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# Immunologic evaluation of 10 different adjuvants for use in vaccines for chickens against highly pathogenic avian influenza virus

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ABSTRACT

Avian influenza viruses (AIV) are a threat to poultry production worldwide. Vaccination is utilized as a component of control programs for both high pathogenicity (HP) and low pathogenicity (LP) AIV. Over 95% of all AIV vaccine used in poultry are inactivated, adjuvanted products. To identify the best formulations for chickens, vaccines were prepared with beta-propiolactone (BPL) inactivated A/British Columbia/314514-1/2004 H7N3 LP AIV using ten commercially available or experimental adjuvants. Each vaccine formulation was evaluated for immunogenicity in chickens. Challenge studies with an antigenically homologous strain of HPAIV were conducted to compare protection against mortality and measure reductions in virus levels in oral swabs. The four best adjuvants from the studies with BPL inactivated antigen were selected and tested identically, but with vaccines prepared from formalin inactivated virus. Mineral and vegetable oil based adjuvants generally induced the highest antibody titers with 100% seroconversion by 3 weeks post vaccination. Chitosan induced positive antibody titers in 100% of the chickens, but the titers were significantly lower than those of most of the oil based adjuvants. Antibody levels from calcium phosphate and alginate adjuvanted groups were similar to those of non-adjuvanted virus. All groups that received adjuvanted vaccines induced similar levels of protection against mortality (0–20%) except the groups vaccinated with calcium phosphate adjuvanted vaccines, where mortality was similar (70%) to groups that received non-adjuvanted inactivated virus or no vaccine (60–100% mortality). Virus shedding in oral swabs was variable among the treatment groups. Formalin inactivated vaccine induced similar antibody titers and protection against challenge compared to BPL inactivated vaccine groups. These studies support the use of oil adjuvanted vaccines for use in the poultry industry for control for AIV.



## 1. Introduction

Avian influenza (AI) is a highly consequential disease of poultry resulting in significant economic losses worldwide due to mortality, morbidity, culling of birds, and lost trade markets. Vaccination is used to help control AI virus (AIV) and limit losses in areas where the virus is endemic. Although vectored vaccines are available and licensed in some countries, 95.5% of the AIV vaccine used for poultry, by dose, is oil emulsion, inactivated whole virus vaccine [1]. Despite the disadvantage that this type of vaccine must be applied to each bird individually, inactivated vaccines are safe, effective, and relatively inexpensive, therefore will remain highly utilized for AIV in poultry particularly in areas where labor costs are low. Individual inoculation does have the advantage that it can ensure high coverage within the vaccinated populated.

Optimal formulations of inactivated vaccines need an appropriate antigen to match the field challenge virus. However, even highly immunogenic AIV strains require adjuvants to elicit a sufficient immune response. Vaccine adjuvants are chemical substances, microbial components or proteins, that enhance the

*Abbreviations:* 70VG, Montanide ISA 70VG adjuvant; 71VG, Montanide ISA 71VG adjuvant; 760VG, Montanide ISA 760VG adjuvant; 780VG, Montanide ISA 780VG adjuvant; AI, avian influenza; AIV, avian influenza virus; BHI, brain heart infusion buffer; BPL, beta-propiolactone; CAP, calcium phosphate adjuvant; DPC, days post-challenge; ECE, embryonating chicken eggs; $EID_{50}$, 50% egg infectious doses; ELISA, enzyme linked immunosorbent assay; GEL01, Montanide GEL01 adjuvant; GMT, geometric mean titer; HA, hemagglutinin protein; HAU, hemagglutinating units; HI, hemagglutination inhibition; HP, highly pathogenic; IFA, Freunds' incomplete adjuvant; IM, intra-muscular; IV, intra-venous; LP, low pathogenic; OP, oropharyngeal; NAIV, non-adjuvanted inactivated virus; NP, nucleoprotein; SPF, specific pathogen free; SQ, sub-cutaneous; WPV, weeks post vaccination.

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# Presence of Virulent Newcastle Disease Virus in Vaccinated Chickens in Farms in Pakistan

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**One year after a virulent Newcastle disease virus (vNDV) outbreak in Pakistan, the causative strain was present in vaccinated chickens of multiple farms despite the existence of high-average NDV-specific antibody titers (>4.75 $\log_2$). The data suggest a possible role of vaccinated birds as reservoirs of vNDV.**

Newcastle disease (ND) is one of the most devastating diseases affecting the poultry industry worldwide (1). It is caused by avian paramyxoviruses of serotype 1 (APMV-1), also known as Newcastle disease virus (NDV) (1). NDV may be classified into two classes and subdivided into up to 18 genotypes based on genetic diversity (2, 3). Currently, pigeons and cormorants are two well-recognized reservoirs of virulent NDV (vNDV) (4, 5); however, those species normally carry viruses of genotypes Va and VI (1), and the reservoir for other highly virulent viruses that commonly circulate in poultry (genotypes Vb, VII, and XII to XVIII) is unknown.

The failure to control ND with vaccination alone in countries where the virus is endemic underscores the importance of understanding the mechanisms of vNDV maintenance. It has been shown under experimental conditions that vaccines do not prevent the replication of highly virulent viruses; thus, circumstantial evidence suggests that the capacity of vaccinated birds to shed the viruses may allow vaccinated poultry to act as a reservoir (1). As culling is the preferred control strategy in the developed world,

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TABLE 1 Selected strain locations and descriptions, accession numbers corresponding to sequenced regions, farm locations and flock sizes, age and number of samples per bird, observed NDV status and mortality, and HI titer data

