.

16. Stone, H., B. Mithe and D. Mabragn, 1997. In ovo vaccination of chicken embryo with experimental Newcastle disease and avian influenza oil emulsion vaccine. Avian Dis.41: 856-863.

17. Wakenell, P.S, J.M Sharma and R.F Slocombo. 1995. Embryo vaccination of chicken with infectious bronchitis virus: histologic and ultrastructural lesion response and immunologic response to vaccination. Avian Dis. 39 (4): 752-765.

18. Wakenell, P.S., J.M. Sharma, 1996. Chicken embryonal vaccination with avian infectious bronchitis virus. Am J. Vet. Res. 47(4) : 933-938.

19. Wakenell, P.S,, T. Bryan, J. Schaeffer, A. Avakian, C. williams and C. Whitfill. 2002. Effect of in ovo Vaccine delivery route on herpesvirus of turkys/ SB-1 efficacy band viremia. Avian Dis. (46) : 247-280.

TESTING OF POULTRY BIRDS WITH FIELD ISOLATED STRAINS OF NEW CASTLE DISEASED INDUCED WITH VITAMIN A

Tanveer M. Anwar, Farzana Rizvi , M. Asad Khan , M. Afzal, Hina Zafar and M. Hafeez
1. Allama Iqbal Open University, 2. Agricultural University Faisalabad, 3. Livestock and Dairy Development Board, 4. PLWO Islamabad.

ABSTRACT

The effect of dietary hypervitaminosis A on experimental production of New Castle Disease in broiler chicks was examined. For this purpose sixty broilers were purchased from local hatchery and divided into two groups A and B having thirty birds in each.
The experimental group A was given Vitamin A diet at the dose level of 15000IU per Kg from day of Ist of age. The control group B received feeding having vitamin A at the level of 1500IU per Kg of feed. Blood samples were taken with anticoagulant from wing vein of seven broilers at random from each groups for hematological studies, hemoglobin concentration and packed cell volume were studied. Broilers of each group were subdivided A1, and A2, B1 and B2 . On the 7th and 23rd day only subgroups A2 and B2 were vaccinated against ND (Lasota strain) intraocularly. Blood were collected from wing vein of broiler after seven days of vaccination for hematological studies.
Keeping in view the importance of vitamin A in production of immunity. This study was planned to investigate the effects of hypervitaminosis A on pathology of New Castle disease in broiler chicks under local conditions.

Key words:- New Castle Disease , Vitamin A , Field isolated Strains , Broilers

INTRODUCTION

Poultry Farming has emerged as one of fast expanding industry in Pakistan. This high intensity of poultry production requires fast growing strains at high stocking density. In this type of husbandry, flocks are highly susceptible to infectious agent either due to reduced immune potential or as result of deteriorating environmental hygiene.Poultry Industry is today encompassing worth 200 Billion rupees (Survey of Pakistan 2008-09)

Among infectious disease, Newcastle Disease is highly contagious viral disease of all ages particularly chicken (Alexander, 1991). This disease occupies its position amongst the most devastating menaces of poultry industry. The latter suffers high economic losses, due to high morbidity and mortality rates (50-100%) in affected birds (Hofstead et al., 1978). Host range of New Castle disease includes chickens, turkeys, pigeons and wide variety of wild birds.

Vitamins are organic compounds required for normal growth and maintenance of life. They are not the major structural components of body and most commonly function as coenzymes or regulators of Metabolism (Crampton, 1959). They are essential components necessary for production of Antibodies against infection (Wolfgang, 1986)

Keeping in view the importance of Vitamin A in production of immunity. Present project was planned to study the effects of hypervitaminosis A on Pathology of New Castle disease in broiler chicks under local condition.

MATERIALS AND METHODS

NCD disease poultry birds were subjected to isolation of ND virus. The organs namely Trachea, spleen, and lungs were collected from these birds for viral isolation .These samples were preserved at -20 C for further procedure.

The samples , prior to their processing were thawed at room temperature Thereafter a 20% solution of the tissues was made in physiological saline and homogenized with the help of electric grinder. Antibiotic of choice (Gentamycin) was added in homogenate (.1mg/l suppress bacterial growth (Senne 1989). This homogenate was centrifuged at 1000x g for 20 minutes at +4C . The supernatant collected was stored in sterilized container at -20 C for further processing.

Isolation and identification of NewCastle Disease Virus

Inoculation of Embryo and Reincubation

Each field isolate was inoculated into ten 9 days old embryonated chicken eggs via allontaic cavity route following the technique described by Hitchner 1980). The inoculated egg were incubated at 37c and candled after every 24hr post inoculation. The embryos found dead thereafter were chilled over night at 4C. The allontoic fluid was then aspired with the help of sterile pasteure pipette and put into sterilized bottles and stored in freezers at 20C. This fluid was tested for haemaggltinating activity with chicken RBCs by spot haemagglutination and microhaemagglutinnation test and later confirmed by haemagglutination inhibition test (Beard, 1989).

Characterization of Pathogenicity of Field isolates

The confirmed isolates of NDV were processed for pathotyping by techniques, described by Alexander (1989) . The isolates were classified as lentogenic, mesogenic and velogenic on basis of

1. Mean Death time (MDT) in day old chick
2. Intracerebral Pathogencity Index (ICPI) in day old chick
3. Intravenous Pathogenecity Index (IVPI) in six week old chick

Mean Death time in Chicken embryos

Fifty Five eggs were collected from the local hatchery and incubated aty 37C with relative humidity 60-70 percent . After confirming fertility. The embryonated eggs were divided into here groups. Each containing fifteen eggs and ten e embryonated eggs were taken as control.

In this test 10^{-2} 10^{-3} and 10^{-4} dilutions were made in sterile isotonic saline and 0.1 ml of each dilution was inoculated into allantoic cavity of 9-10 day old embryonated eggs . Each dilution was inoculated in six more eggs after eight hours. Same quantity of normal saline was inoculated in the allontoic cavity of embryonated eggs of control group. The eggs were marked and placed in incubator at 37C and candling was done twice daily(early morning and late afternoon) for seven days. The time that each embryo found dead was recorded. Allontic fluid was harvested and tested for haemagllutination

activity by spot haemagluutination, microhaemagllutination and haemaglutination inhibition tests.(Beared).

ICPI in day old chicks

One hundred day old cockerels were collected from commercial hatchery and divided three equal groups A , B and C containing thirty chicks each and control group comprising of ten cockerels . A 1: 10 dilution of infective bacteria free allontoic fluid of each isolate was prepared in normal saline. The ICPI was determined by inoculating 0.05ml of each isolate into cerebral cavity of each day old chick of respective group. Same amount of normal saline was inoculated into cerebral cavity of chicks of control group. Clinical signs and mortality was observed in chicks up to eight days. The chicks were scored zero if normal, 1 if sick and 2if dead. The ICPI value is the mean score pe chick per observation (Alexander,1989).

