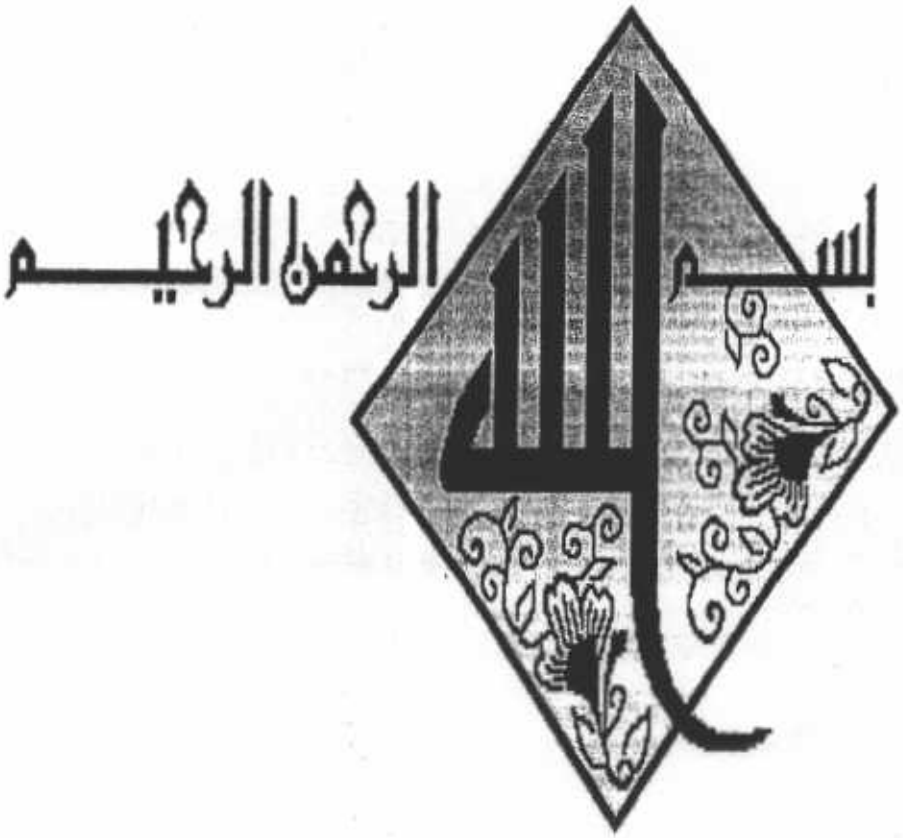

In The Name Of Allah
The Most Gracious, The Most Merciful

Adaptation and Evaluation of Infectious Bursal Disease Virus (Local Isolates) on Embryonated Chicken Eggs for Production of Vaccine

By

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To

The Controller of Examinations,
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We the supervisory committee, certify that the contents and form of the thesis submitted by Mr. Atif Nisar Ahmad Regd. No. 92-ag-926 have found satisfactory and recommend that it be processed for the evaluation by the external examiner(s) for the award of degree.

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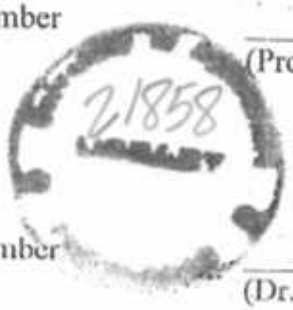

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CHAPTER 1

INTRODUCTION

Pakistan is an agricultural country. It contributes to 23.3 percent in the total GDP. Livestock plays an important role in the agricultural economy of Pakistan, It accounts for 49.1 percent of agricultural value added and about 11.4 percent of the GDP (Anonymous, 2004). Poultry production has emerged as a good substitute of beef and mutton. Its importance can be judged from the fact that every family in rural areas and every fifth family in urban areas is associated with poultry production activities in one way or the other. In Pakistan, we have about 356 million day-old chicks, 280.1 million broilers, 22.1 million layers and 6.5 million breeding stock. The production of poultry meat is 402 thousand tones and of eggs is 8,247 million (Anonymous, 2004).