| Strain | Accession no. | Latitude | Longitude | Flock size | Age of bird (days), host | No. of samples collected | % positive for NDV | % mortality | HI titer | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Mean | SD |
| Chicken/Pak/Bahwalpur/300/2013 | KF761551 | 29.3956 | 71.6836 | 36,000 | 22, broiler | 16 | 94 | 6 | 5.7 | 1.35 |
| Chicken/Pak/Faisal abad/03/2013 | KF761538 | 31.418 | 73.079 | 30,000 | 28, broiler | 7 | 100 | 16 | 6.0 | 0.97 |
| Chicken/Pak/Gujranwala/589/2013 | KF761536 | 32.15 | 74.1833 | 36,000 | 31, layer | 29 | 82 | 15 | 6.9 | 1.06 |
| Chicken/Pak/Gujrat/234/2013 | KF761548 | 32.5738 | 74.0789 | 35,000 | 35, broiler | 55 | 56 | 15 | 6.0 | 0.85 |
| Chicken/Pak/Hafiz abad/611/2013 | KF761535 | 32.07 | 73.68 | 6,000 | 21, broiler | 2 | 100 | 31 | 5.4 | 1.30 |
| Chicken/Pak/Kasur/08/2013 | KF761540 | 31.117 | 74.4502 | 77,000 | 8, broiler | 17 | 53 | 40 | 5.7 | 2.12 |
| Chicken/Pak/Khanewal/55/2013 | KF761552 | 30.303 | 71.9333 | 15,000 | 12, broiler | 23 | 91 | 7 | 6.0 | 0.89 |
| Chicken/Pak/Lahore/02/2013 | KF761537 | 31.545 | 74.3406 | 1,00,000 | 37, broiler | 16 | 100 | 50 | 5.8 | 2.44 |
| Chicken/Pak/Lodhran/62/2013 | KF761543 | 29.5333 | 71.6333 | 2,000 | 28, layer | 6 | 100 | 5 | 5.7 | 0.98 |
| Chicken/Pak/Multan/43/2013 | KF761541 | 30.1978 | 71.4697 | 8,000 | 42, broiler | 48 | 81 | 45 | 6.8 | 1.65 |
| Chicken/Pak/Muzafargarh/160/2013 | KF761549 | 30.0703 | 71.1933 | 22,000 | 21, broiler | 8 | 100 | 23 | 5.4 | 0.81 |
| Chicken/Pak/Nankana Sahib/155/2013 | KF761546 | 31.45 | 73.7 | 9,000 | 25, broiler | 6 | 100 | 50 | 6.8 | 1.69 |
| Chicken/Pak/Norwal/40/2013 | KF761550 | 32.2167 | 74.95 | 17,000 | 26, broiler | 17 | 81 | 33 | 5.3 | 1.13 |
| Chicken/Pak/Okara/118/2013 | KF761547 | 30.8091 | 73.4477 | 62,000 | 29, broiler | 9 | 100 | 27 | 6.5 | 0.63 |
| Chicken/Pak/Rawalpindi/188/2013 | KF761534 | 33.6 | 73.0333 | 16,000 | 22, layer | 4 | 67 | 40 | 4.8 | 1.13 |
| Chicken/Pak/Sahiwal/06/2013 | KF761539 | 30.5833 | 73.3333 | 23,000 | 35, broiler | 7 | 100 | 43 | 6.4 | 1.75 |
| Chicken/Pak/Sargodha/212/2013 | KF761533 | 32.0836 | 72.6711 | 3,000 | 22, broiler | 3 | 100 | 20 | 5.1 | 0.81 |
| Chicken/Pak/Sheikhpura/22/2013 | KF761545 | 31.715 | 73.985 | 29,000 | 24, broiler | 14 | 57 | 10 | 5.0 | 0.73 |
| Chicken/Pak/Sialkut/88/2013 | KF761544 | 32.4972 | 74.5361 | 6,000 | 49, broiler | 7 | 100 | 7 | 5.6 | 1.09 |
| Chicken/Pak/Vehari/46/2013 | KF761542 | 30.0419 | 72.3528 | 7,000 | 22, broiler | 6 | 100 | 7 | 5.9 | 0.85 |

genomeA
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# Complete Genome Sequence of a Recent Panzootic Virulent Newcastle Disease Virus from Pakistan

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**The genome sequence of a new strain of Newcastle disease virus (NDV) (chicken/Pak/Quality Operations Lab/SFR-611/13) is reported here. The strain was isolated from a vaccinated chicken flock in Pakistan in 2013 and has panzootic features. The genome is 15,192 nucleotides in length and is classified in subgenotype VIIi of genotype VII, class II.**

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Newcastle disease (ND) is caused by an avian paramyxovirus of serotype 1 (APMV-1), and it is listed as a serious disease by the World Organization for Animal Health, affecting >236 avian species with wide geographical distribution (1). APMV-1, also designated Newcastle disease virus (NDV), belongs to the genus *Avulavirus* of the *Paramyxoviridae* family, and it has an enveloped, nonsegmented, and negative-sense RNA genome with six transcriptional units (3′-NP-P-M-F-HN-L-5′). Phylogenetic analysis of isolates of the virus worldwide has identified large genetic variability, and at the moment, there are up to 18 different genotypes (2, 3). Recently a new subgenotype of NDV (VIIi) with epizootic characteristics has been identified and characterized (4, 5). Viruses of this genotype have rapidly disseminated from Indonesia, to Pakistan, the Middle East, Europe, and Africa. The 2013 isolate described here is part of this lineage and is important because it has been found to be present in at least 20 Newcastle disease-vaccinated chicken farms in Pakistan, suggesting unique virulence and a capacity to disseminate (4, 5).

The virus was isolated and propagated in 9-day-old chicken embryonated eggs from a flock free from NDV antibody. Viral RNA was isolated from the allantoic fluids using TRIzol LS (Invitrogen, USA), and cDNA was synthesized using the random hexamer primer with the Transcriptor first-strand cDNA synthesis kit (Fermentas, USA), per the manufacturer's recommendations. The complete genome sequence was obtained using an overlapping real-time PCR (RT-PCR) strategy with 22 different primers pairs using Platinum PCR SuperMix high-fidelity polymerase (Invitrogen) that included the ends. The products were purified using the GeneJET gel extraction kit (Fermentas) and cloned into the TOPO TA vector (Invitrogen) accordingly. For sequencing, an ABI 3130 automated sequencer (ABI, Inc., Foster City, CA) was used, and for editing and assembly, the BioEdit software was used (6). Sequence analysis revealed that the full-genome length of the isolated strain currently designated chicken/Pak/Quality Operations Lab/SFR-611/13 is 15,192 nucleotides (nt). Phylogenetic analysis of the complete genome classified this strain into class II, genotype VII, subgenotype VIIi. The nucleotide sequence had the highest homology (99%) with the sequence of Indonesian strain chicken/Banjarmasin/010/10 (GenBank accession no. HQ697254).

The strain chicken/Pak/Quality Operations Lab/SFR-611/13 has a virulent pathotype, with $^{112}$RRQKR$^{116}$ at the C terminus of the F2 protein and F at residue 117. The data presented here provide evidence of a novel strain of NDV in the region, and understanding the prevalence and variation of newly emerging NDV strains has great significance for the prevention and control of ND.

**Nucleotide sequence accession number.** The complete genome sequence of the newly emerging NDV strain chicken/Pak/Quality Operations Lab/SFR-611/13 has been deposited in GenBank under the accession no. KM670337.

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# Characterization of H5N1 highly pathogenic avian influenza viruses isolated from poultry in Pakistan 2006–2008

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**Abstract** Nine avian influenza viruses (AIV), H5N1 subtype, were isolated from dead poultry in the Karachi region of Pakistan from 2006 to 2008. The intravenous pathogenicity indices and HA protein cleavage sites of all nine viruses were consistent with highly pathogenic AIV. Based on phylogenetic analysis of the HA genes, these isolates belong to clade 2.2 and both the HA and NA are closely related to each other (nucleotide identities above 99.0%) and to other Middle Eastern H5N1 AIV isolates (nucleotide identities above 98.0%). The phylogenetic data suggest that the virus in both epornitics of H5N1 HPAIV in commercial poultry in the Karachi region of Pakistan between 2006 and 2008 were from a very closely related source, however, there is inadequate epidemiological data to determine what the reservoir was for the virus between the 2006 and 2007 outbreaks other than that there was a single introduction into the region.