IVPI in six day old chicks

Thirty five day old cockerels were procured from commercial hatchery. The chicks were kept in cages under standard mangement conditions. The birds were raised up to six week of age. A 1:10 dilution of infective bacteria free allontoic fluid of velogenic isolates of NDV was prepared in normal saline. The IVPI was determined by inoculating 0.1ml of velogenic isolate in wing vein of thirty six week old chicks same amount of normal saline was inoculated into wing vein of five chicks which were kept as control. They score zero if normal 1 if sick 2 if paralyzed and 3 dead .

Determination of ELD 50

Thirty fie eggs were purchased from Deptt: of Poultry husbandry UAF Faisalabad and incubated at 37C for 9 days . After candling embronated eggs were inoculated with 0.1ml of 10 folds serial dilutuin of allontoic fluid of velogenic strain of NDV> The eggs were incolated at 37C . Each dilution was inoculated in five eggs and five were kepta s control. Mortality was recorded up to seven days postinoculation. The ELD 50 of the virus was calculated by the method of Reed and Munch as described by Villegas and Purchase(1989)

Proportionate distance (PD) = $\frac{\text{Percentage infected at Dilution next above 50\% - 50}}{\text{Percentage infected at dilution next above 50\%- P.I at ilution next below 50\%}}$

RESULT AND DISCUSSION

Infected Spleen, lungs and trachea were collected from suspected outbreaks of New Castle disease from ten different poultry Farms in and around Faisalabad and were processed for Isolation of virus. Suspected samples were inoculated in 9-10 day old chicken embryos, incubated at 37C for 3-5 days and isolated virus was detected by spot haemagglutination test, haemagglutination test and confirmed by haemagglutination inhibition test.

Out of ten only five samples gave the spot haemagglutination test with 4% chicken Erythrocytes as shown in Table 1. These isolates were characterized as isolates A, B, c, D and E. Haemagglutination test was performed for isolates named as A, B, C D and E giving positive spot agglutination test and their result are shown in the Table 2. Haemagglutination titre for isolates A, B, C, D and E was 1: 512, 1: 256, 1: 32, 1: 64 and 1:128 respectively.Men Death Time of various isolates of NDV is given in Table 3. The Viral isolates were inoculated within allontoic cavity of 9-10 day old embryonated eggs and incubated at 37C for seven days.Intracerebral Pathogenecity indices of various isolates are given in the Table 4.

In Control group no morbidity or mortality was observed and chicks remained healthy and alive upto the end of experimental duration.Comparison of results of MDT , ICPI and IVPI of various isolates is given in Table 6. On its basis various isolates A & B was characterized as velogenic , while isolates C, D and E categorized as mesogenic.For Determination of ELD_{50} different dilution of velogenic field isolate of NDV were used i.e 10^{-1} $10^{-2,}$ 10^{-3} . The calculated ELD_{50} of field isolate A is given in Table 7, which was $10^{-4.81}$ /.1 ml of the virus.

Table 1. Spot Haemagglutination test of the Field

Sample	Spot Agglutination	Isolates
1	-	-
2	+	A
3	+	B
4	-	-
5	+	C
6	-	-
7	+	D
8	-	-
9	+	E
10	-	-

Table: 2 **Haemagglutination Test (Micro titration Plates)**

Isolates	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A	+	+	+	+	+	+	+	+	+	-
B	+	+	+	+	+	+	+	+	-	-
C	+	+	+	+	+	-	-	-	-	-
D	+	+	+	+	+	-	-	-	-	-
E	+	+	+	+	+	+	+	-	-	-

Table: 3 Mean Death Time of Various Isolates

Isolate No	Dilution	No of Embryos died hours post inoculation							
		24	36	48	60	72	84	96	MDT
A	10^{-3}	1	2	-	2	-	-	-	40
	10^{-4}	2	1	-	1	1	-	-	48
B	10^{-3}	2	-	1	2	-	-	-	50
	10^{-4}	1	1	2	1	-	-	-	60
C	10^{-3}	-	1	1	2	-	-	1	66
	10^{-4}	-	-	1	-	2	-	2	72
D	10^{-3}	-	-	-	2	-	2	-	60
	10^{-4}	-	-	1	1	2	-	1	80
E	10^{-3}	-	-	-	-	-	1	3	84
	10^{-4}	-	-	-	2	2	-	2	76

Table: 4 **Intracerebral Pathogenicity Indices (ICPI) of Various Isolates**

Isolates	No of Birds	I/C Dose	No of Birds days Post Inoculation							Total Mortality	Survivals	ICPI
			1	2	3	4	5	6	7			
A	10	.05	-	4	2	4	-	-	-	10	0	2.12
B	10	.05	-	-	5	-	3	2	-	10	0	2.06
C	10	.05	-	-	3	2	-	1	-	6	4	1.49
D	10	.05	-	2	-	-	2	-	1	5	5	1.39
E	10	.05	-	-	-	2	-	3	-	5	5	1.25
Control	10	.05 N. S	-	-	-	-	-	-	-	-	-	-

Table 5 **Intravenous Pathogenecity Indices (IVPI) of Various Isolates**

Isolates	No of Birds	I/C Dose	No of Birds Days Post Inoculation							Total Mortality	Survivals	IVPI
			1	2	3	4	5	6	7			
A	10	0.1	-	2	2	3	2	1	-	10	0	3.14
B	10	0.1	-	-	2	4	2	2	-	8	2	3.0
C	10	0.1	-	-	-	1	-	1	-	4	6	.52
D	10	0.1	-	-	2	-	2	-	-	4	6	.89
E	10	0.1	-	-	1	-	1	2	2	5	5	.94
Control	10	0.1N.S	-	-	-	-	-	-	-	-	-	-

Table 6 : Characterization of various isolates showing MDT, ICPI &IVPI.

Isolate	MDT	ICPI	IVPI	Character of Virus
A	44	2.12	3.0	Velogenic
B	43	2.06	3.14	Velogenic
C	69	1.49	.52	Mesogenic
D	64.5	1.39	.89	Mesogenic
E	80	1.25	.94	Mesogenic

Table 7 Determination of Embryo lethal dose 50 (ELD 50)

Dilutions	Embryos		Accumulated Members		Proportion dead/Total	% age dead
	No of Dead	No of Alive	No of Dead	No of Alive		
10^{-1}	5	0	22	0	22/22	100
10^{-2}	5	0	17	0	17/17	100
10^{-3}	5	0	12	0	12/12	100
10^{-4}	4	1	7	1	7/8	87
10^{-5}	2	3	3	4	3/7	42
10^{-6}	1	1	1	8	1/9	11