Despite efforts at improved management methods, feed formulation and disease preventive measures adopted in the public and private sectors, overall poultry population remained victim to a host of different infectious and noninfectious diseases. Among the infectious diseases, immunosuppressive viral diseases have an important impact on poultry health and production. In this context, infectious bursal disease (IBD) has been a great concern for the poultry industry not only in Pakistan but worldwide during the last decade.

Infectious bursal disease (IBD) is a highly contagious, globally occurring viral poultry disease. The disease was first reported by Cosgrove, who in 1957 observed a disease affecting chicken on farms in the neighborhood of Gumboro, Delware, USA (Cosgrove, 1962). Thus Gumboro disease became synonymous for the condition which was previously known as "avian nephrosis" because of the extreme kidney damage in infected birds. The disease is characterized

by sudden onset, short course, extensive destruction of lymphocytes and profuse watery diarrhoea, followed by death or rapid recovery (Tsukamoto *et al.*, 1992). The economic importance of the disease is manifested in two ways; firstly, some virus strains may cause up to 20-30% mortality in three-week-old chickens and older. The second and the most important manifestation is severe prolonged immunosuppression in chickens infected at an early age (Van den Berg, 2000).

The disease is caused by infectious bursal disease virus (IBDV), which is a member of genus *Avibirnavirus* of family *Birnaviridae*. This is a dsRNA virus with a bisegmented genome which is enclosed within an icosahedral, non-enveloped capsid of 55-65 nm in diameter (Lukert and Saif, 2003). The larger segment A encodes a polyprotein which is cleaved into four viral proteins designated as VP2 (41KDa), VP3 (32KDa), VP4 (28KDa) and VP5 (21KDa). The smaller segment B encodes only VP1 (90KDa) which has polymerase activity. VP2 and VP3 are the major proteins of IBDV (Van den Berg, 2000; Lukert and Saif, 2003). Gumboro disease virus exists in two antigenically distinct serotypes i.e. 1 and 2, and only serotype 1 which displays a wide variation of pathogenic potential is virulent for chickens whereas serotype-2 is nonpathogenic and accurs naturally in turkeys. There are two serotype-1 antigenic types, classic/standard and variant groups. Control of IBD has been complicated by the recognition of these variant strains of serotype-1. These strains differ from standard strain in biological properties and can break maternal immunity against standard strains. Similarly, serotype-2 virus does not protect against challenge with serotype-1 viruses (Jackwood and Saif, 1987; Lukert and Saif, 2003).

Immunization is the principle method used for control of IBD in chickens. The development of vaccines against IBD has been considered as a great triumph of veterinary research. In Pakistan, the poultry industry suffers a major mortality and hence huge economic losses despite the wide use of imported live IBD vaccines. These vaccines are not antigenically similar to those of our field challenge but they differ from indigenous virus in the number and concentration of polypeptides (Anjum *et al.*, 1997).

Vaccination against the disease has been powerful tool in controlling IBD, thus reducing economic losses. Most of the inactivated vaccines against infectious bursal disease are prepared by

destroying the infectivity with chemicals such as formalin, beta propiolactone, phenol and binary ethylenimine (BEI). The beta propiolactone is considered as carcinogen, while phenol induces allergic encephalitis (Mohanty and Dutta 1981). Formalin denatures the protein of the virus that leads to the phenomenon of the membrane effect in which the reaction closes the outer protein shell of the virus before the nucleic acid of the infectious genome is destroyed. BEI member of the group of alkaline substances aziridines reacts very little with proteins and probably does not alter the antigenic components of the virus. BEI has an inactivation reaction that is more specific for the nucleic acid and it produces antigenically superior vaccine (Bahnemann, 1997). By itself BEI is a toxic substance. However, at the end of inactivation process, residual BEI is inactivated through addition of adjusted amount of sodium thiosulfate. So BEI inactivated vaccines are safe to use.