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**Keywords** Avian influenza · High pathogenicity · H5N1 · Pakistan · Hemagglutinin · Neuraminidase

## Introduction

Avian influenza virus (AIV) was first reported in Pakistan in 1995 when an H7N3 high pathogenicity avian influenza virus (HPAIV) infected commercial poultry [1, 2]. Since then there have been sporadic outbreaks of H7N3 HPAIV and low pathogenicity (LP) AIV and H9N2 LPAIV resulting in the loss of large numbers of poultry [2–4]. The H7N3 and H9N2 outbreaks have been controlled primarily by vaccination and biosecurity. In February 2006, the first H5N1 HPAIV outbreaks occurred in the northwest Frontier region of Pakistan in commercial poultry farms and later in 2006 extended to the capital region of Islamabad [5]. In early 2007, the H5N1 HPAIV was detected in wild birds [5], and later that same year the first human cases of H5N1 HPAIV infection in Pakistan were reported [6]. Cases of H5N1 were not reported in commercial poultry in the southern region of Pakistan (Karachi/Sindh region) until March of 2007, although a 2006 AIV isolate (A/chicken/Pakistan/NARC-228/2006) was later identified to be an H5N1 HPAIV. Similar to the other outbreaks in Pakistan, localized vaccination and stamping out procedures were employed to try to contain the virus and were temporarily successful until additional outbreaks were reported in the Karachi region in January of 2008 [5]. Pakistan declared itself free of H5N1 HPAI in September of 2008 with no further H5N1 outbreaks reported to date. In this brief report, we characterize several H5N1 isolates from Pakistan and discuss the epidemiological implications of these isolates.

AVIAN DISEASES 53:306–309, 2009

*Research Note—*

# Molecular Characterization of Pakistani Field Isolates of Infectious Bursal Disease Virus

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SUMMARY. The reverse transcriptase-polymerase chain reaction followed by restriction fragment length polymorphism (RT-PCR/RFLP) technique was used to identify and characterize Pakistani field isolates of infectious bursal disease virus (IBDV). These isolates have caused heavy losses to the poultry industry (mortality up to 60%) during the period between 1999 and 2005. Ten samples (five local isolates and five commercial vaccines) were examined for IBDV. Nine samples were positive for IBDV as evidenced by the amplification of the 743-bp region of the VP2 gene by RT-PCR. The RT-PCR products were subjected to restriction enzyme digestion with *Bst*NI, *Mbo*I, and *Ssp*I. The RFLP profiles of all samples on digestion with the *Mbo*I enzyme yielded a fragment size of 229 and 362 bp except for vaccine strain Bursine Plus, which yielded a profile of 229 and 480 bp. However, digestion with *Bst*NI yielded two distinct RFLP patterns. The first profile was detected in field isolates ML-1/SPVC/2001 and NP2/SPVC/2002 with four fragments of 119, 154, 172, and 209 bp, resembling RFLP profiles of molecular group 4 isolates. NL-3/SPVC/2003, NK-4/SPVC/2004, and NPK-5/SPVC/2005 generated a different RFLP profile with fragments of 119, 172, and 424 bp, resembling the profiles of molecular group 6 isolates. However, all the field and vaccine strains showed the absence of *Ssp*I restriction sites in their genome. It can be concluded that the Pakistani isolates can be grouped in molecular groups 4 and 6 of IBDV.

RESUMEN. *Nota de Investigación*—Caracterización molecular de aislamientos de campo Pakistaníes del virus de la enfermedad infecciosa de la bolsa.

La transcripción reversa y la reacción en cadena de la polimerasa seguida del análisis del polimorfismo de la longitud de los fragmentos de restricción (de las siglas en inglés RT-PCR/RFLP) fueron utilizadas para identificar y caracterizar aislamientos de campo del virus de la enfermedad infecciosa de la bolsa Pakistaníes. Estos aislamientos han causado pérdidas significativas a la industria avícola (mortalidad de hasta 60%) durante el periodo comprendido entre 1999 y 2005. Diez muestras (cinco aislamientos locales y cinco vacunas comerciales) fueron examinadas para detectar al virus de Gumboro. Nueve muestras fueron positivas para el virus de la enfermedad de la bolsa, tal como se evidenció por la amplificación por RT-PCR de un producto de 743 pares de bases que incluye al gene VP2. Los productos de RT-PCR fueron sometidos a la digestión con las enzimas de restricción *Bst*NI, *Mbo*I y *Ssp*I. Los perfiles de RFLP de todas la muestras con la digestión con la enzima *Mbo*I mostraron fragmentos de 229 y 362 pares de bases respectivamente, con excepción de la cepa vacunal Bursine-Plus que indujo un perfil con fragmentos de 229 y 480 pares de bases. Sin embargo, la digestión con *Bst*NI indujo dos patrones de RFLP distintos. El primer perfil fue detectado en los aislamientos de campo ML-1/SPVC/2001 y NP2/SPVC/2002 con cuatro fragmentos de 119, 154, 172 y 209 pares de bases, que es similar al perfil de aislamientos pertenecientes al grupo molecular 4. Mientras que los aislamientos NL-3/SPVC/2003, NK- 4/SPVC/2004, y NPK-5/SPVC/2005 generaron diferentes perfiles de RFLP con fragmentos de 119, 172 y 424 pares de bases semejantes a los perfiles del grupo molecular 6. Sin embargo, todos los aislamientos de campo y las vacunas mostraron la ausencia del sitio de restricción para la enzima *Ssp*I. Se puede concluir que los aislamientos Pakistaníes pueden ser agrupados en los grupos moleculares 4 y 6 del virus de la enfermedad infecciosa de la bolsa.

Key words: IBDV, Pakistan, RT-PCR, RFLD

Abbreviations: IBD = infectious bursal disease; IBDV = infectious bursal disease virus; RFLP = restriction fragment length polymorphism; RT-PCR = reverse transcriptase-polymerase chain reaction

Infectious bursal disease (IBD) is an acute, highly infectious disease of young chickens with significant economic importance. IBD virus (IBDV) is a nonenveloped; double-stranded RNA virus with a bi-segmented (A, 3300 bp, and B, 2800 bp) genome (12). The segment A encodes a precursor poly-protein that is processed in three mature proteins; VP2, VP3, and VP4. The VP2 is the major structural protein that contains antigenic regions, that is, those responsible for the production of neutralizing antibodies in chickens, and a hyper-variable region in terms of amino acid sequence variation between strains (5,14). The segment B of the IBDV genome that is a double-stranded polymer encodes a protein, VP1. There are two serotypes of IBDV, but only serotype I causes infection in poultry. Serotype I has been divided into several groups on the basis of antigenic variation and virulence: classical virulent strains, antigenic variant strains, and very virulent strains. For the characterization of antigenic differences among IBDV strains, several *in vitro* and *in vivo* tests have been used; these include: virus

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RESEARCH ARTICLE

# Efficacy of Live attenuated and Inactivated Oil Emulsion Infectious Bursal Disease Virus Vaccines in Broiler chicks

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**ABSTRACT**

This study was carried out with the aims to evaluate the efficacy of indigenous live and inactivated Infectious bursal disease virus (IBDV) vaccines in broilers. Two hundred and fifty (250), a-day-old broiler chicks divided into five groups (A-E) were immunized with live and inactivated vaccine at varying ages. Live vaccine was given to group A (at 8 days post hatch), B (at 8, 15 days post hatch), C (at 8, 15 and 23 days post hatch) and D (at 8 days post hatch). In addition group D received a booster dose of inactivated vaccine at 21 days of age, while group E served as control. Antibody titers were measured via Agar Gel Precipitation (AGP) test and ELISA, while the degree of protection against the virulent strains of IBDV was also recorded. Results showed that vaccine program adopted for group C and D produced significantly ($P<0.05$) higher antibody titer as compared to other groups. While a significant ($P<0.05$) difference in antibody titers was observed between group A and B while no considerable antibodies were detected in group E. The response to challenge dose was recorded as the difference of lesions in bursa, pectoral muscles or other visceral organs with the exception of group C and D. The study suggests that broiler chicks may be vaccinated at days 8, 15 and 23 with live attenuated vaccine or live attenuated vaccine followed by inactivated vaccine at days 8 and 21 that could provide an adequate protection against the virulent form of IBDV.