References

1. Alexander, J.D., 1989 NewCastle Disease. In a laboratory manual for the isolation and identification of Avian Pathogens.2nd ed Univ. of Pennsylvania.
2. Beared ,C.W and B.C Easter day .1967.J.Infect.Dis 117:55-61
3. Benjamin,M.M., 1978.Outline of veterinary Clinical Pathology .2nd Ed .The Iowa University Press USA
4. Crampton , E.W. and L.E.Lioyd, 1959. Vitamin and General Fundemental of Nutrition , 3rd Ed pp: 158-162.
5. Hofstead,M.S., B.W.Calneck and H.J.Barness,1990 disaes of Poultry .9th Ed Wolf Publishing Limited
6. Mehringer, U.1987.Hypervitaminosis A in Poultry. British Poult Sci.,12:106-12
7. Natt,M.P. and C.A Herrick,1952. A new diluent for counting Erytrocytes and leucocytes of chickens .Poult.Sci., 31:735-738
8. Senne, A.D ., 1989. A laboratory manual for isolation and identification of Avian Pathogens .3rd Ed .AAAP., Univ of Penssylvania
9. Wolfgang Z., D.D.R, Akademie der Wissenchaften , Deustsche Demokratische Republic ,1986 Environmentalimmunomodulatory Fcators Avian pathology ,zootenica International

COMPARISON OF CLINICAL PATHALOGICAL AND IMMUNOLOGICAL CHANGES IN BROILERS SUBJECTED TO FIELD ISOLATED NEW CASTLE DISEASE VIRUS

Tanveer M. Anwar, Farzana Rizvi ,M. Afzal M. Asad Khan
1.Allama Iqbal Open University, 2. Agricultural University Faisalabad
3. and 4. Livestock and Dairy Development Board Islamabad.

ABSTRACT

The effect of dietary hypervitaminosis A on experimental production of NewCastle Disease in broiler chicks was examined. For this purpose sixty broilers were purchased from local hatchery and divided into two groups A and B having thirty birds in each.
The experimental group A was given Vitamin A diet at the dose level of 15000IU per Kg from day of 1st of age. The control group B received feeding having vitamin A at the level of 1500IU per Kg of feed. On the 7th and 23rd day only subgroups A2 and B2 were vaccinated against ND (Lasota strain) intraocularly. Various Tissues like spleen, lungs, liver and Kidney were subjected to histopathological examination
Keeping in view the importance of vitamin A and its effect on immunity and pathology of Broiler chicks. This study was planned to investigate the comparison of clinical Pathological and Immunological changes in Broiler chicks subjected to Field Isolated ND Virus.

Key words: pathology , Immunology , NCD , Field Isolates , Broilers ,

INTRODUCTION

Poultry Farming has emerged as one of fast expanding industry in Pakistan. This high intensity of poultry production requires fast growing strains at high stocking density. In this type of husbandry, flocks are highly susceptible to infectious agent either due to reduced immune potential or as result of deteriorating environmental hygiene. Poultry Industry is today encompassing worth 200 Billion rupees (Survey of Pakistan 2008-09)

Among infectious disease, Newcastle Disease is highly contagious viral disease of all ages particularly chicken (Alexander, 1991). This disease occupies its position amongst the most devastating menaces of poultry industry. The latter suffers high economic losses, due to high morbidity and mortality rates (50-100%) in affected birds (Hofstead et al., 1978). Host range of New Castle disease includes chickens, turkeys, pigeons and wide variety of wild birds.

Vitamins are organic compounds required for normal growth and maintenance of life. They are not the major structural components of body and most commonly function as coenzymes or regulators of Metabolism (Crampton, 1959). They are essential components necessary for production of Antibodies against infection (Wolfgang, 1986)

Keeping in view the importance of Vitamin A in production of immunity. Present project was planned to study the effects of hypervitaminosis A on Pathology of New Castle disease in broiler chicks under local condition.

Methodology

Determination of antibody Titre against NewCastle disease vaccine by haemagglutination and haemagglutination test and Determination of antibody titre against IBD vaccine by indirect Haemagglutination test.

Result and Discussion

Geometric Mean titre against NDV of various groups is shown in Table 1. The data shows that the GMT of broilers kept on high dose of vitamin A was higher as compared to control group.. However GMT of control unvaccinated broilers was almost same as in broiler of unvaccinated experimental group Geometric Mean titre against IBD of experimental and control group shown in Table. The data shows that GMT of broilers fed with Vitamin A at 15, 000IU/Kg of feed was higher as compared to control group.

After giving infection, gross lesion were observed only in broilers kept on vitamin A excess diet. the postmortem lesion of slaughtered and dead chicks showed proventriculus and tracheal hemorrhages. Liver was friable. Intestine showed button ulcers in different areas. Spleen was enlarged and hemmorhagic. Lungs were congested and discolored.

Clinical Signs(Vaccinated Group)

The chickens were given experimental diet

The chickens were given experimental vitamin A excess diet (15000 IU /Kg) from Ist day of the trial. The signs of hypervitaminosis A include conjunctivitis, concomitant crusty exudates around naries and eyes were observed. Red Inflammatory lesion appeared on the upper and lower eyelid and commisures of mouth. After giving challenge of NDV, the signs of NDV appeared three days post infection in birds. Two birds were off feed and paralyzed. On next day these birds were found dead. On the 4rth day three chicks died showing nervous signs. There was greenish white diarrhea in two birds. After 7^{th} day of post Infection rest of ten birds were slaughtered.

Unvaccinated Group

In this group mortality started 2^{nd} day post infection. Two birds were dead without showing any signs. On 3^{rd} day three birds showed paralysis of both legs. These birds were found dead on 4rth day. On 5^{th} day, two birds showing torticollis of neck and one bird found dead. On 7^{th} day remaining birds were slaughtered to observe various changes in various organs.

Histopathological Changes

Pseudostratified ciliated corner epithelium was changed into pseudostratified squamous epithelium . Blood vessels in muscular mucosa were congested.

Proventriculus

Epithelial cells of Proventriculus showed coagulative necrosis ,congestion and mucosal haemorrhages were also seen./

Intestine

There was sloughing of mucosal epithelium. Inflammatory cells were present in mucosa and sub mucosa of intestine. Nucleus became pyknotic and there were heamorrhagic spots in the intestine.

The geometric mean titer (GMT) post vaccination with primary and secondary doses of Vitamin A is available in table-1 & table-2 below:-

Table 1. **GMT of experimental Broilers Chicks challenged with NDV**

Response	Control		Vitamin A 15,00 IU/Kg	
	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated
Primary	125.8	5.60	150.4	4.40
Secondary	120.5	7.90	156.0	3.90
7 Days Pos Infection	98.6	6.0	102.8	5.40

Table 2. **GMT of experimental Broilers Chicks against IBD Virus**

Response	Control Vaccinated	Vitamin 15,000I.U/Kg
Primary	142.4	170.04
Secondry	138.5	160.5

The liver indices of experimental broilers depicted through this study on day 38 post vaccination are presented In table-3 below:-

Table 3 **Liver indices of experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
38th	4.31±0.14	5.07 ± 0.15**	5.65±.10**

REFERENCES

1. Alexander, J.D., 1989 Newcastle Disease. In a laboratory manual for the isolation and identification of Avian Pathogens.2nd ed Univ. of Pennsylvania.
2. Beared ,C.W and B.C Easter day .1967.J.Infect.Dis 117:55-61
3. Benjamin,M.M., 1978.Outline of veterinary Clinical Pathology .2nd Ed .The Iowa University Press USA
4. Natt,M.P. and C.A Herrick,1952. A new diluents for counting Erythrocytes and leucocytes of chickens .Poult.Sci.,31:735-738
5. Wolfgang Z., D.D.R, Akademie der Wissenchaften , Deustsche Demokratische Republic ,1986 Environmentalimmunomodulatory Fcators Avian pathology ,zootenica International

HAEMATOLOGICAL STUDIES IN ND INDUCED BROILER CHICKS WITH TWO DOSE REGIMEN OF VITAMIN A.

