Chapter 3
Exposition of the study

Rotavirus, the leading cause of acute infectious diarrhea in infants and young children is an important public health problem worldwide. Each year rotavirus causes 600,000 deaths, 2 million hospital admissions and 111 million cases of gastroenteritis requiring only home care in children below 5 years of age. As a result, by the age of 5, nearly every child will have an episode of rotavirus gastroenteritis, 1 in 5 will visit a clinic, 1 in 50 will be hospitalized, and approximately 1 in 205 will die (Glass et al. 2006). In both developed and developing countries alike, these cases lead to an annual economic loss of several millions US$ in direct and indirect medical care (Zimmerman et al. 2001; Nelson et al., 2005; Chun et al., 2006). However, the major burden of rotavirus illness is currently experienced by the developing world accounting for majority of the deaths due to rotavirus (Parashar et al 2006). According to a recent report, India alone accounts for 122,000 to 153,000 deaths, 457,000 to 884,000 hospitalizations and 2 million out patient visits due to rotavirus infection in children aged below 5 years and spends Rs.2 to 3.4 billion in treatment of rotavirus diarrhea annually (Tate et al. 2009). Further, evidences are emerging for long term consequences of early childhood diarrhea (in the first 2 years of life) on physical growth and cognitive development that may translate into impairment of human potential and productivity (Niehaus et al., 2002).

Given the magnitude of the disease and associated economic burden of rotavirus infections, different strategies, including both active and passive immunization/immunotherapy, have continuously been attempted to develop a safe and effective intervention to achieve reduction in the mortality, severity of disease and associated hospitalizations (Kirkwood and Buttery 2003; Weiner et al. 1999; Pant et al. 2006). Two new generation rotavirus vaccines, RotaTeq and Rotarix have been introduced in the global market. The vaccines are reported to have good safety and efficacy records with an immunization regimen of two (at 2 and 4 month of age) and three (at 2, 4 and 6 month of age) doses of Rotarix and RotaTeq respectively (Ruiz- Palacios et al. 2006; Vesikari et al. 2006). In developed countries, the efficacy of these vaccines has been reported to be significant against rotavirus gastroenteritis of any severity (74- 79%), severe
rotavirus gastroenteritis (90-98%) and hospitalization due to rotavirus (96%) (Dennehy 2008). As against this, in impoverished children from Asian and African countries, a low level (39.3-48.3%) of efficacy of rotavirus vaccine probably attributable to high concentration of transplacentally acquired maternal antibodies, interfering gut flora and diarrheal diseases of enteric infections other than rotavirus, micronutrient malnutrition and associated immunodeficiency and concomitantly administered oral poliovirus vaccine is described recently (Armah et al., 2010; Zaman et al., 2010). While inclusion of rotavirus vaccine in national childhood immunization program is under consideration in these settings (Esposito et al., 2011), exploration of the alternate and adjunct strategies such as oral immunotherapy and antivirals carries valued significance for management of rotavirus infection.

Neutralizing antibodies (NAbs) generated against two outer capsid proteins, VP7 and VP4 of rotavirus have been described as correlates of protection from rotavirus disease (Offit et al.1986a, b; Matsui et al., 1989; Green and Kapikian, 1992). These antibodies prevent infection locally by inhibiting rotavirus attachment to susceptible cells in the intestinal lumen (Ruggeri et al., 1998; Westerman et al., 2005). As a corollary to this, oral delivery of rotavirus specific neutralizing milk, serum antibodies and monoclonal antibodies have been demonstrated to impart local passive protection against rotavirus infection (Hammerstrom 1999). However, the practical application of these antibodies as oral immunotherapy may be limited because of difficulties in large-scale production.

In recent years, a considerable attention has been drawn towards chicken eggs as a good source of conveniently packaged antibodies useful for immunotherapeutics and diagnostics. Chicken produce a unique immunoglobulin molecule called IgY that is functionally homologous to mammalian IgG (Warr et al., 1995). IgY, the serum antibodies of chicken are passed from hens to embryo via the egg yolk. Laying hens immunized against different antigens, transport high concentration of specific IgY to the egg yolk. Nonreactivity of IgY with mammalian Fc receptors, an evolutionarily distinctive biochemical property makes IgY antibodies appropriate for per oral immunotherapy (Larsson and Sjöquist, 1990; Carlander et al., 2000). In addition, IgY is phylogenetically nearer to mammalian secretory IgA, making it a natural part of the mucosal epithelial environment (Hadge and Ambrosius, 1984). They are resistant to high
temperature and low pH and have a half-life of 3-5 days in the environment of gut (http://www.hyperimmunegg.org/questions.htm). Hence, hyperimmune egg stands excellent source of antibody as well as functional food for children and immunocompromized patients. Further, a hen lays a large number of eggs per year from which a good amount (~40 g) of IgY antibody can be obtained. Since the housing and caring for millions of chickens is well developed in poultry industry of India, feasibility of economic production of yolk antibodies can be envisaged.

The preformed neutralizing antibodies derived from egg yolk of hens hyperimmunized against rotavirus antigens can serve as effective and affordable choice of prophylaxis and therapy to reduce rotavirus disease incidence and severity of infection that may lead to fatal disease. Introduction of the hyperimmunized egg yolk (HEY) antibody therapy could act as a readily available biological drug against rotavirus infection in economically weaker section of society that practically remains out of reach of prompt medical care. For the evaluation of potential of this strategy as an intervention against rotavirus infection, preparation of high titered, good quality IgY antibodies and examination of their protective efficacy in rotavirus diarrhea at laboratory stage are highly required. Although, the beneficial effect of specific IgY antibodies has previously been described against rotaviruses (Kuroki et al., 1993; Ebina 1996; Sarkar et al., 2007), these studies have rarely included the production and evaluation of IgYs against globally prevalent rotavirus serotypes, G1-G4 and G9, essentially required for practicability of the approach. Further, the effect of orally supplied rotavirus specific IgY antibodies on the intestinal viral load and cellular pathological changes has not been examined. In view of this, the present study was designed to achieve the following objectives:

1) Preparation of immunoglobulins (IgY) against HRVs in egg yolk
2) Evaluation of protective efficacy of IgY immunoglobulins by
   (a) Cell culture based in-vitro neutralization and
   (b) In-vivo studies using small animal model

The study was divided into three parts that are presented in the upcoming sections of this thesis.