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## INTRODUCTION

Infectious bursal disease (IBD), an immune-suppressive disease of chickens leads to heavy economical losses to poultry industry (Lukert and Saif, 2003; Mahmood *et al*., 2006; Uddin *et al*., 2010). IBD was successfully controlled through vaccination using classical strains, however, in 1988 the emergence of very virulent form in Europe and variant strains in United States caused a sub-clinical immune-suppression despite of vaccination (Rautenshlein *et al.,* 2005). The variant isolates differ pathologically and serologically from classical IBDV strains and they contain different neutralizing epitopes which causes vaccination failures. Therefore vaccines prepared from indigenous strains have been observed to provide better protection due to more antigenic relatedness (Hsieh *et al*., 2010; Rojs *et al.,* 2011). A part from vaccines, the vaccination programs also play an important role in providing adequate protection but may vary from country to country and area to area (Block *et al.,* 2007). In addition, the vaccination program is also influenced by pathogenicity of viral challenge, placement program, density and diversity of the poultry population in the area of operation, level of biosecurity and ability of a vaccine to produce stress (Tsukamoto *et al.*, 1995; Alam *et al.*, 2002; De Wit, 2003). Maternal antibodies (MA) have also been reported to interfere with the vaccination program against IBD (Al-Natour *et al.*, 2004). Despite of heavy vaccination clinical outbreaks are reported in Pakistan and only 10% of the farmers use laboratory services for monitoring the immune status in their flocks.

Present study was designed to determine the efficacy of indigenous live attenuated and inactivated oil emulsion IBDV vaccines and to recommend an effective vaccination program for broilers to suit poultry industry in Pakistan. Besides these, another criterion of the present study was to reveal the maternal immune status in local broiler chickens.

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*Full Length Article*

# Molecular Identification of Agents Causing Respiratory Infections in Chickens from Southern Region of Pakistan from October 2007 to February 2008

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## ABSTRACT

Livestock and poultry contribute more than 49% of the agricultural GDP of Pakistan and play an important part in the alleviation of poverty and improvement in quality of life. Poultry industry is the second largest and organized industry of the country, progressing with an average rate of 8-10% yearly. However, viral and bacterial diseases are the major constraints in its growth. Respiratory infections are of paramount importance as high mortality may occur in poor management. Therefore, the etiology of respiratory disease is complex, often involving more than one pathogen at the same time. A wide variety of pathogens have been associated with respiratory infection in poultry, including Avian Pneumovirus (APV), Avian Influenza Virus (AIV), Infectious Bronchitis (IBV), Newcastle Disease Virus (NDV), Infectious Laryngotracheal virus (ILTV) and *Mycoplasma gallisepticum* (MG). As the Avian Influenza and Newcastle disease are endemic in the country, therefore, this study was planned to evaluate their status during the winter outbreaks. Tracheal samples were collected from poultry farms in southern region of Pakistan, experiencing heavy mortality of poultry flock. The samples were subjected to RNA extraction followed by RT-PCR. Out of 50 samples, 20 samples were positive for NDV, 28 for AIV and 2 were negative for both. Further, serotyping of 28 AIV isolates showed that, 6 were positive for H9, 20 for H5 and 2 for H7. Thus, it can be concluded that molecular techniques help in rapid identification of the agents causing infections. Further, the southern region of Pakistan had major infection resulting from AIV (H5) during October 2007 to February 2008.

**Key Words**: Newcastle disease virus (NDV); Avian influenza virus (AIV); Reverse transcription-polymerase chain reaction (RT-PCR); Avian influenza virus serotype H5; Avian influenza virus serotype H7; Avian influenza virus serotype H9

## INTRODUCTION

Poultry sector is one of the most vibrant segments of agriculture sector of Pakistan that generates an employment (direct/indirect) and income for about 1.5 million people. The current investment in this industry is about Rs. 200 billion with an annual growth of 8-10% (Government of Pakistan, 2007). The sector has faced tough challenges through its viral infections especially Avian Influenza (AI) and Newcastle disease (ND). In addition to these other infections such as infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV) and pneumovirus are also of major concern to the respiratory tract of chickens. Pneumovirus and ILTV have been found in tissues of the respiratory tract, whereas IBV, NDV and AIV also invade other tissues such as the kidneys, reproductive system and the gastrointestinal tract and the central nervous system by NDV and AIV (Swayne *et al.*, 1998). To control most respiratory viruses, live and inactivated vaccines have been developed and used by the poultry industry for many years. In most cases, live vaccines are prepared with attenuated strains of the respective virus and therefore, these viruses replicate in the tissues of the respiratory tract, inducing a reaction that is known as post vaccination reaction. Moreover, promising results have also been obtained using hyper immune yolk to protect against viral infection (Muhammad *et al.*, 2001).

The etiology of respiratory organisms is complex often involving more than one pathogen (Yashpal *et al.*, 2004). Therefore, these are of most importance as they cause diseases independently, in association with other or with bacterial organisms (Yashpal *et al.*, 2004). Poultry industry in Pakistan during the year 2004-05 had suffered an enormous loss of about Rs. 5.4 billion due to these respiratory viral infections. The NDV and AIV are endemic in the country with a quiet different situation in comparison

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# Characterization of Newcastle Disease Virus Isolated During 1995-2009 From Suburbs of Karachi-Pakistan

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**Abstract.**- Newcastle disease (ND) which is caused by *Avian Paramyxovirus* type 1 is among the major concerns of poultry production worldwide. In developing countries especially where backyard poultry are not monitored for infection, ND remains a threat. The use of vaccines (live and inactivated) for the last 50 years in South Asia has not been able to control the infection. The poultry mortality rate worsened after the emergence of Avian Influenza in commercial poultry during early 1990's. The present study was conducted on 84 samples during 1995-2009, from commercial poultry flocks in the suburbs of Karachi to isolate and characterize strains of Newcastle disease virus circulating in the region. The samples were passaged in 9-10 day old chicken embryonated eggs. Haemagglutinating activity was observed in eighty samples which were cross checked using ND virus (NDV) polyclonal serum employed in haemagglutination inhibition assay and only forty seven were positive for NDV. The infective allantoic fluid was later used for pathotyping of isolates by employing biological characterization tools. Results demonstrated that the majority of isolates were either mesogenic or velogenic strains based on the mean death time and intra cerebral pathogenicity indices. Molecular identification was done using reverse transcription polymerase chain reaction (RT-PCR) performed using viral RNA extracted from infective allantoic fluid. Three sets of previously reported primers were employed and primer set BK1/BK2 was found to be the most sensitive. The results suggest that Newcastle disease remains endemic and requires similar attention to that given to highly pathogenic avian influenza.