Tanveer M. Anwar, Farzana Rizvi and M. Asad Khan and M. Afzal
1. Allama Iqbal Open Unversity, 2. Agricultural University Faisabad, 3. and 4. Livestock and Dairy Development Board Islamabad.

ABSTRACT

The effect of dietary hypervitaminosis A on experimental production of New Castle Disease in broiler chicks was examined. For this purpose sixty broilers were purchased from local hatchery and divided into two groups A and B having thirty birds in each.
The experimental group A was given Vitamin A diet at the dose level of 15000IU per Kg from day of Ist of age. The control group B received feeding having vitamin A at the level of 1500IU per Kg of feed. Blood samples were taken with anticoagulant from wing vein of seven broilers at random from each groups for hematological studies, hemoglobin concentration and packed cell volume were studied. Broilers of each group were subdivided A1, and A2, B1 and B2 . On the 7th and 23rd day only subgroups A2 and B2 were vaccinated against ND (Lasota strain) intraocularly. Blood were collected from wing vein of broiler after seven days of vaccination for hematological studies.
Keeping in view the importance of vitamin A in production of immunity. This study was planned to investigate the effects of various blood parameter in relation to effect of hypervitaminosis A on pathology of New Castle disease in broiler chicks under local conditions.

Key words : NCD , Broilers , Heamatology, Vitamin A , Poultry.

INTRODUCTION

Poultry Farming has emerged as one of fast expanding industry in Pakistan. This high intensity of poultry production requires fast growing strains at high stocking density. In this type of husbandry, flocks are highly susceptible to infectious agent either due to reduced immune potential or as result of deteriorating environmental hygiene. Poultry Industry is today encompassing worth 200 Billion rupees (Survey of Pakistan 2008-09)

Among infectious disease, Newcastle Disease is highly contagious viral disease of all ages particularly chicken (Alexander, 1991). This disease occupies its position amongst the most devastating menaces of poultry industry. The latter suffers high economic losses, due to high morbidity and mortality rates (50-100%) in affected birds (Hofstead et al., 1978). Host range of New Castle disease includes chickens, turkeys, pigeons and wide variety of wild birds.

Vitamins are organic compounds required for normal growth and maintenance of life. They are not the major structural components of body and most commonly function as coenzymes or regulators of Metabolism (Crampton, 1959). They are essential components necessary for production of Antibodies against infection (Wolfgang, 1986)

Keeping in view the importance of Vitamin A in production of immunity. Present project was planned to study the effects of hypervitaminosis A on Pathology of New Castle disease in broiler chicks under local condition.

Methodology

Hematological Observation

Blood samples were collected from wing vein of seven birds of each group using anticoagulant (EDTA 1mg/ml). Blood was examined for total erythrocyte, leucocytes count (Natt and Harrick, 1952) hemoglobin Concentration, Packed Cell volume and differential leucocytic count.(Benjamin 1978).

Total Erythrocytic Count

Blood 20ul was mixed 2ml (dilution 1:1000 Natt and Harrick Fluid (1952). Erythrocytes were counted in improved Neubar counting chamber in 80 small squares of central large square.

Total Leucocytic Count (TLC)

Blood 20ul was mixed with 2ml (dilution 1:1000 Natt and Harrick fluid (1952). Nuclei of leucocytes were stained Bluish . Leucocytes were continued in central counting squares representing 400 small squares.

Hemoglobin Concentration

Hemoglobin Concentration was determined by acid haematin method using sahlis apparatus (Benjamin, 1978)

Packed Cell Volume (PCV)

Packed Cell volume was measured by Microhaematocrit (Capillary Tube Method).

Differential Leucocytic Count (DLC)

Blood smears were stained with Giemsa stain and relative values of various types of leucocytes including hetrophils, lymphocytes, monocytes and Eosinophils were counted as described by Benjamin (1978).

Organ Index

$$\text{Organ Index} = \frac{\text{Actual Organ weight}}{\text{Live body weight}}$$

Feed Conversion Ratios

$$\text{FCR} = \frac{\text{Total feed Consumed}}{\text{Total weight gained}} \times 100$$

Results and Discussion

Erythrocyte Count of Broilers of different group were shown in Table No 1. The data indicates that there was significant increase in erthrocyte count in birds kept on vitamin A in excess feed plus infection as compared to control group.

Hemoglobin Concentration of birds are shown in table 2 . It indicates that there was significant increase in Hb Concentration of broilers kept on excess diet at age of 18, 28, and 38 days.

Packed Cell Volume (PCV) in different group were shown in Table 3. There was significant decrease in PCV in experimental birds as compared to control at 18, 28 and 38 days of age. Total leucocyte counts of the broilers of different group were shown in Table 3(A, B and C). the data shows that there was significant increase in TLC of broilers kept on higher doses of Vitamin a plus vaccinated as compared to control group. TLC of broilers of unvaccinated group differs non significantly from control group.

Table-1.Erythrocyte Count (millions/ul) of Experimental Broilers

Age (Days)	Control	Vitamin A 15,00	
18th	1.50+ 0.17	2,57+ .26	2.66+ 0.22**
28th	1.28+ 0.12	1.97+ .14	2.33+ 0.22**
38th	1.82+ 0.26	2.01+ 0.09	2.18+ 0.277**

Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.
** Significant difference as compared to Control group

Table-2 . Hemoglobin Concentration (g/dl) of experimental Broilers

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	6.15+ 0.24	7.54+ .25**	7.11+ 0.09**
28th	5.90+ 0.08	6.65+ .33**	6.94+ 0.13**
38th	5.86+ 0.20	7.41+ 0.39**	7.18+ 0.26**

Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.
** Significant difference as compared to Control group

Table 3 **Packed Cell Volumes (%) of Experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	21.14+ 0.91	18.85+ .76**	17.14+ 1.39**
28th	25.28+ 1.61	20.14+ .85**	19.00+ 0.81**
38th	28.14+ 1.62	7.41+ 1.81**	21.14+ 1.40**

Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.
** Significant difference as compared to Control group

Table 4 **Total Leucocytes Count (1000/ul) of Experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	5.44± 0.18	6.80± .18**	5.04± .18
28th	6.30± .18	8.92± .24**	6.47± 0.24
38th	5.67± .20	7.98± .12**	5.45± .12

Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.
** Significant difference as compared to Control group

Table 4.1 (A) **Hetrophils Count (1000/ul) of Experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	25.28± 2.14	30.57± .89**	29.95± 1.69**
28th	24.14± 1.53	8.92± .71**	28.71± 1.68**
38th	27.14± 1.05	28.28± 1.76**	30.85± 1.71**

Each figure represent mean (standard deviation) of seven chicks.
Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.
** Significant difference as compared to Control group

Table 4.1(B) **Lymhocyte Count (1000/ul) of Experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	27.14± .50	26.28± 1.28	24.1± 1.88
28th	25.57± .60	25.14± 1.35	26.42± 1.08
38th	28.14± 1.16	27.57± 1.27	28.71± 1.47

Each figure represent mean (standard deviation) of seven chicks.
Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.