Commercial vaccines against IBD available in Pakistan are imported. Antigenic variation have been observed between local field isolates and imported IBD vaccines virus (Anjum *et al.*, 1997). A huge amount of money is being spent for the import of IBD vaccines in Pakistan, but the disease outbreaks even occur in spite of vaccination as these vaccines have pathogenic and immunosuppressive effects. The need exists for an effective infectious bursal disease vaccine, low in virulence which can be applied by a mass vaccination procedure such a vaccine would minimize immunosuppression and also immunized young chicks possessing passively conferred IBD immunity (Parveen *et al.*, 1995; Hussain *et al.*, 2001).

IBDV was grown in the embryonated chicken eggs (Hitchner, 1970; Yamaguchi *et al.*, 1996; Abdel Alim and Saif, 2002) and cell culture (Jackwood *et al.*, 1987; Abdel Alim and Saif, 2001) for the production of vaccine (Jeffrey and Jackwood, 2001). Strains of IBDV show reduced virulence during passage in embryonated eggs and chicken embryo fibroblast (CEF) cells. Most strains of wild type IBDV recovered from infected bursa do not replicate in CEF cells (Izawa *et al.*, 1978). With successive passages, however the virus becomes progressively adapted to growth in vitro has been associated with attenuation of virulence as evidence by a reduction in ability of the virus to induce bursal lesions. Recently the highly virulent IBD virus strains were adapted to serial passages in embryonated eggs. The ECE adapted strains showed considerable reduction in virulence (Yamaguchi *et al.*, 1996). The embryonated chicken eggs or cell adapted strains showed the good

protection against the fatal infection of IBDV. Extended in vitro passage of virus has been proposed as an approach to the development of attenuated live vaccine.

In Pakistan no work has been reported for the adaptation of local IBDV on embryonated chicken eggs for the production of vaccine. Keeping in view the tremendous import of IBD vaccine in poultry industry, and to save precious foreign exchange, the present work was designed to achieve the following objectives.

1. Adaptation and attenuation of field isolates of IBDV in chicken embryos
2. To detect the antigen titer in various passages of chicken embryos
3. To detect the pathogenecity of attenuated virus in the chicken
4. To ascertain the comparative immunogenicity and pathogenicity of locally isolated virus inactivated with BEI and formalin with and without adjuvant
5. To compare the attenuated and field isolates with commercial vaccine using SDS-PAGE
6. To evaluate the vaccines on the basis of humoral, cellular and challenge response

CHAPTER 2

REVIEW OF LITERATURE

Review of the previous literature and previous work done in a field provides a guideline in designing the scientific studies through the identification of the weaknesses of the previous studies. This chapter furnishes a review of some relevant literature about the history, morphology of virus, epidemiology, pathogenesis, clinical signs, gross pathology, histopathology, immunosuppression, diagnosis, isolation (embryonated eggs and cell culture), serology and immunization against infectious bursal disease (IBD).

1. HISTORY

Infectious bursal disease (IBD) also known as Gumboro disease was first recognized in 1957, in Southern Delware, USA by Cosgrove (Cosgrove, 1962). Winterfield and Hitchner (1962) described a virus isolate (Gray) that came from a field case of nephrosis not unlike the newly reported syndrome. Because of the similarity between kidney lesions induced by Gray virus and those seen in avian nephrosis as described by Cosgrove (1962), it was believed that Gray virus was the causative agent. Winterfield *et al.* (1969) succeeded in isolating an agent in embryonated eggs. The isolate was referred to as 'infectious bursal agent' and was identified as the true cause of IBD. Gray virus was identified as an isolate of infectious bronchitis virus with nephrotoxic tendencies. The term infectious bursal disease was proposed by Hitchner (1970), as the name of the disease causing specific pathognomonic lesions of the cloacal bursa.

Allan *et al.* (1972) reported that IBD virus (IBDV) infection at an early age was immunosuppressive. The existence of a second serotype was reported in 1980 (McFerran *et al.*, 1980). Control of IBD viral infections has been complicated by the recognition of 'variant' strains of serotype-1 IBDV, which were found in the Delmarva poultry producing area (Saif,