**Key words:** Newcastle disease, avian paramyxovirus type I, haemagglutinating activity, RT-PCR.

## INTRODUCTION

Newcastle disease (ND), an infection caused by avian paramyxovirus type 1 (APMV-1), was a major threat for the developed world in early 60's and late 70's (Alexander, 1998). The disease remains a problem for the developing world. Newcastle disease virus (NDV) continues to trigger backyard poultry outbreaks in developed countries such as U.S.A. and UK because of the importation of exotic birds (Alexander *et al.*, 1999a; Pedersen *et al.*, 2004) and racing pigeons (Kaleta *et al.*, 1988), respectively.

The disease is highly contagious and can infect approximately 236 avian species (Alexander, 1998). It exists as a single serotype based on neutralizing antibody test and cross protective analysis (Alexander *et al.*, 1999a,b). It has been endemic in this region for six decades and outbreaks are still reported in Pakistan (Afzal, 1992; Akhtar and Zahid, 1995; Arsha, 2000). Live vaccines developed by local producers using indigenous strains has contributed to controlling the spread of disease (Rehmani, 1996). However the lack of regulatory statues on importation of live birds and poultry vaccines, have limited the impact of control efforts. In practice, infection and periodic outbreaks are not necessarily prevented through vaccination (Khalafall *et al.*, 1992; Oncel *et al.*, 1997; Roy *et al.*, 2000; Yang *et al.*, 1997, 1999), therefore, emerging NDV strains are of concern and may overcome vaccination barriers (Panshin *et al.*, 2002).

Avian influenza virus (AIV) infection from significant strains (*i.e.* H5, H7, H9) in combination with NDV has been reported to be negatively impacting the poultry industry in Pakistan (Naeem and Siddiqui, 2006; Naeem *et al.*, 1999, 2007).

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Natural Resources Management: Reviews and Research in the Himalayan Watersheds II

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## Prevalence of Asthenozoospermia and Piyospermia in District Gilgit, Gilgit Baltistan

**Riaz Ali[1], Sajida Asghar[1], Dr.Nazir Ahmed Lone[2] and Imran[1]**

**Abstract**

Infertility is a reproductive health problem that affects many couples in human population; the affected couples undergo enormous emotional and psychological distress and constitute a major life crisis from social point of view. Asthenozoospermia is reduction in the vitality of spermatozoa; a high percentage of spermatozoa in the sample have less than normal motility, i.e., 50% fast progression. Piyospermia is abnormal presence of leukocytes in human ejaculate. Perspective study of male infertility to determine the prevalence of asthenozoospermia and piyospermia started from July 2010 to Feb 2011 in district hospital Gilgit and different clinics in Gilgit district. The total patient investigated were 87, affected cases were 58 and number of asthenozoospermia was 35 (40.22%), and the number of piyospermia and normozoospermic were 11 (12.64%,) and 29 (33.33%.) respectively. Thus, asthenozoosperima was higher in ratio than piyospermia in Gilgit district.

**Key Words:** Infertility, semen analysis, reproduction, motility, sperm count

### Introduction

Infertility is defined as the state in which a couple wanting a child cannot conceive after 12 month of unprotected intercourse (Mueller and Daling 1989). Whether it is primary or secondary infertility, the affected couples go through huge emotional and psychological misery and socially it comprises a big problem. Internationally the incidence of infertility is estimated to about 13 -18% (Hull et al. 1985). About 67-71% and 29-33% of patients have primary and secondary infertility respectively (Thonneau et al. 1991).

Globally, approximately 10-15% couples seek medical help for the problem of infertility. In 20-25% cases the problems are attributed to the male partner, while, 30-40% symbolize female factor. Approximately 30% of cases are associated with both partners and in 15% no specific factor can be identified (WHO 1992). Infertility is a common disorder and nearly one out of every six to eight couples suffers from it at any given time. Infertility among couples in their respective age is more common than hypertension, diabetes, heart diseases and even the common flu (Khan et al. 2005).

Agrawal (2008) assessed the sperm function, and noted good history, physical examination, and at least two semen analyses. Pryor et al. (1997) conducted a study on some infertile men with azoospermia or severe oligospermia who have small deletions in regions of Y chromosomes. They sought to determine the prevalence of Y chromosome microdeletion among infertile couples. Makker et al. (2009) studied why the male factor is considered a major contributory factor to infertility. Jarvi (1994) studied piyospermia and male infertility. Piyospermia is found in the semen analysis of up to 23% of men who are being investigated for infertility. The presence of significant numbers of white blood cells in the semen is correlated with poorer sperm parameters and diminished fertility.

The present study was conducted during July 2010 to February 2011 to investigate prevalence of asthenozoospermia and piyospermia in District Gilgit. Earlier to this no such studies on male infertility were conducted in Gilgit-Baltistan.

### Methodology

#### *Study area*

Gilgit-Baltistan is the northernmost political entity within Pakistan. Its administrative center is the city of Gilgit (population 216,760). It has seven districts namely Gilgit, Skardu, Ghizer, Diamer, Hunza Nagar, Astore and Ghanche. The health sector in Gilgit Baltistan is not much developed.

#### *Methods*

The total number of patients investigated was 87. The semen of suspected infertile patients referred by physicians/gynecologists of district hospital Gilgit, family health hospital and private maternity clinics for male infertility investigation in Gilgit was collected in sterilized open mouth container from July 2010 to February 2011. The most common way to collect a semen sample was through masturbation, directing the sample into a clean cup (Essig 2007).

A sample may also be collected during intercourse in a special type of condom known as a collection condom. Collection condoms are made of silicone or polyurethane, as latex is somewhat harmful to sperm (Ellington 2005). Many men prefer collection condoms to masturbation, and some religions prohibit

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# LACK OF ASSOCIATION BETWEEN VITAMIN D RECEPTOR *FOK*1 POLYMORPHISM AND URINARY TRACT INFECTION AMONG KARACHI POPULATION

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**ABSTRACT**

Present investigation was the first of its type carried out on Pakistani population with respect to vitamin D receptor (VDR) gene polymorphism in Urinary tract infection (UTI) patients. Vitamin D, a secosteroid belongs to the nuclear receptor family. In urinary tract it produces its action by binding with vitamin D receptor in bladder epithelial cells inducing the expression of cathelicidin, a potent antimicrobial peptide. Polymorphism in VDR gene may results in lowering the activity of vitamin D that leads to the susceptibility of UTI. Here, we evaluated VDR *Fok*1 gene polymorphism with its correlation to the risk of UTI in Karachites. Genomic DNA through proteinase-K method was extracted using mouth epithelial cells from 31 infected and 62 controls of both genders. VDR *Fok*1 gene polymorphism was evaluated though PCR-RFLP. A non-significant (p=0.699) association of VDR *Fok*1 gene polymorphism among cases and control was observed, however risk may be observed for Ff genotype with an odds-ratio of 1.255 (95%CI=0.476-3.310). Moreover, in controls the gene was in Hardy Weinberg Equilibrium (p=0.859).