Table 4.1(C) **Monocyte Count % of Experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	24.85± .82	24.00± .87	24.00± 1.95
28th	26.57± 1.30	28.00± 1.48	26.00± 1.17
38th	29.00± 1.49	28.85± 1.58	24.28± 1.40

Each figure represent mean (standard deviation) of seven chicks.
Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.

Table 4.1(D) **Eosinophils Count % of Experimental Broilers**

Age (Days)	Control	Vitamin A 15,00 IU/Kg	
		Vaccinated	Unvaccinated
18th	13.00+.64	13.42+.94	14.00+2.38
28th	10.85+ 1.58	10.57+ 1.66	11.28+.80
38th	7.42+ .81	7.28+1.68	10.85+1.24

Each figure represent mean (standard deviation) of seven chicks.
Each figure represent mean (standard deviation) of seven chicks.
Data subjected to analysis of variance revealed significant difference among the groups.

REFERENCES

1. Alexander, J.D., 1989 NewCastle Disease. In a laboratory manual for the isolation and identification of Avian Pathogens.2nd ed Univ. of Pennsylvania.
2. Beared ,C.W and B.C Easter day .1967.J.Infect.Dis 117:55-61
3. Benjamin,M.M., 1978.Outline of veterinary Clinical Pathology .2nd Ed .The Iowa University Press USA
4. Hofstead,M.S., B.W.Calneck and H.J.Barness,1990 disaes of Poutry .9th Ed Wolf Publishing Limited
5. Mehringer, U.1987.Hypervitaminosis A in poultry . British Poult Sci.,12:106-12
6. Natt,M.P. and C.A Herrick,1952. A new diluent for counting Erytrocytes and leucocytes of chickens .Poult.Sci.,31:735-738
7. Wolfgang Z., D.D.R, Akademie der Wissenchaften , Deustsche Demokratische Republic ,1986 Environmentalimmunomodulatory Fcators Avian pathology ,zootenica International

GENERALIZED CAMEL-POX ASSOCIATED WITH HIGH RATE OF MORTALITY IN CAMELS

Muhammad Abubakar[1], M. Sarfaraz[2], Muhammad Javed Arshed[1] and Qurban Ali[1]
1.3, and 4. National Veterinary Laboratories, Islamabad, 2. Veterinary Officer Fateh-Jung, Attock

Abstract

Following is a detailed description of an outbreak resulting high mortality in camels. There were a total of 26 deaths (both young and adult) during the time period of one and half month. Mostly disease appeared in a generalized form, affecting the mouth and throat region and then spreading to other parts of the body. Major lesions were pustules on mouth (in and outside) and swelling of throat region affecting the mouth later. The most significant lesion was the swelling of eyes which was leading to blindness in some cases. Deaths were mainly due to blindness, anorexia and respiratory arrest. Both adult (Mortality= 23) and young-ones (Mortality= 03) were affected with the disease. As the condition was affecting the large scale area and huge population of camels, it was great economic loss for the poor villagers. It was recommended to treat the disease in its early phase to check secondary infection as later on the bacterial invaders made the disease situation worst and unable to treat and mortality occurred in high rates.

Keywords: Outbreak, Generalized camel-pox, Mortality and camels

INTRODUCTION

Mortality among camels (Camelus dromedarius) is one of the most serious problems faced by camel herdsmen and, although there are several reasons for this mortality, diseases play a major role.

Alhendi *et al.,* (1) described a slow-spreading mild form of camel-pox involving range camels. The morbidity rate was 10% while the case fatality rate was zero percent. Camel pox virus was isolated and identified, and the histo-pathological picture of the lesions was also described.

Following is a detailed description of an outbreak of generalized camel-pox. The effected area is of malal union council, situated 35 km away from Tehsil Fatehjung (District Attock); it is an arid area in north of Punjab province. It has a scattered population into many villages. The veterinary services are provided by Veterinary Dispensary Dhoryal (under the tehsil fatehjung veterinary officer) which is 10 km away from the affected area.

CASE HISTORY

General Management Practices of the Area

- The area has approximate 250-300 camels which are very much scattered through out the area.
- Disease mainly affected the adults but few cases of young ones also reported.
- The early reports of the disease started in may 2007.
- Disease also had a pattern of reoccurrence.

- Few animals were recovered but the later disease occurred in severe fatal form.
- People used the local made and herbal medicines to treat but with no effects.
- The allopathic treatment was started a few days back with few improved cases.

Total Exposure area

- The camels were 300 in the effected area (Total population in all the surrounding area was approximate 1500-2000)
- A total of 26 deaths in two months (15 males and 11 females)
- 25 animals were also having the clinical signs.

MATERIALS AND METHODS

Disease pattern

Disease usually exhibited in two patterns, firstly, the swelling of the face and throat area in starting which spread to other part of body. Secondly, vesicles and pustules develop on mouth, neck and then spread to other body parts. In some cases, it was reverse as these vesicles and pustules developed first and later swelling appeared. Disease occurred both in per-acute and acute forms, as animals were surviving for a few hours to a week with the onset of the disease. The animals were having fever, lassitude, diarrhea and anorexia. In the later the eruptions were distributed to the entire body. Death mainly occurred due to arrest of respiration. In few cases, the myiasis occurred due to the rainy weather. In most of the cases bad odour from the pustules was coming.

Outbreak description

Earlier vesicles and pustules develop on mouth and thoracic region and later inside the mouth on gums and buccal mucosa. The lesions were so severe that animal was unable to eat. There was so severe swelling of the face and thoracic area that it closed the eyes of animals and blindness developed.

Myiasis developed in the pustules giving bad odour from the lesions. Few owners reported postmortem findings that were blisters in the mouth. There was watery discharge from mouth. Lungs were found congested and watery at post mortem. Liver and heart were also affected. Disease affected both adult (23) and young-ones (03).

Dead animals belong to 09 owners from different villages. The maximum number of dead animals with the same owner was five. All of these five animals died within a time period of twelve days difference.

RESULTS AND DISCUSSION

Laboratory Diagnosis

Two blood samples from clinical cases of were examined for hematology using Backman Coulter USA (Model Coulter A^{c}T Diff), results are given in table. While the swab samples from the pustules, nasal and ocular discharges produced the *Staphylococcus aureus* on the blood agar and nutrient agar which proved the presence of secondary bacterial invasion.

Table showing Hematology of Two Suspected Samples

Sr No.	Test Name	Units	Results of Sample 1	Status of Sample 1	Results of Sample 2	Status of Sample 2	Normal Range
1)	WBC Counts	/ul	7500	L	8100	N	8000-18000
2)	RBC Counts	M/ul	5.9	L	6.2	N	6-8
3)	Hemoglobin	g/dl	10.2	L	8.7	L	12.0-18.3
4)	Platelets	/ul	325000	N	370000	N	120000-400000
5)	Neutrophils	%	15	L	25	L	40-60
6)	Lymphocytes *	%	70	H	70	H	20-45
7)	Monocytes	%	07	H	02	N	02-05
8)	Eosinophils	%	08	H	03	N	02-06
9)	ESR	mm/1 hour	05	H	10	H	1.0-4.0
10)	RBC Morphology		-Microcytic hypochromic -Macrocytic hypochromic -Anisocytosis -Ovalocytosis	(++++) (Trace) (++) (+)	-Microcytic hypochromic -Macrocytic hypochromic -Anisocytosis -Ovalocytosis	(++++) (Trace) (++) (++++)	

* High levels of lymphocytes and iron deficiency are indicative of viral diseases.

Pox is the most frequent infectious viral disease of the camel and therefore the most widely reported. Differences in the virulence of camelpox strains have also been suggested Munz et al., (2); Otterbein et al., (3) and Wernery et al., (4), which may explain the phenomenon that some strains produce generalized pox infections and others only a localized form but in the instant case, we observed the generalized form and also invasion by bacterial infection.

Camelpox is most probably not a zoonosis, although clinical observations in various articles have reported the possibility of transmission of Orthopoxvirus cameli to humans. Even very recent reports of skin eruptions in camel herdsmen could not identify camelpox as the causative agent using current laboratory methods Kirz (5) and Wernery and Zachariah (4) but our observation was the camel handlers (No = 03) were having lesions on hands and they were given treatment with penicillin.

Camelids may become infected with the poxvirus through small abrasions of skin, by aerosol infection of the respiratory tract or mechanical transmission by biting arthropods. Differences in the virulence of camel pox strains have also been suggested by Munz et al., (2) and Wernery and Zachariah (4).

In another report from Pakistan, Ghulam *et al.*, (6) reported an outbreak of generalized pox among the draught camels. They reported the severity of disease but with less mortality and also claimed the poor nutrition and common water source to be the

main causes of outbreak but in the above description, the disease is generalized with more than 10 % mortality but we also associate it with the late onset of treatment. Alhendi *et al.,* (1) described a slow-spreading mild form of camel-pox involving range camels in the Eastern region of Saudi Arabia. The morbidity rate was 10% while the case fatality rate was zero per cent but in our case we had 20% morbidity and nearly 40% case fatality.

In both localized and systemic pox virus diseases, the initial multiplication of the virus occurs at the site of entry. In case of systemic disease, further viral multiplication in the draining lymph-nodes is followed by the primary viremia and multiplication of virus in organs and tissues. This resulted in a secondary viremia and subsequent infection of the skin as described by Wernery and Kadeen (7) and same was the finding in above case. But generally no or even low mortality is reported from camelpox but here this rate is much higher so the attention must be given to provide animals with early treatment and better environment to avoid secondary infections.

REFERENCES

1. Alhendi, A. B., E. M. Abuelzein, A. A. Gameel, M. M. Hassanein: A slow-spreading mild form of camel pox infection. Zentralbl Veterinarmed B. 1994; 41: 71-73.

2. Munz, E., C. K. Otterbein, H. Meyer and I. Renner-Mueller: Laboratory investigation to demonstrate a decreased virulence of two cell adapted African camel pox virus isolates as possible vaccine candidates. J. Camel Prac. and Res., 1997; 4: 169-175.

3. Otterbein, C., H. Meyer, I. Renner-Mueller and E. Munz: Charakterisierung zweier afrikanischer Kamelpockenvirus-Isolate. Mitt. Oesterr. Ges. Tropenmed. Parasitol., 1995; 17: 7-16.

4. Wernery, U. and R. Zachariah: Experimental camelpox infection in vaccinated and unvaccinated dromedaries. J. Vet. Med. B., 1999; 46: 131-135.

5. Kriz, B.: A study of camelpox in Somalia. J. Comp. Path., 1982; 92: 1-8

6. Ghulam, M., M. Z. Khan and M. Ather: An outbreak of generalized pox among draught camels in Faisalabad city. J. Camel Prac. and Res., 1998; 5: 127-129.

7. Wernery, U., and O. R. Kaaden: Infectious Diseases in Camelids. 2nd ed. Blackwell Science Berlin-Vienna, pp.177-178.

SERO-PREVALENCE OF AVIAN INFLUENZA IN WILD BIRDS IN NORTHERN PAKISTAN.

Ayaz, M, K.Naeem, A. Qayyum, M. Sajid, I.Ahmad., A.Hussain, M.Hussain

1. Veterinary Research Center Mandian, Abbottabad NWFP Pakistan, 2. National Reference Laboratory for Poultry Diseases NARC Islamabad Pakistan. 3. Hazara University Mansehra NWFP Pakistan.

ABSTRACT

Prevalence of antibodies to avian influenza virus subtypes H5, H7 and H9 was studied in wild birds of northern parts of Pakistan. The wild birds included 240 pheasants, 1500 common crows, 300 passerine and 60 migratory water fowl. The serum samples taken from these birds were subjected to haem_agglutination inhibition test (HAI). The HI antibody titres were recorded as 0.8 % against H5, 2.2 % against H7 and 3.06 % against H9 of 1500 examined common crows (Corvus splendens in 300 examined house sparrows) prevalence of antibodies of avian influenza viruses was recorded as 1.33 % against H7 and 8.33 % against H9 in whereas prevalence of H7 antibody in 3.33 % and H9 antibody in 10 % of the migratory water fowl was recorded. Comparative prevalence of H9 antibodies was the highest in migratory water fowl.

Key words: avian influenza viruses, antibodies, wild birds.

INTRODUCTION:

Influenza virus infections have been previously reported in many species including human, pigs, horses, marine animals and variety of domestic and wild birds (Webster et al., 1992). It is generally accepted that in human influenza pandemics from the last centuries and numerous outbreaks in domestic and wild animals, interspecies transmission of avian influenza viruses have played a crucial role (Frend and Franson, 1999). Predominantly water associated wild birds such as Ducks, Geese, Gulls and shore birds form the reservoir of influenza A viruses in nature (Webster et al., 1992).

In 2003 Senne detected serum antibodies against Avian Influenza Virus (AIV) H5N2 in chicken, quail and ostrich. The antibodies to H9N2 in chicken, turkey, wild duck, domestic duck and ostrich. Stevena and co-workers (2005) reported the incidence of H5N1 in smuggled Thai Eagles in Belgium.