**Key words:** *Fok*1, UTI, VDR, polymorphism

**9th International Symposium on Avian Influenza**
**The University of Georgia, Athens GA**
**April 12-15, 2015**

**1. Title:**

**Characterization of 10 adjuvants for inactivated Avian Influenza Virus (AIV) vaccines against challenge with highly pathogenic AIV in chickens**

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**Abstract:**

Inactivated vaccines comprise 95% of all vaccine used for avian influenza virus (AIV) by dose. Optimizing the adjuvant is one way to improve vaccine efficacy. Inactivated vaccines were produced with beta-propiolactone inactivated A/chicken/BC/314514-1/2004 H7N3 low pathogenicity AIV and standardized to 384 hemagglutinating units per dose. Ten commercial and experimental adjuvants were evaluated for antibody levels induced by 3 weeks post vaccination of specific pathogen free chickens. The adjuvants were also characterized for protection against morbidity and mortality, and reduction of oral virus shed after homologous challenge with a highly pathogenic (HP) AIV. The highest antibody titers were induced by the 3 mineral oil based adjuvants and a synthetic polymer based product. All of which resulted in 100% seroconversion. Incomplete Freunds' adjuvant, Chitosan, and a synthetic ester based polymer commercial product induced lower levels of antibody and 40-70% of the chickens had detectable antibody. Calcium phosphate, seaweed derived algenate and un-adjuvanted virus injected intramuscularly or subcutaneously produced no detectable antibody. One of 10 birds inoculated intravenously with un-adjuvanted virus did seroconvert to a low titer. Mortality was reduced to 10% or less by all adjuvanted groups except calcium phosphate (20% morality). Mortality in non-vaccinated and the groups which received un-adjuvanted virus was 60-100%. Virus shed was substantially reduced in all groups compared to non-vaccinated controls except those vaccinated with calcium phosphate, seaweed derived algenate and un-adjuvanted virus.

research article

# Differential Viral Load of HBV and HCV in Co-infected Patients: A Potential Battle between the Viruses

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## Abstract

Viral hepatitis is one of the major health problems in which Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) have the highest affinity to cause chronic liver disease. 5% of the people are infected from HBV and 1% are infected from HCV worldwide. In Pakistan 7.4% of the population is suffering with HBV and HCV (An Overview of Hepatitis B and C in Pakistan). Chronic infection of HBV and HCV leads to slow progressive liver diseases. In a period of 30 years or more, infection can lead to liver cirrhosis, chronic liver failure and hepatocellular carcinoma. Co-infection is more frequent than single infection particularly in areas where these two viruses are endemic. To observe and compare the viral loads of HBV and HCV in co-infected and mono infected patients in Karachi, Pakistan. A total 419 serum samples were collected from suspected patients with HBV and HCV infection. 276 were males and 143 were females, with the age ranging from 10 to 40 years. HBV DNA and HCV RNA were extracted and amplified by Abbott sp2000 and Abbott rt2000 respectively as per the manufacturing protocol. 419 serum samples were screened for HBV and HCV viral load by real time PCR. 276 (66%) were male and 143 (34%) were female. HBV viral load was detected in 157 patients (37.4%), HCV viral load was detected in 86 patients (20%), whereas HBV/HCV Co-infected patients were detected in 89 (21%) patients. In co-infected patients, an HCV viral load of <1000 was found in 37 (41%) patients while the viral load >1000 was 52 (58%) patients. The HBV viral load of <1000 was found in 32 (35.95%) patients and >1000 was in 57 (64.04%). 87 (20.76%) of the patients tested negative for both HBV and HCV. Pakistan is a developing country with limited economic and health care resources, low literacy rates and limited awareness about diseases. As HBV and HCV share the same routes of transmission, the chances of co-infection of HBV and HCV are increased. The incidence of co-infection also increases the chances of cancer possibly due to the combined oncogenic action of both viruses. Co-infected patients ranging from 10-40 years old showed that the incidence of infection is higher in younger males as compared to females. This may correlate with more exposure to high risk factors. The HBV viral load was higher than HCV viral load in co-infected patients, indicating that there may be a competition between the two viruses in which HCV is suppressed by HBV.

**Keyword:** HBV/HCV; Viral load; Pakistan.

## Introduction

Globally HBV and HCV are important human pathogens that can cause chronic liver disease which can lead to the degradation of life quality and death. It is estimated that nearly 350 million people around the world are infected with the Hepatitis B virus (HVB) and 170 million people are infected with hepatitis C virus (HCV)[1].

The viruses are classified as hepatotropic viruses because they replicate primarily in the liver. Both viruses belong to different families and therefore have different life cycles. HBV belongs to a Hepadnaviridae family and HCV belongs to a Flaviviridae family[2]. Co-infection of these two viruses is prevalent in areas where these viruses are endemic.

In non-endemic areas, co-infection of HBV/HCV is found in the high risk population which includes injection drug users, immunocompromised patients, organ transplantation recipients, and persons having multiple sex partners3. Some cross-sectional studies have shown that in comparison with mono-infection of HBV and HCV the co-infection has a

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# The Current Status of Research Bioethics in Pakistan

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## Introduction

The Islamic Republic of Pakistan is a developing country and has a population of more than 171 million people [1], although it has some large cities such as Karachi, Lahore, Peshawar and Quetta but almost 65% of the population is still living in rural areas, with a per capita income of $ 408 per year. The overall literacy rate is estimated to be 41.5% but is much lower for women particularly in small of the provinces [1,2]. Majority of the population take advantage of the free healthcare facility provided by the government in the public sector hospitals and health care units

due to low income, but these facilities are generally short-staffed, congested, overburden and incompetent which cannot meet the requirements of the people visiting these facilities however due to poverty and lack of national health coverage and health insurance schemes, a huge section of the population is deprived to have even access to any healthcare facility [3,4].

According to economic Survey 2009-2010, in Pakistan, Government has allocated only 2.0 per cent for the education which is very less in comparison with the other South Asian countries rather one of the lowest in the developing countries. 65 percent of enrolled students attend the public sector schools where education supposed to be free or less expensive. The quality of education in the public sector schools is usually very poor. The well privileged or those who can afford, go to the private sector schools where most of the cases quality education is provided but usually very expensive and beyond the reach of the masses, however education in low fee private sector schools is also not satisfactory [5]

Another segment of society who usually cannot afford to go to school usually go the Madrasas (institutions where usually religious education is provided) where they get free boarding and lodging along with education.

Still not all children in Pakistan are in school and yet another majority of the children who don't go or cannot afford to go to any of the institutions rather they are on the streets or support their family by earning in different vocational palaces. According to the Pakistan Labour Force Survey (2007-2008), there are over 21 million children between 10 to 14 years of age out of which 2.68 million are employed [6]

There are too many local issues and problems including terrorism, poverty, joblessness, and tension on the eastern and western borders, political and economical instability in the country. All these and other problems put the important sectors like education and health comparative in less priority and the government invests very less in these areas rather spends more in defense and to control the law and order situation in the country. Due to very less investment in education particularly in higher education and science the research bioethics seems to be luxury and no one speaks about this rather most of the time the politicians and the policy makers and even masses talk about the availability of basic needs and necessities. Bioethics or ethics in research is usually not thought as a separate subject at college or at university level, due which there is very less awareness and practices among the researches and professionals.

Research is mandatory element to define success of a nation. The most challenging task is maintenance of highest ethical standards in research. Research on human subjects is very challenging in developed & under developed countries [7]. People are less willing to participate in research subjects unless well compensated in developed countries, this made research very expensive compared to developing countries. So the pool of research is diverted towards developing countries but unfortunately outcome of the action has raised serious concerns to ensure the rights & ethical issues being well protected [8].