Chen et al., (2004) reported the circulation of H5N1 among apparently healthy ducks in China. Sims at al (2005) detected H5N1 virus in southern China first time in domestic Geese during 1996. By 2000, host range extended to domestic Ducks which played a key role in the genesis of 2003/2004 outbreak in China. Chen et al., (2006) claimed that source of outbreak of H5N1 in Qingai lake in migratory water fowl was poultry. In 2005, Terregino et al., reported the isolation of H7N7 and H7N4 from water fowl in Italy. They reported nine isolates of H7 subtypes, five from wild Mallard, two from Teals, one from domestic Duck and one from domestic Geese.

The first out break appeared in Pakistan during 1995, when Highly Pathogenic Avian Influenza (HPAI) virus of H7 occurred in Northern Pakistan (Naeem and Hussain, 1995). An outbreak of Avian Influenza caused by Low Pathogenic Avian Influenza

(LPAI) virus H9N2 appeared during 1998 among broilers in Pakistan (Naeem et al., 1999).

During 2003-2004, Pakistan was affected by a devastating epidemic of Highly Pathogenic Avian Influenza (HPAI) H7N3 where as the first outbreak of H5N1 was recorded in February, 2006 in northern parts of Pakistan (Naeem, et al., 2007).

The present study was designed to examine the role of wild birds in the spread of AIV infection in the North Western Frontier Province (NWFP) of the country.

MATERIALS AND METHODS:

During 2005-06 2100 serum samples were collected from the wild birds species; namely migratory water fowl (60), Pheasants (240), common crows (1500) and house sparrows (300) at different locations of NWFP of Pakistan. The distribution of sampling is further detailed in Table-1.

Similarly, 60 blood samples were collected from migratory water fowl from Tarbela Lake. The water fowl examined were Mallard Ducks [Anas platyrhynchos] 25, Coots [Fulica atra] 20 , Shovelar [Anas clypeata] 4, Teal [Anas crecia] 4, Pochard [Aythya ferina] 3 and Tern [Sterna albifrons] 4.

Blood samples of 240 pheasants were collected from seven aviaries of North West Frontier Province (NWFP) Wild Life Department, each at different location in the province. The species examined were, Ring Neck Pheasant [Phasianus colchicus] 130, Cheer Pheasant [Catreus wallichii] 50, Leady Amherst's Pheasant [Chrysolophus amherstiae] 20, Reeve's Pheasant [Syrmaticus reevesi] 20, and Silver Pheasant [Lophura nycthemera] 20. No sick bird reported at the time of sampling.

Table –I. Distribution of samples collected from various species of wild birds

Pheasants (240)	Migratory Birds(60)	Common Crows (1500)	House Sparrow (300)
LeadyAmherst's Pheasant (20)	Mallard duck (25)		
Reeve's Pheasant (20)	Coot (20)		
Silver Pheasant (20)	Shovelar (04)		
Ring necked (130)	Teal (04)		
Cheer Pheasant (50)	Pochard (03)		
	Tern (04)		

Pheasants 240, Migratory birds 60, Common Crows 1500 and House sparrows 300

Serum obtained from the blood samples was analyzed for detection of antibody against Avian Influenza Virus subtypes H5, H7 and H9, employing Haemagglutination Inhibition (HI) test following the procedure described in OIE- Manual of Diagnostic tests and Vaccines for Terrestrial Animals (2005). All the serum samples were pre sensitized with 10 percent chicken red blood cells for 30 minutes before conducting the HI procedure. Antibody titer of equal or above 1:16 was considered positive against corresponding antigen.

RESULTS:

The prevalence of antibodies against Avian Influenza Viruses subtypes H5, H7 and H9 in the tested subgroups of wild birds is described as below.

Table-II Presence of seroconversion against Avian Influenza Viruses in Wild Birds.

		No. of samples showing GMT of HI Antibody titres against subtypes		
Type of birds	No of birds tested	H5	H7	H9
Pheasants	240	-	-	-
Crows (Corvus splendens	1500	12	34	46
House Sparrows (Passer domesticus)	300	-	04	25
Migratory birds	60	-	02 (3.33)	06

Table-III. Distribution of HI antibody titres against Avian Influenza viruses in different species of Water Fowl.

Species	No of Birds	H5	H7	H9
Mallard Ducks (Anas platyrhynchos	25	-	2 (8.00)	3 (12.00)
Coots (Fulica arta)	20	-	-	2 (10.00)
Shovelar (Ana clypeata)	4	-	-	-
Teal (Anas crecca)	4	-	-	-
Pochard (Aythya ferina)	3	-	-	-
Common Tern (Storna albifrns)	4	-	-	1 (25.00)

DISCUSSIONS:

Wild birds specially water fowl had long been a focus of concern as a source for Avian Influenza infection in poultry. Avian Influenza is usually a non clinical viral infection in wild bird. The viruses are maintained in wild birds through faeco-oral route of transmission. These viruses change rapidly in nature by mixing of their genetic components to form slightly different virus subtypes. Avian influenza is caused by these slightly different viruses rather than a single virus (Easterday and Hinshaw, 1991). In the present study, sero-prevalence of antibodies against Avian Influenza Virus (AIV) subtypes were found in majority of the test subgroups of wild birds, except Pheasants (table-II). These results were also in line with those reported by Terrigeno et al., (2005) and Stallknecht and Shane (1988). The present findings were also supported by Alexander (1988), who reported Avian Influenza Viruses (AIV) infection in Pheasant and passerine. However, the absence to any subtype of Avian Influenza infection in Pheasants in present study was contrary to the findings of Jennifer and co-workers (2006) and to that of Alexander (1988). This may be due to the reason that Pheasants in the Aviaries understudy were maintained under strict management standards and the infection could not be transmitted during captivity.

The high prevalence of AIV subtypes H9 and h7 antibodies among migratory water fowl, House Sparrow are in line with those previously reported by Naeem, et al., (2006), where the similar types of results were obtained in the Punjab area.

The antibodies to different AIV in different types of migratory water fowl indicated that Mallard ducks had been the major type harboring these viruses (Table-III). This was supported by earlier study of Terregino, et al., (2005).

The work presented here indicates that different serotypes of Avian Influenza viruses circulate in the poultry population and also get transmitted from the endemic areas of NWFP to other parts of the country through the movement of the transitory wild birds. This has been clearly found during the out break of H5N1 in 2007 when this virus was recorded from the dead crows in the affected areas. Similarly, the virus H9N2 was reportedly recovered from the specimens collected from wild birds by Naeem, et al., (2006). The presence of antibodies against H5, H9 and H7 viruses of AI indicates that the species of wild birds were involved in this regard as reservoirs for AIVs in nature.

The study of Naeem, et al.,(2006) indicated that the Avian Influenza outbreak (H7N3), occurred in commercial poultry in one region (South East Asia Region) during 1995 and spread rapidly to other parts of the country as well as situation complicated by the occurrence of different subtypes of Avian Influenza Viruses. The circulation of Avian Influenza viruses as reported in the present study in wild birds both native and migratory may be an important link for circulation of these viruses. The role of native wild birds in propagation of Avian Influenza Viruses and transfer of these viruses to the poultry and on the other side to migratory water fowl may be of concern and needed to elaborate further in this direction.

REFERENCES:

1. Born,S.V., I.Thomes, G.Hamquet, B.Lambrecht, M.Boschmans, et al., 2005.
2. Highly Pathogenic H5N1 Avian Influenza Virus in smuggled Thai Eagles, Belgium. Emerging Infectious Diseases, 11(50: 702.
3. Capua,I., B.Grossele, E.Bertoli, 2000. Monitoring of highly pathogenic Avian Influenza In wild birds in Italy. Veterinary record, (November): 640.
4. Chen,H., G.deng, Z.Li, G.Tian, Y.Li, et al., 2004. The evolution of H5N1
5. Influenza Virus in Ducks in Southern China. PNAS, 10(28)10452-10457.
6. Chen,H., G.J.D.Smith, K.S.Li, J.Wang, X.H.Fan, et al., 2006. Establishment of multiple Sub lineages of H5N1 Influenza Viruses in Asia. Implications for pandemic control. PNAS, 103 (8): 2845-2850.
7. Field Manual of Wild life Diseases, 1999. General field Procedures and a. DiseasesOf Wild Life: Avian Influenza: Milton Friend and J.C.Franson eds.US Department of the Interior, US Geological Survey. 181-184.
8. Naeem,K., and N.Siddique, 2006. Use of strategic vaccination for the control of Avian Influenza in Pakistan. Develop. Biol., 124: 145-150.
9. Naeem,k., N.Siddique, M.Ayaz, M.A.Jalali, 2007. Avian Influenza in Pakistan: Outbreaks of low and high pathogenicity Avian Influenza in Pakistan during 2003-2006. Avian Diseases, 51: 189-193.
10. Office International Des Epizootic (OIE) 2005. Manual of Diagnostic Tests and Vaccines for Terrestrial animals, 5th edition, 2.7.12.
11. Senne,D.A., 2003. Avian Influenza in the western hemisphere including the pacific Islands and Australia. Avian Diseases, 47: 798-805.
12. Sims,L.D., J.Domenech, C.Bonigno, S.Kohns, A.Kamata, J.Lubroth, V.Martin, P.Roeder, 2005. Origin and Evolution of highly pathogenic H5N1 Avian Influenza in Asia. Veterinary Record, 157: 159-164.
13. Terrigeno,C., G.Cattoli, R.De.Nardi, m.S.Beato, I.Capua, 2005. Isolation of Influenza A Viruses subtype H7N7 and H7N4 from Water Fowl in Italy. Veterinary record, 6: 292.

This study was partially sponsored by FAO/TCP/PAK/3002-E project which was gratefully acknowledged.

PAKISTAN JOURNAL OF LIVESTOCK SCIENCES (PJLSc)

RECENT INSTRUCTIONS / GUIDE LINES FOR CONTRIBUTORS

1. The original Articles/Research Notes be sent on A-4 paper with one Inch Margin on left side. The Text should be on Font No. 11.
2. The standard format should be abstract, introduction, review of literature, material and methods, results and discussions, conclusions and recommendations followed by references/literature cited (in alphabetical order)
3. Number of tables be restricted to minimum possible.
4. Alongwith Two printed (hard copies) a CD (Soft copy) may also be enclosed to quicken the process of Referees) evaluation.
5. Colour prints, photographs, if indispensable, (include 100 prints/100 photographs with colour scheme, advised). This is negotiable.
6. References be kept limited (Not more than ten) prepferably for the last 5-10 years. Standard format be adopted.
7. Please attach CVs if you are a New comer to Pakistan Journal of Livestock Sciences (PJLSc) Islamabad.
8. Contribution of Rs.500/- per article/paper be enclosed upto four pages each Extra page will cost Rs.100/- each.
9. Abstracts be limited to one para of 100-150 words in between the A-4 paper supported in separate line with key words for example:-
   Microbiology ; Coliform bacilli ; E-coli ; incidence of food contamination, Pakistan.
   Chemistry ; Physico – Chemical analysis; algae; lotus lake water – Pakistan.
10. First Screening of the papers will be within one month and acceptance/or other wise will be communicated after a period of 30 (THIRTY) Days.
11. Changes/Amendments /Reviewers comments and advises must be attended by the contributer(s) and final draft with CDs be re-submitted to the Chief Editor within 14 days.
12. Duplications be avoided.
13. Advertisements be sent according to subscribed rates.
14. Selected Scientific Papers/Articles will be subjected to PEER REVIEWING simultaneously by the local as well as Foreign Referees, in accordance with the guidelines of HEC Islamabad Pakistan.

EDITORIAL BOARD

Pak. J.L.Sc.Vol .(1) No.1-2009

PAKISTAN JOURNAL OF LIVESTOCK SCIENCES (PJLSc)

RECENT INSTRUCTIONS / GUIDE LINES FOR CONTRIBUTORS

1. The original Articles/Research Notes be sent on A-4 paper with one Inch Margin on left side. The Text should be on Font No. 11.
2. The standard format should be abstract, introduction, review of literature, material and methods, results and discussions, conclusions and recommendations followed by references/literature cited (in alphabetical order)
3. Number of tables be restricted to minimum possible.
4. Alongwith Two printed (hard copies) a CD (Soft copy) may also be enclosed to quicken the process of Referees) evaluation.
5. Colour prints, photographs, if indispensable, (include 100 prints/100 photographs with colour scheme, advised). This is negotiable.
6. References be kept limited (Not more than ten) prepferably for the last 5-10 years. Standard format be adopted.
7. Please attach CVs if you are a New comer to Pakistan Journal of Livestock Sciences (PJLSc) Islamabad.
8. Contribution of Rs.500/- per article/paper be enclosed upto four pages each Extra page will cost Rs.100/- each.
9. Abstracts be limited to one para of 100-150 words in between the A-4 paper supported in separate line with key words for example:-
   Microbiology ; Coliform bacilli ; E-coli ; incidence of food contamination, Pakistan.
   Chemistry ; Physico – Chemical analysis; algae; lotus lake water -- Pakistan.
10. First Screening of the papers will be within one month and acceptance/or other wise will be communicated after a period of 30 (THIRTY) Days.
11. Changes/Amendments /Reviewers comments and advises must be attended by the contributer(s) and final draft with CDs be re-submitted to the Chief Editor within 14 days.
12. Duplications be avoided.
13. Advertisements be sent according to subscribed rates.
14. Selected Scientific Papers/Articles will be subjected to PEER REVIEWING simultaneously by the local as well as Foreign Referees, in accordance with the guidelines of HEC Islamabad Pakistan.

EDITORIAL BOARD