The primary aim of this paper is to report the current status of bioethics in Pakistan and try to propose guidelines to help to resolve controversial issues in a practical way that would lead to both scientific and ethical progress in research in the country. Issues administered during bioethics research -unethical application of science & modern medical technologies, collusion of health professionals, the physician-pharma nexus quiet an unholy alliance, international organ trade & transplantation. Finally, we will look at the need to implement guidelines in structural policy changes.

The history of bioethics in Pakistan is a rather short one though in our country, there is a need for a major capacity building exercise in Bioethics and Research Bioethics. In Pakistan it is primarily members of the medical community, many trained in Western institutions, who take the lead in introducing modern bioethics into the country. The first proper move in this direction occurred in 1984 at the Aga Khan University (AKU) in Karachi, where biomedical ethics was introduced in the curriculum of medical students at AKU by the Chairman of the Department of Community Health Sciences and later extended into the courses of the University's School of Nursing.

In Pakistan as other developing countries, there has been a great demand for research-ethics related programs. In 2001, the Pakistan Medical and Dental Council (PMDC), the certifying body for all graduating physicians and dentists, stipulated that biomedical ethics must be included in the medical curricula. However, this is still not the case in most of the country's research institutions, medical colleges, universities and many still limit themselves to a few informative lectures on the subject during rotation of students through community

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**(Technical base line studies conducted for UNDP for Pakistan GLOF Project Ministry of Climate Change as a Consultant).**

1. **Title: A study on the socio-economic impact of the GLOFs in Bagrot valley, at Gilgit-Baltistan**
   **(Dr. Nazir Ahmed, Consultant UNDP, GLOF Project).**


Summary

The report provides an overview and analysis of the impact of climate change on local livelihood in Bagrot valley, Gilgit. This report has been written based on information gathered through direct field survey using questionnaire forms, which were filled by the respondents. Field staff only assisted the individuals where literacy or other limitations made it necessary. The scope of the study was to conduct the socio-economic impact assessment of GLOFs (Glacial Lake Outburst Floods) on the local communities of Bagrot valley at Gilgit presently suffering from the disaster risks created by Bagrot Glacier and GLOFs. The Himalayas, Karakoram and Hindukush (HKH) lofty mountain ranges meet each other in Northern Pakistan hosting more than 5000 glaciers which feed snow/ice melt water to the Indus River System. Due to global warming and climatic changes the glaciers are melting very fastly and frozen water resources have been losing their reserves at an unprecedented rate, resulting in increased number of glacial lakes.

The GLOFs are the devastating hazards of mountains which have started occurring with increased frequency during the recent years in Gilgit-Baltistan. Glacial lakes pose a potential threat to downstream communities, but they are also a potential source of water storage for sustaining agriculture and forest-based livelihoods. The challenge is to minimize the risk of outburst and to reduce the vulnerability of nearby communities while securing the potential benefits of the lakes. Scientific information about existing glacial lakes, enhanced by monitoring and early warning systems, and mitigation measures to reduce the impact of glacial melting is essential. The potential

for serious losses due to GLOFs is likely to increase as climate warming progresses, new lakes are forming and existing ones are enlarging. Monitoring ice and water resources, promoting community resilience and preparedness for disaster risk reduction, and ensuring the sharing of upstream-downstream benefits are pivotal importance and needs detailed research. As glaciers and glacial lakes are related both to water resources and to water-related natural hazards, they need to be mapped, monitored and field surveys are required to be carried-out.

In the present survey the people of Gilgit in general and Bagrote valley in particular were found comparatively more educated then national average, and were open to discuss environmental hazards & their solutions. The population growth rate is very high in these areas and people generally have large family size, 80 % have a family size larger than five & at least 40% have a greater family size than eight members. The natural recourses are shrinking due to over population. The people of Bagrote valley have a modest income (35 % respondents have annual income, Rs. 100,000-200,000); class difference is rare, they want to work for better earnings, and people are easy to engage if financial incentives are offered. According to the survey data most of the people are working in government sector (48.9 %) or engaged in private sector, the traditional occupations like farming are on decline, and less educated or illiterate people are involved in agriculture and animal rearing. The majority of the respondents (92 %) think that agriculture is the main source of locally available livelihood. Traditional and local livelihood options are seasonal in nature and farmers are without most of the work in winters. The earthquakes, landslides, floods and GOLFs are the major natural hazard faced by local community in the area. The majority of the respondents agreed that the GOLFs, floods, landslides and earthquakes are causing damage of infrastructure, reducing land holding, and increasing poverty. In some cases it also caused loss of lives. Post-disaster affectees of GLOFS mostly want rehabilitation in their own lands and want to start a new

life, adjusting themselves with the nature again. The majority of respondents (46.6 %) perceived that rainfall & temperature have increased over the period of last decade and is likely to increase further causing melting of existing glaciers. The research data also indicates that 70 respondents (79.5 %) suggested to use fertilizers and animal manure for multiplying crops / obtaining better yield from crops where as 15 respondents (17 %) suggested technological improvement and 2 respondents (2.3 %) suggested certified seed for better yield of crops. The research also indicated that mostly traditional methods of farming are being used, however, chemical fertilizers are also commonly used.

Some of the farmers have resorted to cultivation of new crops like potato, however large no of farmers have taken no remedial measures. In the valley natural disasters have caused damage /reduction in agriculture livestock, forestry, telecommunication, tourism, schooling, health, and hydroelectric projects. The women and children, elderly and disabled are the most affected group by natural disasters. It was noticed that community leaders/ local medical practitioner/religious scholars/teachers are involved in disaster preparedness programs but their contribution is limited. There is a dire need for launching a comprehensive program for disaster management. It was found that lack of preparedness measures and early warning system is the major cause of damage to life & property. Majority of the respondents indicated that the risk reduction measures, both at the community level and by the Government are very limited. The rate to access to the facilities of health care, education, communication/transportation and electricity are poor. The majority (100 %) of the respondents strongly support the idea of organizing trainings / workshops / seminars to increase awareness in community and Government departments regarding risk assessment and management from natural hazards including GLOFs, and research to identify such risk and capacity building and establishment of institutional set up to address such issues. Furthermore, it

is very important that Government should formulate short term and long-term policies at local and national level and collaborate with international organizations which are already working and international knowledge is required to be downscaled to the local communities through academic institutions and NGOs so that the risk and losses to lives of people and agriculture could be minimized.

**(A Technical base line study conducted for UNDP for Pakistan GLOF Project)**

2. **Title: KAP studies on GLOFs at Bagrot Valley, Gilgit-Baltistan**

**(Dr. Nazir Ahmed, PhD)**

**Executive Summary**

The present study was a pre-intervention survey to assess the knowledge, attitude and practices (KAP studies) of local community of Bagrote valley, awareness and knowledge of media personnel, NGOs, government departments and members of Gilgit- Baltistan legislative assembly about GLOF (Glacial Lake Outburst Floods), climate change and its impact on livelihood of local community of Bagrote, Gilgit-Baltistan. Environmental sustainability is increasingly threatened by large-scale changes to the natural environment due to rapid industrialization, over population and un-wise use of natural resources and has adversely affected human, animal and plant health and ecosystem. Sustainable behaviour change is needed to reduce greenhouse gas emissions, reduce natural disaster risk including GLOFs, mitigate related impacts, and develop the capacity to adapt to future climate and environmental changes. Towards these ends, it is necessary to understand how members of the public perceive and behave in relation to global environmental change. In particular, this study focuses on results from qualitative, semi-structured in-depth interviews (n=91) with adults in Gilgit, Baltistan Pakistan. Participants were asked questions about climate change, its impact on GLOFs, floods, draught, temperature, forest core, agriculture, health

and environment. The participants were also asked questions to know their knowledge and attitudes of global and local environmental changes, actions taken to mitigate environmental change, and potential behaviour change mechanisms. Results indicate that although participants are environmentally aware and concerned to some extent about local environmental issues (eg. floods, draughts, deforestation, air pollution, GLOFs, global warming and ozone depletion), however, detailed knowledge of specific causes, impacts and risks of climate change and GLOFs is very limited.

In the present survey the people of Baltistan in general and Bagrote valley in particular were found to be more educated then national average, and people are very cooperative and open to discuss environmental hazards & their possible solutions. The population growth rate is very high in the valley (80 % of population have family size larger than five & about 40% have a greater family size than eight members) and the natural recourses are shrinking due to increased population. The people of Bagrote valley have very rare class difference and have limited earnings and the people of the area want to work hard for better earnings. The results of the study indicates that most of the educated people are working in government sector (48.9 %) or engaged in private sector, the traditional occupations like farming are declining day by day, and less educated people are mostly working in agriculture and animal rearing. The majority of the respondents (92 %) are off the opinion that agriculture is the main source of their livelihood and seasonal in nature as most of the farmers are without work during winter season. The natural disasters including earthquakes, landslides, floods and GOLFs are the major hazards faced by local community in Bagrot valley. The majority of the respondents (52.7 % and 53.8 %) agreed that the highland glacial lakes are very sensitive and mountain areas lakes are sensitive to GLOFs and climate change. The natural disasters like GOLFs, floods, landslides and earthquakes are causing loss of human lives,

damaging infrastructure, agriculture and livestock and resultantly increasing poverty. Most of the post-disaster affectees of GLOFs and other disasters want rehabilitation in their own lands. The majority of respondents (71.4 % and 56 %) think that the temperature and rainfall will increase in future, which may cause melting of existing glaciers and will result increased GLOFs, draughts, and may affect livelihood (food sufficiency, health, income and standard of living) of the local community. The study shows that 89.0 % of respondents think that climate changes may entail many problems in daily life. The majority of the respondents (41.8 %) and (40.7 %); observed that the major causes of climate change are global warming, and environmental pollution subsequently while 12 % showed no knowledge and 5 % of the respondents think it is due to deforestation. Besides afore mentioned causes the majority of the respondents (72.5 %) are of the opinion that the industrialized nations have played major role inclimate change and global warming. The respondents have great expectations from international community to play a leading role and support the local community to reduce the risk of GLOFs and climate change induced disasters.

The study also indicated that the farmers and other organizations are not able to cope with climate change induced disasters and the community do not have any concrete plans, there is no institutional set –up- regarding disaster management (97.8 % respondents). The community using traditional methods for protecting forest core, preventing soil erosions, and managing natural resources. The majority of the local people are using chemical fertilizers (68.1 %), animal manure (22.0 %), and technologies (9.9 %) to increase their crop yield. The changing weather pattern and consequent impact has also contributed to a change in the cropping pattern (cropping calendar, crop variety, yield and diseases). In livestock sector, the impact of climate change was perceived to be the drying up of grass in pastures thus reducing the availability of natural fodder, and the

shifting of some pasture sites to higher altitude. The respondents also indicated that the altered climatic conditions have also significant impact on the overall environment, in the form of depletion of resources, such as natural vegetation, forest cover and medicinal plants. Many species of birds (e.g. chakor , ram chakor (partridge) and animals (e.g. black bear, keil, leopard, markhor and wolf) are not being sighted anymore. Since communities have long experience of coping with variable climate, local knowledge and existing coping strategies have been considered when planning responses targeted towards cultural and resource conservation, and management. Traditional adaptation strategies adopted by the community have been noticed in the construction of buildings, clothing, agriculture and fuel wood use. However, coordinated efforts by concerned authorities is required to institute an effective and planned adaptation response, which constitutes local knowledge, but also builds capacity of the community to adopt scientifically suggested practices. The rate to access to the basic needs of life including health care, education, communication/ transportation and electricity are poor in the valley. The majority (100 %) of the respondents strongly support the idea of education local community, government departments and relevant organizations about risk assessment and management from natural hazards including GLOFs. The respondents also indicated that there is dire need to build and establish institutional set up to address such issues in Gilgit and Bagrote Valley.

The survey also indicated that more than 91 % respondents felt it necessary that policy – including new/ revised legislation, bills, act of parliaments / assembly etc should be formulated and implemented to reduce risk from natural disasters. The respondents (91.2 %) also agreed that economic incentives and financial mechanisms should be introduced and awareness –raising campaigns should also be increased by organizing workshops, seminars and conferences on these vital issues. The respondents also felt that electronic and print media is not paying due attention to

risk associated with GLOFs and other natural disasters and it can play a leading role in sensitizing all stakeholders. Furthermore, it was also observed during discussions with village notables at Bagrot and they demanded that the Government should formulate short term and long term policies at local and national level and collaborate with international organizations so that scientific knowledge and international experience could be transferred / downscaled to the local communities which may help to reduce the risk of GLOFs and other climate change induces natural disasters. Majority of the participant's demonstrated willingness to act in environment friendly ways, and respondents described possible environment-friendly activities such as use of less firewood, reforestation, controlled use of energy sources etc. The respondents also urged the constructions of check dams to restrain floods and GLOFs, awareness raising campaigns, and training of local people about early warning systems. There is a dire need for launching a comprehensive program for climate change induced disaster management. It was found that lack of preparedness measures and early warning system is the major cause of damage to life & property. Majority of the respondents indicated that the risk reduction measures, both at the community level and at the Government level are very limited and there is absence of formal disaster related institutional arrangements, no early warning system is in-place, and no adaptation structure at community level is existing at Bagrot and other GLOF or natural disaster prone valleys of Gilgit-Baltistan and there is lack of disaster response cells at district level.

The majority of the respondents 91-98 % felt it necessary that adaption measures including identification of GLOF vulnerable areas, review of community values and identification of root causes of GLOFs, landscape planning, less firewood and re-forestation, educate and encourage environment friendly agriculture and human activities, restriction on illegal mining, improved forecasting, monitoring information and early warning system have not been planned yet and these

adaptation measures should be planned and implemented to reduce the risk and losses of GLOFs and climate change induced disasters. Considering the present gap in addressing climate change, this report attempts to suggest a sector wise action plan (for mitigation and adaptation) and management implications, to integrate climate change in the Bagrote valley and other valleys of Gilgit- Baltistan. In doing so, more specifically Bagrote valley, can transform into a model for conserving other vulnerable ecosystems and heritage, as part of a regional climate change induced management strategies.